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REVIEW

ANATOMY OF THE ANTIBODY MOLECULE

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Abstract—The structures of the various regions of an antibody molecule are analysed and correlated with biological function. The structural features which relate to potential applications are detailed.

Key words: X-ray structures, antibody–ligand interactions, polyreactivity, humanization strategies.

INTRODUCTION

In terms of structure, antibodies are probably now the most studied of all proteins. Amino acid and nucleotide sequence data are available for thousands of different chains (Kabat *et al.*, 1991) and three-dimensional structures, obtained by X-ray crystallographic analysis, are available for whole antibodies and for a variety of fragments. Very recently, all parts of an antibody molecule have been visualized (Harris *et al.*, 1992), including the hinge which, previously, either was only partly discernible in the electron-density map because of its flexibility (Marquart *et al.*, 1980), or was deleted (Sarma *et al.*, 1971; Silverton *et al.*, 1977; Sarma and Laudin, 1982; Rajan *et al.*, 1983) in the intact antibodies analysed by X-ray diffraction.

The antibody fragments whose structures have been elucidated crystallographically include several human and murine V_L dimers (Epp *et al.*, 1974, 1975; Fehlhammer *et al.*, 1975; Colman *et al.*, 1977; Furey *et al.*, 1983; Stevens *et al.*, 1981; Steipe *et al.*, 1992), Fv from murine and human antibodies (Bhat *et al.*, 1990; Fan *et al.*, 1992) including a "humanized" murine Fv (Eigenbrot *et al.*, 1993), several Bence–Jones light-chain dimers (Schiffer *et al.*, 1973, 1989; Ely *et al.*, 1985), Fc from human and rabbit IgG (Deisenhofer, 1981; Sutton and Phillips, 1983) and pFc′ from guinea pig IgG (Bryant *et al.*, 1985). The structure of the complex of human IgG1 Fc and fragment B of protein A from *Staphylococcus aureus* has also been determined (Deisenhofer, 1981).

By far, the fragment which has been the most studied is the Fab, with the structures of several dozen Fabs of different ligand-binding specificities having been elucidated by X-ray diffraction, many with bound specific ligand. The ligands range in size from small haptens to macromolecules. The small ligands include phosphocholine (Padlan *et al.*, 1973, 1985; Segal *et al.*, 1974), a hydroxy derivative of vit. K_1 (Amzel *et al.*, 1974), a dinitrophenyl-spin-label hapten (Bruenger *et al.*, 1991), fluorescein (Herron *et al.*, 1989), digoxin (Bruenger, 1991) and progesterone (Wilson *et al.*, 1991). The bigger ligands include oligopeptides, like cyclosporin A (Altschuh *et al.*, 1992; Vix *et al.*, 1993), angiotensin II (Garcia *et al.*, 1992), a 19-amino acid peptide homolog of a myohemerythrin helix (Stanfield *et al.*, 1990), a 15-residue long peptide derived from cholera toxin (Shoham *et al.*, 1991) and a nonapeptide from influenza virus hemagglutinin (Rini *et al.*, 1992), and fragments of other macromolecular antigens, like a trinucleotide of deoxythymidylic acid (Herron *et al.*, 1991) and a trisaccharide epitope of a branched bacterial lipopolysaccharide (Cygler *et al.*, 1991). The macromolecular ligands include lysozyme (Amit *et al.*, 1986; Sheriff *et al.*, 1987; Padlan *et al.*, 1989; Fischmann *et al.*, 1991; Lescar *et al.*, 1993), influenza virus neuraminidase (Colman *et al.*, 1987, 1989; Tulip *et al.*, 1989, 1992*a*, *b*), HIV-1 reverse transcriptase (Arnold *et al.*, 1992), and even another Fab in an idiotope–anti-idiotope complex (Bentley *et al.*, 1990). In addition, X-ray structures are available for an Fab complexed with streptococcal protein G (Derrick and Wigley, 1992), for a chimeric Fab in which the variable domains were from a murine antibody and the constant domains were from human molecules (Brady *et al.*, 1992), and for two versions of a "humanized" murine Fab (Eigenbrot *et al.*, 1993).

The availability, in some cases, of both complexed and uncomplexed structures for the same antibody permits the evaluation of the possibility of conformational changes occurring upon ligand binding. Previously, with phosphocholine and vit. K_1OH, no conformational changes in the Fabs were observed when these small ligands were bound in the crystal (Padlan *et al.*, 1973, 1985; Segal *et al.*, 1974; Amzel *et al.*, 1974). More recent studies with larger ligands reveal that significant changes can occur upon complexation (Bhat *et al.*, 1990; Stanfield *et al.*, 1990; Herron *et al.*, 1991; Wilson *et al.*, 1991; Rini *et al.*, 1992).

Starting soon after crystallographic data became available, various aspects of antibody structure and function have been the subject of analysis, including the distribution of the different amino acid types in relation to ligand-binding specificity (e.g. Kabat *et al.*, 1977;

Table 1. Immunoglobulin structures which have been determined by X-ray crystallography

Antibody	Isotype	Fragment	PDB Code	Resolution	R-value	Ligand	Reference
(Human)							
Dob	(IgG1,κ)	whole Ig		4.0			Sarma and Laudin (1982)
Kol	(IgG1,λ)	whole Ig	2IG2	3.0	0.207		Marquart *et al.* (1980)
Mcg	(IgG1,λ)	whole Ig		2.8			[a]
NEW	(IgG1,λ)	Fab	7FAB	2.0	0.169		Saul and Poljak (1992)
		Fab		3.5		vit. K_1OH	Amzel *et al.* (1974)
Kol	(IgG1,λ)	Fab	2FB4	1.9	0.189		Marquart *et al.* (1980)
Hil	(IgG1,λ)	Fab	8FAB	1.8	0.173		[b]
3D6	(IgG1,κ)	Fab	1DFB	2.7	0.177		He *et al.* (1992)
POT	(IgM,κ)	Fv	1IGM	2.3	0.201		Fan *et al.* (1992)
	(IgG1)	Fc	1FC1	2.9	0.22		Deisenhofer (1981)
	(IgG1)	Fc	1FC2	2.8	0.24	fragment B of Protein A	Deisenhofer (1981)
Mcg	(λ)	L-dimer	2MCG	2.0	0.187		Ely *et al.* (1989)
			3MCG	2.0	0.208		Ely *et al.* (1989)
LOC	(λ)	L-dimer	1BJL	3.0	0.194		Chang *et al.* (1985)
		L-dimer	2BJL	2.8	0.216		Schiffer *et al.* (1989)
Mcg-Weir	(λ)	L-dimer	1MCW	3.5	0.170		Ely *et al.* (1985)
REI	(κ)	V_L-dimer	1REI	2.0	0.24		Epp *et al.* (1975)
Au	(κ)	V_L-dimer		2.5	0.31		Fehlhammer *et al.* (1975)
Rhe	(λ)	V_L-dimer	2RHE	1.6	0.149		Furey *et al.* (1983)
ROY	(κ)	V_L-dimer		3.0	0.33		Colman *et al.* (1977)
Wat	(κ)	V_L-dimer					Stevens *et al.* (1981)
("Humanized" murine)							
4D5		Fv	1FVC	2.2	0.183		Eigenbrot *et al.* (1993)
		Fab	1FVD	2.5	0.179		Eigenbrot *et al.* (1993)
		Fab	1FVE	2.7	0.171		Eigenbrot *et al.* (1993)
(Murine–human chimera)							
B72.3		Fab	1BBJ	3.1	0.176		Brady *et al.* (1992)
(Murine)							
Mab231	(IgG2a)	whole Ig		3.5	0.188		Harris *et al.* (1992)
McPC603	(IgA,κ)	Fab	1MCP	2.7	0.225		Satow *et al.* (1986)
		Fab		3.1	0.196	phosphocholine	[c]
J539	(IgA,κ)	Fab	2FBJ	1.95	0.194		[d]
D1.3	(IgG1,κ)	Fab	1FDL	2.5	0.184	lysozyme	Fischmann *et al.* (1991)
		Fab		2.6	0.27		Bentley *et al.* (1989)
NC41	(IgG2a,κ)	Fab	1NCA	2.5	0.191	influenza virus neuraminidase	Tulip *et al.* (1992a)
		Fab	1NCC	2.5	0.212	neuraminidase I368R mutant	Tulip *et al.* (1992b)
		Fab	1NCB	2.5	0.165	neuraminidase N329D mutant	Tulip *et al.* (1992b)
		Fab	1NCD	2.9	0.157	influenza virus neuraminidase	Tulip *et al.* (1992a)
HyHEL-5	(IgG1,κ)	Fab	2HFL	2.54	0.245	lysozyme	Sheriff *et al.* (1987)
HED10		Fab		3.0	0.272		Cygler *et al.* (1987)
CF4C4	(IgG1,κ)	Fab		4.0	0.470		Vitali *et al.* (1987)
Jel42		Fab		3.5	0.282		Prasad *et al.* (1988)
NC10		Fab		3.0	0.20	influenza virus neuraminidase	Tulip *et al.* (1989)
HyHEL-10	(IgG1,κ)	Fab	3HFM	3.0	0.246	lysozyme	Padlan *et al.* (1989)
4-4-20	(IgG2a,κ)	Fab	4FAB	2.7	0.215	fluorescein	Herron *et al.* (1989)
R19.9	(IgG2b,κ)	Fab	2F19	2.8	0.182		Lascombe *et al.* (1992)
		Fab	1FAI	2.7	0.189		Lascombe *et al.* (1992)
E225	(IgG2b,κ)	Fab		2.5	0.179	D1.3 Fab	Bentley *et al.* (1990)
B13I2	(IgG1,κ)	Fab	2IGF	2.8	0.22	myohemerythrin peptide	Stanfield *et al.* (1990)
		Fab	1IGF	2.8	0.18		Stanfield *et al.* (1990)
MAb131	(IgG1,κ)	Fab		3.0	0.25	angiotensin II	Garcia *et al.* (1991)
BV04-01	(IgG2b,κ)	Fab		2.66	0.191	$d(pT)_3$	Herron *et al.* (1991)
		Fab		2.0	0.246		Herron *et al.* (1991)

Table 1. Continued overleaf.

Table 1.—Continued

36-71	(IgG1,κ)	Fab	6FAB	1.85	0.248		Strong *et al.* (1991)
AN02	(IgG1,κ)	Fab	1BAF	2.9	0.195	DNP-spin-label hapten	Bruenger *et al.* (1991)
NQ10/12.5	(IgG1,κ)	Fab		2.8	0.182		Alzari *et al.* (1990)
		Fab		3.0	0.19	2-phenyl-oxazolone	Alzari *et al.* (1990)
TE33		Fab		3.0	0.386	cholera toxin peptide	Shoham *et al.* (1991)
Se155-4	(IgG1,λ)	Fab		2.05	0.185	dodecasaccharide	Cygler *et al.* (1991)
26-10		Fab		2.7	0.17	digoxin	Bruenger (1991)
		mutant Fab		2.5	0.21		Bruenger (1991)
B13A2		Fab					Wilson *et al.* (1991)
HC19	(IgG1,λ)	Fab		3.5	0.176		Bizebard *et al.* (1991)
DB3	(IgG1,κ)	Fab		2.8			Wilson *et al.* (1991)
		Fab		2.4		progesterone	Wilson *et al.* (1991)
		Fab		2.8		iodobenzoyl progesterone	Wilson *et al.* (1991)
		Fab		3.0		11α-hemisuccinyl progesterone	Wilson *et al.* (1991)
R454511	(IgG1,κ)	Fab		2.65	0.185	cyclosporin A	Altschuh *et al.* (1992)
mAb28		Fab		7		HIV-1 reverse transcriptase and DNA	Arnold *et al.* (1992)
Jel72		Fab					[e]
Jel318		Fab					[f]
		Fab				protein G	Derrick and Wigley (1992)
Yst9-1	(IgG2b,κ)	Fab	1MAM	2.5	0.215		[g]
17/9	(IgG2a,κ)	Fab	1HIN	3.1	0.22	hemagglutinin peptide	Rini *et al.* (1992)
		Fab	1HIM	2.9	0.20	hemagglutinin peptide	Rini *et al.* (1992)
		Fab	1HIL	2.0	0.19		Rini *et al.* (1992)
8F5	(IgG2a,κ)	Fab	1BBD	2.8	0.190		Tormo *et al.* (1992)
F9.13.7		Fab		4.0	0.409	guinea-fowl lysozyme	Lescar *et al.* (1993)
D1.3		Fv		2.4	0.19	lysozyme	Bhat *et al.* (1990)
		Fv		1.9	0.19		
McPC603		V_L	1IMM	2.00	0.149		Steipe *et al.* (1992)
		V_L,CDR-1 replaced	1IMN	1.97	0.149		Steipe *et al.* (1992)
(Rabbit)							
	(IgG)	Fc		2.7			Sutton and Phillips, (1983)
(Guinea pig)							
	(IgG1)	pFc′	1PFC	3.125	0.303		Bryant *et al.* (1985)

[a]Guddat, Edmundson and Herron (to be published), cited in Kabat *et al.* (1991).
[b]Saul and Poljak (to be published), cited in PDB Entry: 8FAB.
[c]Padlan, Cohen and Davies (in preparation).
[d]Bhat, Padlan and Davies (in preparation).
[e]Mol, Muir, Lee and Anderson (unpublished), cited in Kabat *et al.* (1991).
[f]Muir, Cygler, Mol, Lee and Anderson (unpublished), cited in Kabat *et al.* (1991).
[g]Rose, Przybylska, To, Kayden, Oomen, Vorberg, Young and Bundle (to be published), cited in PDB Entry: 1MAM.

Padlan, 1990*a*; Mian *et al.*, 1991), interdomain interactions (e.g. Poljak *et al.*, 1975*a*, *b*, 1976; Davies *et al.*, 1975*a*, *b*; Amzel and Poljak, 1979; Davies and Metzger, 1983; Novotny *et al.*, 1983; Novotny and Haber, 1985; Chothia *et al.*, 1986; Padlan *et al.*, 1986; Schiffer *et al.*, 1988; Miller, 1990), flexibility (e.g. Edmundson *et al.*, 1974, 1978, 1987; Burton, 1985, 1990*a*, *b*; Huber and Bennett, 1987; Lesk and Chothia, 1988; Ely *et al.*, 1989; Rini *et al.*, 1992; Davies and Padlan, 1992), hypervariable region structures (e.g. Padlan and Davies, 1975; Potter *et al.*, 1976; Padlan, 1977*a*; De la Paz *et al.*, 1986; Chothia and Lesk, 1987; Chothia *et al.*, 1989, 1992; Tramontano *et al.*, 1990; Wu *et al.*, 1993), disulfide bridges (e.g. Steiner, 1985), and antibody–ligand interactions (e.g. Padlan *et al.*, 1976; Huber, 1986; Mariuzza *et al.*, 1987; Alzari *et al.*, 1988; Colman, 1988; Davies *et al.*, 1988, 1990; Wilson *et al.*, 1991; Davies and Chacko, 1993). In addition to X-ray crystallography, other techniques have been used to study antibody structure, including two-dimensional NMR (e.g. Anglister, 1990; Theriault *et al.*, 1991), genetic engineering (e.g. Morrison *et al.*, 1984; Morrison and Oi,

1988; Bird *et al.*, 1988; Huston *et al.*, 1988; Winter, 1989; Tan *et al.*, 1990; Helm *et al.*, 1991; Glockshuber *et al.*, 1992; Jin *et al.*, 1992) and theoretical analysis (e.g. Novotny *et al.*, 1989; Novotny and Sharp, 1992).

Here, we will analyse the currently available three-dimensional data on antibodies which have been obtained by crystallographic analysis, focusing on those structural features which relate to antibody function and potential applications. The crystallographically-determined structures, which are known to the author, are listed in Table 1. The present analysis is limited to those structures for which atomic coordinates are available at the time of writing from the Protein Data Bank (PDB) (Bernstein *et al.*, 1977; Abola *et al.*, 1987), or have been kindly made available by the original investigators.

The studies on antibody structure are too many to enumerate and only a limited number of references could reasonably be cited. It is hoped that the citations given can serve as starting points for those who are interested in particular aspects of antibody structure. Several comprehensive reviews have been written recently on the subject (e.g. Mariuzza *et al.*, 1987; Alzari *et al.*, 1988; Colman, 1988; Davies *et al.*, 1988, 1990; Wilson *et al.*, 1991; Davies and Chacko, 1993) and the reader is encouraged to consult those also.

Caveat

Only a few of the structures listed in Table 1 have been determined to high resolution (2.0 Å or better) and refined extensively (to crystallographic R-values of 0.20 or better), so that the errors associated with many of the structures could be large. An example of a highly-refined structure is J539 Fab, which has been determined to 1.95-Å resolution and refined to an R-value of 0.194 (PDB Entry: 2FBJ); for J539 Fab, the error in the atomic coordinates, estimated by the method of Luzzati (1953), is 0.25 Å (Bhat, Padlan and Davies, unpublished results). An example of a structure determined at medium resolution is HyHEL-5 Fab, which has been determined to 2.54-Å resolution and refined to an R-value of 0.245 (Sheriff *et al.*, 1987); the estimated error for HyHEL-5 Fab is 0.40 Å. These error estimates are for the most ordered (usually, interior) regions of the molecule; the errors will be larger for exposed segments, especially for the side chain atoms. For structures that have been determined to lower resolution or that have been refined less extensively, the errors are probably higher. In view of the potentially large errors in the individual structures, it would be prudent to look for trends from the analysis of the whole set of structures than to draw conclusions on the basis of only one.

THE STRUCTURE OF ANTIBODIES

An antibody molecule is composed of three major fragments: the two Fabs, which are identical and each of which contains the light chain and the first two domains of the heavy chain, and the Fc, which contains the C-terminal constant domains of the two heavy chains. The Fabs are linked to the Fc by the hinge region, which varies in length and flexibility in the different antibody classes and isotypes. The antigen-binding sites (paratopes) are located at the tips of the Fabs. A representation of human IgG1 antibody is shown in Fig. 1.

From the earliest studies of antibody structure (Poljak *et al.*, 1973, 1974; Schiffer *et al.*, 1973; Epp *et al.*, 1974; Segal *et al.*, 1974), it was clear that all antibody domains, whether variable or constant, form compact globular structures with a characteristic fold, termed the *Immunoglobulin Fold* by Poljak *et al.* (1973). Each domain consists of a stable arrangement of hydrogen-bonded, anti-parallel β-strands which form a bilayer structure, further stabilized by a disulfide bond between the two layers. In the variable domains, the bilayer structure is formed by nine strands; in the constant domains, the bilayer is formed by seven strands. Bends of different sizes and conformations connect the strands. The predominant secondary structure in antibodies is the anti-parallel β-sheet, with short stretches of α-helix found in some bends.

The variable domains of the light and heavy chains, the V_L and V_H, are similar to each other in three-dimensional structure, as are the constant domains: the C_L of the light chain and the C_H1 of the heavy chain in the Fab, and the C_H2 and the C_H3 domains in Fcγ. Homologous domains from different species are very similar and are essentially superposable. Even in the variable domains, where as much as 30% of the residues could be different among different antibodies, the differences in structure are almost entirely confined to the hypervariable regions (Wu and Kabat, 1970), and usually only if length differences exist in those regions (Padlan and Davies, 1975).

The availability of three-dimensional data for a variety of fragments from different antibody classes and isotypes and from different species, helps in the identification of structurally important features and of analogous structural elements. An attempt to align representative sequences from the various human antibody chain types on the basis of structure is made in Table 2. For purposes of this review, the C-terminus of the Fd, the heavy-chain component of the Fab, is defined as the Cys residue at position 220 [Eu numbering (Edelman *et al.*, 1969)] in human IgG1, or the analogous residue in the other heavy chain classes; the disulfide bridge, which Cys220 forms with the Cys at 214 in the light chain, creates a natural (structural) boundary for the Fab. Accordingly, the hinge is defined as starting with the Asp221 in human IgG1, or analogous residue. In the only available Fc structure (PDB Entries: 1FC1 and 1FC2), the first visible residue, which is probably the first residue in the compact portion of the Fc, is Pro238. Again, for purposes of this review only, we will define the Fc as starting with the Pro238 in human IgG1, or analogous residue in the other heavy chain classes, and define the end of the hinge as the residue preceding Pro238.

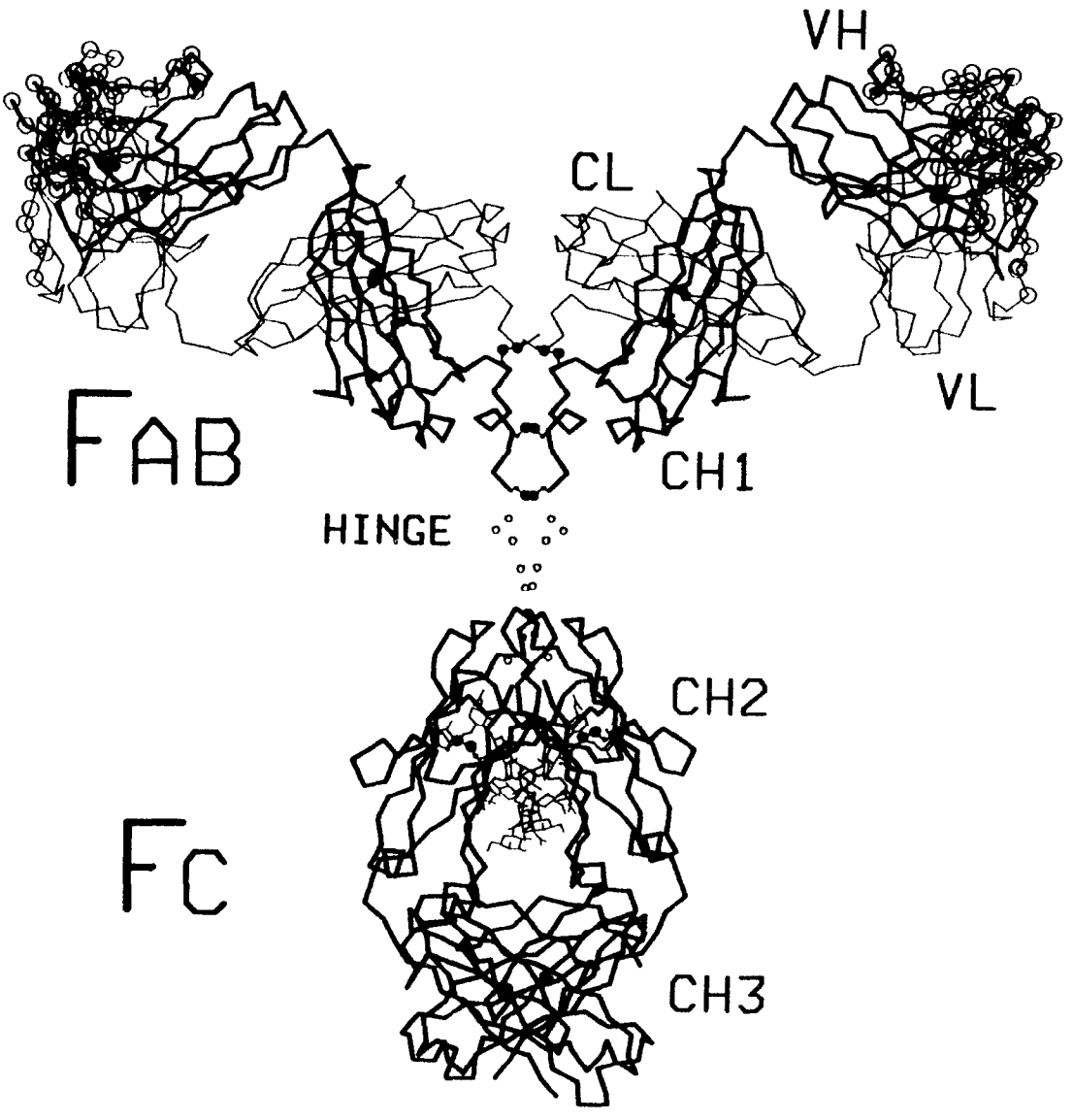
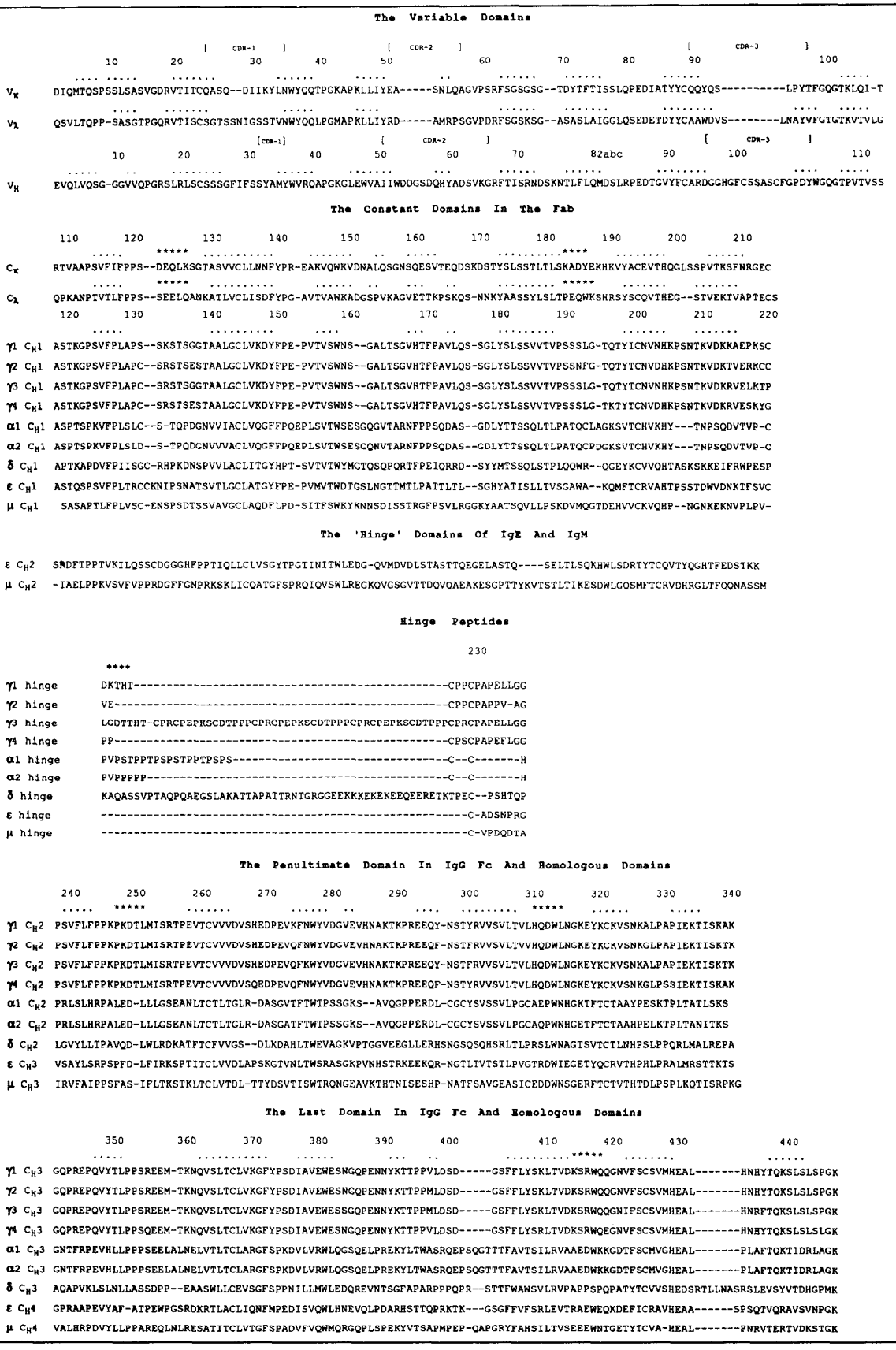


Fig. 1. Line drawing representation of a possible structure for human IgG1. The drawing is a composite of the crystal structures of human IgG1, Kol [Marquart *et al.* (1980); PDB Entry: 21G2], which is visible only to the inter-heavy chain cystine at 229 (Eu numbering), and of human IgG1 Fc [Deisenhofer (1981); PDB Entry: 1FC2], which is visible only from Pro238 to Leu443 (Eu numbering). The octapeptide, PAPELLGG, between Cys229 and Pro238 has not been visualized and its probable location is indicated by the unconnected circles in the middle of the figure. Only the α-carbons are traced. The heavy chains are drawn with thick lines, the light chains with thin lines. The domains of the light chain (V_L and C_L) and those of the heavy chain (V_H, C_H1, C_H2 and C_H3) are labeled. Also indicated is the hinge, the region that lies between the Fabs and the Fc and which varies considerably in size in the different antibody types. The CDR residues are drawn with larger open circles at the tips of the Fabs. The disulfide bridges are indicated by filled circles. The carbohydrate moieties attached to the asparagines at position 297 (Eu numbering) are drawn with thin lines between the two C_H2 domains.

The structure of Fabs

The Fab structures which are examined here are those of the murine J539 (PDB Entry: 2FBJ), McPC603 with and without bound phosphocholine (PDB Entry: 1MCP and unpublished results of Padlan, Cohen and Davies), HyHEL-10 (PDB Entry: 3HFM), HyHEL-5 (PDB Entry: 2HFL), R19.9 (PDB Entry: 2F19), 4-4-20 (PDB Entry: 4FAB), BV04-01 with and without bound $d(pT)_3$ (atomic coordinates kindly made available by Dr Allen B. Edmundson and coworkers), 36-71 (PDB Entry: 6FAB), B13I2 with and without bound peptide (PDB Entries: 2IGF and 1IGF, respectively), D1.3 (PDB Entry: 1FDL), Yst9-1 (PDB Entry: 1MAM), AN02 (PDB Entry: 1BAF), 17/9 with ligand in two crystal forms (PDB Entry: 1HIN and 1HIM, the latter with two Fabs in the asymmetric unit of the crystal) and without ligand (PDB Entry: 1HIL, with two Fabs in the asymmetric unit of the crystal), NC41 with bound neuraminidase (PDB Entry: 1NCA) and 8F5 (PDB Entry: 1BBD), and those of the human Kol (PDB Entry: 2FB4), NEW (PDB Entry: 7FAB), Hil (PDB Entry: 8FAB, with two

Table 2. Structural alignment of human antibody domains



Secondary structure from program DSSP of Kabsch and Sanders (1983) on 3D6 (PDB Entry: 1DFB) for Vκ and Cκ, on Kol (PDB Entry: 2FB4) for Vλ, Cλ, V_H and C_H1, and on human IgG1 Fc (PDB Entry: 1FC1) for C_H2 and C_H3. Residues which participate in the β-sheets are indicated by (.); those which are in α-helices are indicated by (*). The numbering scheme for the V_L, V_H and C_L domains follows the convention of Kabat *et al.* (1991); Eu numbering (Edelman *et al.*, 1969) is used for the IgG1 constant domains. The domains of J539 and Kol Fab, which are two of the most highly-refined Fab structures listed in Table 1, were used in the structural alignment; the other sequences were aligned on the basis of their sequence similarity to these two.

Fabs in the asymmetric unit of the crystal) and 3D6 (PDB Entry: 1DFB). The human Fv, POT (PDB Entry: 1IGM), was included in the analysis where appropriate.

In the Fab (Fig. 2), V_L and V_H associate closely to form a compact module, the Fv, and are related by a pseudo-dyad; the constant domains in the Fab, the C_L and the C_H1, likewise form a compact module and are also related by a pseudo-dyad (Table 3). On average, the V_L–V_H contact buries approximately 1420 Å^2 of surface area (720 in V_L and 700 in V_H). The V_L–V_H contact varies from antibody to antibody (Table 4) and differences are found even for the same Fab but crystallized in different crystal forms. The C_L–C_H1 contact (Table 4) buries approximately 1710 Å^2 of surface area (870 in C_L and 840 in C_H1) and, here also, some variation is found in the contacts present in supposedly identical C_L–C_H1 pairings. The variation seen in the identical C_L–C_H1 pairings may reflect the different lattice forces to which the molecules are subjected in the different crystal forms, the errors in the crystallographic analysis of these structures, or both.

In both light and heavy chains, the variable and constant domains are linked by a short segment of polypeptide chain, called the switch. Visual inspection of the Fab structures (for example, Fig. 2) identifies the switch peptides as residues 107, 108 and 109 in the light chains and residues 113, 114 and 115 in the heavy chains [numbering scheme of Kabat *et al.* (1991)]. Residue 106a is missing in κ chains so that the switch region in κ chains is shorter by one residue than those in λ chains and heavy chains. The relative disposition of the variable and constant modules is usually described by the angle between the V_L–V_H and C_L–C_H1 pseudo-dyads. This angle is called the "elbow bend" of the Fab and is variable, in view of the flexibility of the switch. The Fab bend ranges from a tight 127.2° (in Fab 8F5, PDB Entry: 1BBD) to an almost straight 176.2° (in Fab R19.9, PDB Entry: 2F19) (Table 3, Fig. 3). In all cases known to date where the Fab is bent, the Fd is more bent than the light chain, i.e. the angle between V_H and C_H1 is less than that between V_L and C_L; this has been attributed to the presence of smaller side chains in the interface between V_H and C_H1 (Segal *et al.*, 1974).

The Fv

On the basis of sequence variation, the residues in the variable domains are assigned either to hypervariable or complementarity-determining regions (CDRs), or to nonhypervariable or framework regions (Wu and Kabat, 1970). The CDRs of the light chain are defined as being comprised of residues 24-34 (CDR1-L), 50-56 (CDR2-L) and 88-97 (CDR3-L); those of the heavy chain contain residues 31-35 (CDR1-H), 50-65 (CDR2-H) and 95-101 (CDR3-H) [numbering convention of Kabat *et al.* (1991)]. Variations in length accompany the variability in sequence in these CDRs, with CDR3-H displaying particularly large length variations (Kabat *et al.*, 1991). The framework regions in V_L are defined as being comprised of residues 1-23 (FR1-L), 35-49 (FR2-L), 57-87 (FR3-L) and 98-107 (FR4-L); those in V_H contain residues 1-30 (FR1-H), 36-49 (FR2-H), 66-94 (FR3-H); and 102-112 (FR4-H).

In three-dimensions, the CDRs are seen as loops mainly situated at the N-terminal tip of the Fab (Fig. 4), where they form a continuous surface approximately 2800 Å^2 in area. The extent and conformation of each CDR are primarily determined by the nature and number of amino acids in the segment, and the variability in sequence and size seen in the CDRs results in a large variation in the topography of the CDR surface. The

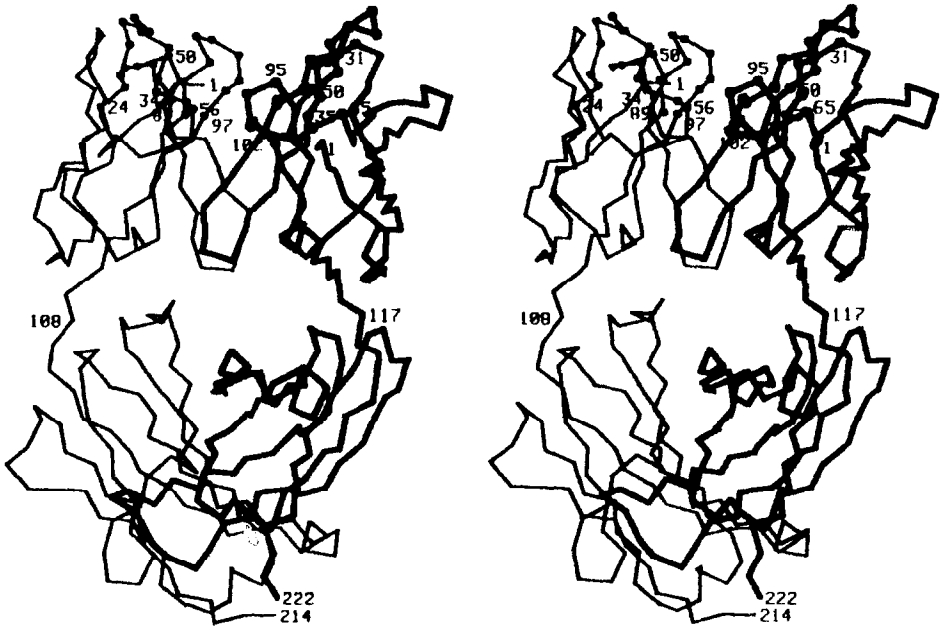


Fig. 2. Stereodrawing of the α-carbon trace of the Fab of the murine antibody, HyHEL-10. The light chain is drawn with thinner lines. The variable domains are on top and the constant domains are at the bottom. The N- and C-termini of the two chains, residues in the switch regions, and the first and last residues of the six CDRs are labeled. The CDR residues are indicated by filled circles.

Table 3. Symmetry in the quaternary structure of murine and human antigen-binding regions of known three-dimensional structure

Antibody	PDB Code	V_L–V_H symmetry Rotation (degrees)	V_L–V_H symmetry Translation (Å)	C_L–C_H1 symmetry Rotation (degrees)	C_L–C_H1 symmetry Translation (Å)	Fab bend (degrees)
(Murine)						
J539	2FBJ	168.7	0.2	173.9	−2.0	143.8
McPC603	1MCP	173.4	0.1	171.8	−2.8	131.3
		173.6	0.3	171.9	−2.7	131.8[a]
HyHEL-10	3HFM	170.6	−0.3	167.7	−2.0	145.6
HyHEL-5	2HFL	171.4	0.1	169.1	−2.0	161.7
R19.9	2F19	175.2	0.3	166.8	−2.0	176.2
	1FAI	175.0	0.3	167.8	−1.8	176.1
4-4-20	4FAB	175.5	−0.3	174.3	1.6	174.9
BV04-01	[d]	176.8	−0.1	170.6	−1.8	173.5[a]
	[d]	173.5	0.3	172.3	1.8	172.5
36-71	6FAB	176.2	−0.1	171.5	−2.0	166.6
B13I2	2IGF	172.4	0.1	170.6	−2.0	155.8[a]
	1IGF	172.5	0.0	170.0	−2.0	153.0[b]
		172.7	0.1	170.9	−2.0	155.4[c]
D1.3	1FDL	166.2	0.7	171.1	−1.9	173.0
Yst9-1	1MAM	175.1	−0.1	172.4	−1.5	148.1
AN02	1BAF	173.3	−0.5	167.4	−2.2	153.8
17/9	1HIN	168.9	−0.3	172.3	1.9	175.1[a]
	1HIM	169.1	0.2	171.3	−1.7	172.7[a,b]
		169.5	−0.3	171.2	1.8	171.6[a,c]
	1HIL	170.3	−0.1	171.8	−1.9	159.7[b]
		168.9	0.2	171.7	2.0	160.5[c]
8F5	1BBD	173.7	−0.7	171.0	−2.1	127.2
NC41	1NCA	177.6	0.4	171.2	2.1	146.6
(Human)						
Kol	2FB4	167.9	−0.1	170.4	3.4	165.2
NEW	7FAB	164.3	−0.5	170.4	−3.8	129.9
Hil	8FAB	171.2	0.3	174.6	2.7	147.2[b]
		174.8	0.1	168.8	−3.3	135.7[c]
3D6	1DFB	166.8	0.2	169.5	−2.1	174.9
POT	1IGM	174.4	0.0	—	—	—

[a]Liganded form.

[b]For the first Fab in the asymmetric unit of the crystal.

[c]For the second Fab in the asymmetric unit of the crystal.

[d]Kindly provided by Dr Allen B. Edmundson and coworkers.

The rotation and translation parameters which relate V_L to V_H and C_L to C_H1 were obtained with program ALIGN (G. H. Cohen, NIH) using the α-carbon positions of residues: 3-7, 11-26, 31-38, 44-48, 60-66, 70-90 and 97-106 in V_L; residues: 3-7, 10-25, 32-39, 45-49, 65-71, 77-94 and 102-111 in V_H; residues 109-117, 129-142, 144-149, 158-162, 170-186, 192-198 and 204-208 in C_L; and residues: 119-127, 139-152, 154-159, 166-170, 177-193, 198-204 and 210-214 in C_H1. The numbering convention of Kabat *et al.* (1991) is used for V_L, V_H and C_L; Eu numbering (Edelman *et al.*, 1969) is used for C_H1. These residues represent the structurally equivalent positions in the homologous domains of J539 Fab (Suh *et al.*, 1986; Bhat, Padlan and Davies, in preparation). The Fab bend is the angle between the rotation axes which relate V_L to V_H and C_L to C_H1.

CDR surface is characterized by depressions and protrusions and may contain a deep pocket [for example, in McPC603 (Segal *et al.*, 1974), or cleft for example, in MAb131 (Garcia *et al.*, 1992)], or a protruberance [for example, in HyHEL-10 (Padlan *et al.*, 1989)]. Crystallographic analysis of several antibody–antigen complexes and other studies have repeatedly shown that antigen binding primarily involves this surface. The CDR surface is therefore usually equated with the combining site of the antibody (the paratope).

The six CDRs are disposed (Fig. 4) such that the N-terminal part of CDR1-L and the C-terminal parts of CDR2-L and CDR2-H are farther from the center of the CDR surface, while CDR1-H, CDR3-H, CDR3-L, the C-terminal part of CDR1-L, and the N-terminal parts of CDR2-L and CDR2-H are closer to the center.

Table 4. V_L–V_H and C_L–C_H1 interactions in murine and human antigen-binding regions of known three-dimensional structure

		V_L–V_H interactions					C_L–C_H1 interactions				
	PDB	Surface	Buried				Surface	Buried			
Antibody	Code	V_L	V_H	vdW	H.b.	I.p.	C_L	C_H1	vdW	H.b.	I.p.
(Murine)											
J539	2FBJ	773	740	190	9	0	858	835	147	3	0
McPC603	1MCP	874	825	170	6	0	765	750	120	1	0
		874	825	170	6	0	837	790	111	3	0
HyHEL-10	3HFM	718	676	122	3	0	945	981	153	6	1
HyHEL-5	2HFL	655	627	119	9	1	836	810	139	6	1
R19.9	2F19	827	846	168	9	0	872	835	159	5	0
	1FAI	744	751	153	5	0	868	861	165	6	0
4-4-20	4FAB	670	696	119	6	1	864	870	131	5	1
BV04-01		702	671	105	3	0	913	867[a]	149	7	1
		662	688	123	6	0	958	930	182	6	1
36-71	6FAB	726	729	131	4	0	1068	1056	217	17	2
B13I2	2IGF	715	682	132	8	0	1045	973[a]	145	4	1
	1IGF	756	731	163	11	1	876	836[b]	166	6	0
		786	752	159	7	0	868	911[c]	161	7	0
D1.3	1FDL	732	684	195	11	1	866	864	161	12	1
Yst9-1	1MAM	644	626	80	3	0	918	837	159	4	0
AN02	1BAF	642	615	120	7	0	1043	1000	188	11	2
17/9	1HIN	730	706	137	10	2	788	758[a]	162	5	0
	1HIM	760	742	132	6	1	799	761[a,b]	151	8	1
		740	730	143	9	1	834	796[a,c]	169	4	0
	1HIL	757	741	166	14	1	788	765[b]	163	6	1
		744	744	163	11	1	870	809[c]	172	8	1
8F5	1BBD	665	635	140	12	0	906	849	161	4	0
NC41	1NCA	614	631	141	5	0	1074	991	191	8	0
(Human)											
Kol	2FB4	800	790	162	4	0	894	816	125	9	2
NEW	7FAB	623	594	119	6	0	709	648	98	6	1
Hil	8FAB	583	592	132	9	1	676	623[b]	96	4	0
		714	694	153	6	0	829	755[c]	129	9	1
3D6	1DFB	716	683	133	2	1	841	888	116	6	0
POT	1IGM	687	687	130	9	1	—	—	—	—	—

[a]Liganded form.
[b]First Fab in the entry.
[c]Second Fab in the entry.
Two atoms are said to be in van der Waals' contact (vdW) if the distance between them is at most the sum of their van der Waals' radii plus 0.5 Å. Polar atoms are said to be hydrogen-bonded (H.b.) if the distance between them is at most 2.90 Å (taken to be the standard hydrogen-bond distance) plus 0.5 Å. Oppositely-charged atoms are said to form an ion pair (I.p.) if the distance between them is at most 2.85 Å (taken to be the standard ion-pair distance) plus 0.5. An average error in the atomic positions of 0.35 Å is assumed for all the structures included in the analysis; the assumed error in the interatomic distances is then 0.5 Å [$=0.35 \times \text{sqrt}(2.0)$]. Surface areas were computed using program MS of Connolly (1983); a probe radius of 1.7 Å was used and four points per square Ångstrom of surface area were computed.

The nonhypervariable or framework regions, by and large, show conserved amino acid substitutions and very similar three-dimensional structures. The different antibody combining sites, therefore, can be pictured as being constructed with CDRs of varied shapes and sizes, which are grafted onto a scaffolding of basically conserved structure.

The contact between V_L and V_H involves both framework and CDR residues and features framework–framework, framework–CDR and CDR–CDR interactions. The involvement of CDR residues in the V_L–V_H contact is significant, ranging from 26 to 57% of all atomic interactions in the structures analysed (Table 5). This involvement undoubtedly contributes to the variation seen in the quaternary association of the variable domains. The contribution to the contact from the individual CDRs is not the same (Table 6); in V_L, only CDR3-L interacts with all the CDRs of the heavy chain (on average, 5% of its contact is with CDR1-H, 28% is with CDR2-H and 67% is with CDR3-H); in V_H,

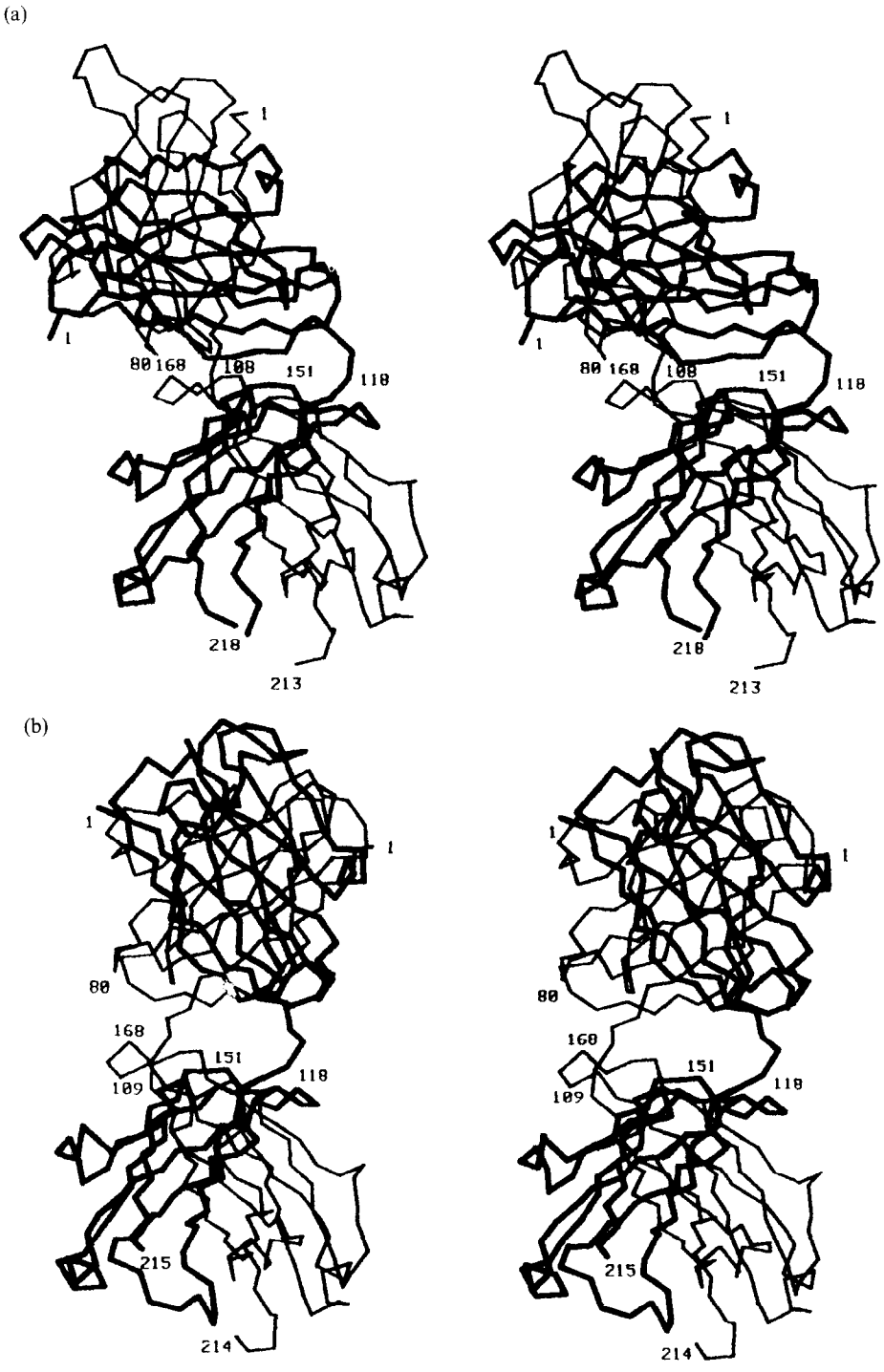


Fig. 3. Stereodrawings of the α-carbon trace of the Fabs of (a) 8F5 (PDB Entry: 1BBD), which is the most bent (Fab bend = 127.2°), and (b) R19.9 (PDB Entry: 2F19), which is almost straight (Fab bend = 176.2°). In these drawings, the Fabs are oriented such that their C_L–C_H1 modules are maximally superposed. The light chains are drawn with thinner lines and the heavy chains with thicker lines. The variable domains are on top and the constant domains are at the bottom.

only CDR3-H interacts with all the CDRs of the light chain (on average, 22% of its contact is with CDR1-L, 18% is with CDR2-L, and 60% is with CDR3-L). The framework residues of V_L which interact with the CDRs in the heavy chain are mostly from FR2-L and FR4-L, and occasionally from FR1-L; the framework residues of V_H which interact with the CDRs in the light chain are exclusively from FR2-H and, in almost all cases, the only residue that is involved is that at position 47 (usually a Trp). Details of the V_L–V_H contacts in Kol and J539 are presented in Table 7 and Fig. 5. The CDR and framework residues, which are involved in the V_L–V_H interaction in the various antigen-binding regions of known three-dimensional structure, are given in Table 8.

Intradomain framework–CDR interactions can influence the conformation of the CDRs. Indeed, canonical

Fig. 4. Stereodrawing of the α-carbon trace of the Fv of antibody HyHEL-10 viewed end on. The figure is rotated 90° relative to Fig. 2. The V_L is on the left (thinner lines). The CDR residues are indicated by filled circles and their ends are labeled.

structures have been observed for most CDRs and those structures appear to be determined by the nature of a small number of framework residues that interact with the CDRs (Chothia and Lesk, 1987; Chothia *et al.*, 1989; Tramontano *et al.*, 1990). The framework, therefore, does not simply provide a foundation for the

Table 5. Involvement of framework (FRM) and complementarity-determining (CDR) regions in the V_L–V_H interactions in murine and human antigen-binding regions of known three-dimensional structure

		Number of interatomic contacts between:				
Antibody	PDB Code	V_L and V_H FRMs	V_L FRM and V_H CDRs	V_H FRM and V_L CDRs	V_L CDRs and V_H CDRs	Total
(Murine)						
J539	2FBJ	65	36	24	65	190
McPC603	1MCP	56	27	27	60	170
		56	27	27	60	170
HyHEL-10	3HFM	51	8	17	46	122
HyHEL-5	2HFL	65	25	12	17	119
R19.9	2F19	50	27	17	74	168
	1FAI	48	40	15	50	153
4-4-20	4FAB	40	30	22	27	119
BV04-01	[a]	55	14	8	28	105
		49	32	15	27	123
36-71	6FAB	53	31	21	26	131
B13I2	2IGF	41	25	11	55	132
	1IGF[b]	68	26	11	58	163
	[c]	66	30	10	53	159
D1.3	1FDL	68	36	21	70	195
YST9-1	1MAM	47	11	13	9	80
AN02	1BAF	53	19	17	31	120
17/9	1HIN	54	26	18	39	137
	1HIM[b]	47	29	17	39	132
	[c]	50	24	17	52	143
	1HIL[b]	57	36	19	54	166
	[c]	57	33	15	58	163
8F5	1BBD	66	21	22	31	140
NC41	1NCA	58	17	15	51	141
(Human)						
Kol	2FB4	74	23	21	44	162
NEW	7FAB	51	14	22	32	119
Hil	8FAB[b]	59	23	12	38	132
	[c]	55	24	10	64	153
3D6	1DFB	49	14	16	54	133
POT	1IGM	55	28	11	36	130

[a]Liganded form.
[b]For the first Fab in the asymmetric unit of the crystal.
[c]For the second Fab in the asymmetric unit of the crystal.
See footnote to Table 4 for the definition of contacts.

Table 6. van der Waals' contacts among the CDRs of murine and human antigen-binding regions of known three-dimensional structure

	Interatomic contacts								
	CDR1-L with:			CDR2-L with:			CDR3-L with:		
Antibody	CDR1-H	CDR2-H	CDR3-H	CDR1-H	CDR2-H	CDR3-H	CDR1-H	CDR2-H	CDR3-H
J539	0	0	23	0	0	7	1	12	22
McPC603	0	0	15	0	0	2	13	4	26
HyHEL-10	0	0	5	0	0	0	0	33	8
HyHEL-5	0	0	0	0	0	0	4	9	0
R19.9	0	0	5	0	0	6	1	10	52
4-4-20	0	0	9	0	0	17	1	0	0
BV04-01	0	0	0	0	0	9	0	0	19
36-71	0	0	11	0	0	6	0	3	6
B13I2	0	0	36	0	0	3	0	0	16
D1.3	0	0	5	0	0	0	1	19	45
Yst9-1	0	0	0	0	0	4	0	3	2
AN02	0	0	2	0	0	0	0	26	3
17/9	0	0	9	0	0	10	0	4	16
8F5	0	0	0	0	0	16	1	14	0
NC41	0	0	3	0	0	6	0	0	42
Kol	0	0	5	0	0	0	3	8	28
NEW	0	0	4	—	—	—	3	12	13
Hil	0	0	10	0	0	1	0	1	26
3D6	0	0	2	0	0	18	1	0	33
POT	0	0	0	0	0	7	0	2	27
Totals	0	0	144	0	0	112	29	160	384

The coordinates used in the computations were for the liganded forms of McPC603, B13I2, BV04-01 and 17/9 (PDB Entry: 1HIM, first Fab in the entry); and (here and in subsequent calculations) those in PDB Entry 2F19 for R19.9, the first Fab in PDB Entry 8FAB for Hil, and those in PDB Entry 1NCA for NC41. The 7 residue deletion around the CDR2-L of NEW V_L is interpreted here as a deletion of all of CDR2-L in view of the minigene hypothesis of Kabat *et al.* (1978); this region in NEW has unusual amino acids and a different structure compared to the other V_Ls. See footnote to Table 4 for the definition of contacts.

construction of the combining site; small variations in the architecture of the framework can affect the topography of the CDR surface.

Crystallographic analysis of antibody structures, with and without bound ligand, has shown that, in some cases, the combining site structure could change on

Table 7a. V_L–V_H contacts in the human (IgG1,λ) Kol (PDB Entry: 2FB4)

	V_L																	
V_H	T32	N34	Y36	Q38	G41	M42	A43	P44	L46	Y49	Y87	W91	V93	N95a	A95b	Y96	F98	Totals
Y35																3		3
V37																	4	4
Q39				6[h]	2						1							9
G44											4							4
L45				2				2			7						9	20
W47														1	6	14	5	26
I50												3						3
H58														5				5
F91					2	3	2											7
C100a												7						7
S100b												2						2
S100c													2					2
C100f	1											6				2		9
F100g		3[h]								10								13
G100h		1														3		4
P100i			10[h]													6		16
D101									3									3
W103			5				1	15									2	23
G104							2											2
Totals	1	4	15	8	4	3	5	17	3	10	12	18	2	6	6	28	20	162

Table 7b. V_L–V_H contacts in the murine (IgA,κ) J539 (PDB Entry: 2FBJ)

	V_L																		
V_H	S32	H34	Y36	Q38	S43	P44	P46	Y49	E50	A55	Y87	Q89	W91	Y93	P94	L95	I96	F98	Totals
W33														1					1
V37																		4	4
Q39				9^h							1^h								10
K43											1								1
G44											3								3
L45						3					5							6	14
W47															2	1	21	1	24
E50														1					1
T56														1					1
N58														5^h	4				9
P61																1			1
Y91				1	4	3													8
L95																	1		1
Y97									1										1
Y98	4	6^h							5				11						26
G99		3										3^h	4				2		12
Y100		10	5				3	16		1									35
N100a			6^h				2					1						1	10
A101							3												3
W103			3			6	3											1	13
G104					3^h														3
Q105					8^h														8
Totals	4	19	14	10	15	12	11	16	6	1	10	4	15	8	6	2	24	13	190

The residues on the horizontal lines are from the V_L and those along the vertical are from the V_H. In these matrices of contacts, the element $c(i,j)$ represents the number of interacting atom pairs, one from residue i and the other from residue j. The superscript ^{h} that is found after some matrix elements signifies that the contact involves at least one hydrogen bond. See footnote to Table 4 for the definition of contacts.

Fig. 5. Stereodrawings of the α-carbon trace of the Fvs of Kol (a) and of J539 (b) showing the residues which are in the V_L–V_H contact. The V_Ls are drawn with thinner lines on the left. The side chains of the residues involved in the contact are shown in full.

Table 8a. V_L residues involved in the V_L–V_H contact in murine and human antigen-binding regions of known three-dimensional structure

```
                                          CDR1                        CDR2                                     CDR3
                10        20      27abcdef 30          40         50         60        70        80              95ab   100
                                                  *  * *    **    *                                     * *       **  *  *
HyHEL-10  DIVLTQSPATLSVTPGNSVSLSC RASQ------SIGNNLH WYQQKSHESPRLLIK YASQSIS GIPSRFSGSGSGTDFTLSINSVETEDFGMYFC QQSNSWP--YT FGGGTKLEIK
          *                                          * *    ** *  * *                                     *    *  *   *  **
HyHEL-5   DIVLTQSPAIMSASPGEKVTMTC SASS-------SVNYMY WYQQKSGTSPKRWIY DTSKLAS GVPVRFSGSGSGTSYSLTISSMETEDAAEYYC QQWGRN---PT FGGGTKLEIK
          *                             *    * *    ** *  *                                     *  * *  **  *  * *
D1.3      DIQMTQSPASLSASVGETVTITC RASG------NIHNYLA WYQQKQGKSPQLLVY YTTILAD GVPSRFSGSGSGTQYSLKINSLQPEDFGSYYC QHFWSTP--RT FGGGTKLEIK
                                      * *** *  * *    ** *  * *                                     *  * *  **  *  ***
McPC603   DIVMTQSPSSLSVSAGERVTMSC KSSQSLLNSGNQKNFLA WYQQKPGQPPKLLIY GASTRES GVPDRFTGSGSGTDFTLTISSVQAEDLAVYYC QNDHSYP--LT FGAGTKLEIK
                                          * *  * *    ** *  * *    *                                *  * * ***  *  *
J539      EIVLTQSPAITAASLGQKVTITC SASS-------SVSSLH WYQQKSGTSPKPWIY EISKLAS GVPARFSGSGSGTSYSLTINTMEAEDAAIYYC QQWTYPL--IT FGAGTKLELK
                                          * *  * *    ****  * *    *                                *  * *****  *  * *
R19.9     DIQMTQTTSSLSASLGDRVTISC RASQ------DISNYLN WYQQKPDGTVKLLIY YTSRLHS GVPSRFSGSGSGTDYSLTISNLEHEDIATYFC QQGSTLP--RT FGGGTKLEIK
                                          * *  * *    ****  * *    *                                          **  *  *
4-4-20    DVVMTQTPLSLPVSLGDQASISC RSSQSLVHS-QGNTYLR WYLQKPGQSPKVLIY KVSNRFS GVPDRFSGSGSGTDFTLKISRVEAEDLGVYFC SQSTHVP--WT FGGGTKLEIK
                                                  *  * *  **** *  *                                     *       * * *  * *
AN02      QIVLTQSPAIMSASPGEKVTMTC SASS-------SVYYMY WYQQKPGSSPRLLIY DTSNLAS GVPVRFSGSGSGTSYSLTISRMEAEDAATYYC QQWSSYPP-IT FGVGTKLELK
                                          * *  * *   * * *  * *                                     *    *  **  *  *
36-71     DIQMTQIPSSLSASLGDRVSISC RASQ------DINNFLN WYQQKPDGTIKLLIY FTSRSQS GVPSRFSGSGSGTDYSLTISNLEQEDIATYFC QQGNALP--RT FGGGTKLEIK
                                   *      * *  * *    ** *  * *    *                                *  * *   *  *  *
B13I2     DVLMTQTPLSLPVSLGDQASISC RSNQTILLS-DGDTYLE WYLQKPGQSPKLLIY KVSNRFS GVPDRFSGSGSGTDFTLKISRVEAEDLGVYYC FQGSHVP--PT FGGGTKLEIK
                                                     * *   * * *  *    *                                *       **  *  **
Yst9-1    DIQMTQTTSSLSASLGDRVTISC RASQ------DIYNYLN WYQQKPDGTVKLLIY YTSRLHS GVPSRFSGSGSGTDYSLTISNLNQEDMATYIC QQGNTLP--FT FGSGTKLEIK
                                                     * *   *** *  *    *                                *    *   *  *  * *
BV04-01   DVVMTQTPLSLPVSLGDQASISC RSSQSLVHS-NGNTYLH WYLQKPGQSPKLLIY KVSNRFS GVPDRFSGSGSGTDFTLKISRVEAEDLGVYFC SQSTHVP--LT FGAGTKLELK
                                              *   * *  * *  * ** *  * *                                     *  * *  **  *  *
17/9      DIVMTQSPSSLTVTAGEKVTMSC TSSQSLFNSGKQKNYLT WYQQKPGQPPKVLIY WASTRES GVPDRFTGSGSGTDFTLTISSVQAEDLAVYYC QNDYSNP--LT FGGGTKLELK
                                                     * *    ** *  *         **                                *       **  *  *
8F5       DIVMTQSPSSLTVTTGEKVTMTC KSSQSLLNSRTQKNYLT WYQQKPGQSPKLLIY WASTRES GVPDRFTGSGSGTDFTLSISGVQAEDLAVYYC QNNYNYP--LT FGAGTKLELK
                                                  *  * *    ** *  *         **                                *  * *   *  *  *
NC41      DIVMTQSPKFMSTSVGDRVTITC KASQ------DVSTAVV WYQQKPGQSPKLLIY WASTRHI GVPDRFAGSGSGTDYTLTISSVQAEDLALYYC QQHYSPP--WT FGGGTKLEIK

                                                  *  * *    ** *  * *                                     *  *    **  *  *
Hil         ELTQPPS-VSVSPGQTARITC SANA------LPNQYAY WYQQKPGRAPVMVIY KDTQRPS GIPQRFSSSTSGTTVTLTISGVQAEDEADYYC QAWDNSA--SI FGGGTKLTVLG
                                          * *  * *  **** *  *                                     *    * *  ***  *
Kol       QSVLTQPPS-ASGTPGQRVTISC SGTSSN----IGSSTVN WYQQLPGMAPKLLIY RDAMRPS GVPDRFSGSKSGASASLAIGGLQSEDETDYYC AAWDVSLNAYV FGTGTKVTVLG
                                                  *  * *   *** *                                        *    *  **  *  *
NEW       ASVLTQPPS-VSGAPGQRVTISC TGSSSNI---GAGHNVK WYQQLPGTAPKLLIF ------- HNNARFSVSKSGTSATLAITGLQAEDEADYYC QSYDRSL--RV FGGGTKLTVLR
                                          *    * *    ** *  * *    *                                *  * * *    *  *
3D6       DIQMTQSPSTLSASVGDRVTITC RASQ------SISRWLA WYQQKPGKVPKLLIY KASSLES GVPSRFSGSGSGTEFTLTISSLQPDDFATYYC QQYNS----YS FGPGTKVDIK
                                                     * *    ** *  *    *                                *  * * ***  *  *
POT       DIQMTQSPSSLSASVGDRVTITC QASQ------DISNYLA WYQQKPGKAPELRIY DASNLET GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC QQYQNLP--LT FGPGTKVDIK
```

Table 8b. V_H residues involved in the V_L–V_H contact in murine and human antigen-binding regions of known three-dimensional structure

```
                                          CDR1                         CDR2                                               CDR3
                  10        20        30           40             52abc    60         70            82abc    90       100abcdefghi          110
                                                   * *   * * ***** *          ****                                 *    ***               **
HyHEL-10  DVQLQESGPSLVKPSQTLSLTCSVTGDSIT SDYWS- WIRKFPGNRLEYMG YVS---YSGSTYYNPSLKS RISITRDTSKNQYYLDLNSVTTEDTATYYCAN WDG------------DY WGQGTLVTVSA
                                             *   * *    ** *                **                                 *    ***            *  **
HyHEL-5   ZVQLQQSGAELMKPGASVKISCKASGYTFS DYWIE- WVKQRPGHGLEWIG EILP--GSGSTNYHERFKG KATFTADTSSSTAYMQLNSLTSEDSGVYYCLH GNYDF----------DG WGQGTTLTVSS
                                             *   * *    ** *    * *         ** *                                  *    *  ***          *  ***
D1.3      QVQLKESGPGLVAPSQSLSITCTVSGFSLT GYGVN- WVRQPPGKGLEWLG MIW---GDGNTDYNSALKS RLSISKDNSKSQVFLKMNSLHTDDTARYYCAR ERDYRL---------DY WGQGTTLTVSS
                                           * *     *    ** *   *          *                                    *    *   *****         *  **
McPC603   EVKLVESGGGLVQPGGSLRLSCATSGFTFS DFYME- WVRQPPGKRLEWIA ASRNKGNKYTTEYSASVKG RFIVSRDTSQSILYLQMNALRAEDTAIYYCAR NYYGSTWYF------DV WGAGTTVTVSS
                                           *     * *   *** *   *        * *  *                                 *    * *****            *  ***
J539      EVKLLESGGGLVQPGGSLKLSCAASGFDFS KYWMS- WVRQAPGKGLEWIG EIHP--DSGTINYTPSLKD KFIISRDNAKNSLYLQMSKVRSEDTALYYCAR LHYYGYN--------AY WGQGTLVTVSA
                                             *   * *    ****   *           **                                  *         * ******  *  ***
R19.9     QVQLQQSGAELVRAGSSVKMSCKASGYTFT SYGVN- WVKQRPGQGLEWIG YINP--GKGYLSYNEKFKG KTTLTVDRSSSTAYMQLRSLTSEDAAVYFCAR SFYGGSDLAVYYF--DS WGQGTTLTLVSS
                                             *   * *     * *                                                   *      ***          ** ***
4-4-20    EVKLDETGGGLVQPGRPMKLSCVASGFTFS DYWMN- WVRQSPEKGLEWVA QIRNKPYNYETYYSDSVKG RFTISRDDSKSSVYLQMNNLRVEDMGIYYCTG SYYGM----------DY WGQGTSVTVSS
                                                 * *   *** *              ****  *                              *     ***           *  ***
AN02      DVQLQESGPGLVKPSQSQSLTCTVTGYSIT SDYAWN WIRQFPGNKLEWMG YMS---YSGSTRYNPSLRS RISITRDTSKNQFFLQLKSVTTEDTATYFCAR GWPL-----------AY WGQGTQVSVSE
                                                 * *   * * *                *                                  *       * *****     *  *
36-71     EVQLQQSGVELVRAGSSVKMSCKASGYTFT SNGIN- WVKQRPGQGLEWIG YNNP--GNGYIAYNEKFKG KTTLTVDKSSSTAYMQLRSLTSEDSAVYFCAR SEYYGGSYKF-----DY WGQGTTLTVSS
                                                 * *   * * *                                                   *       * ***       *  **
B13I2     EVQLVESGGDLVKPGGSLKLSCAASGFTFS RCAMS- WVRQTPEKRLEWVA GISS--GGSYTFYPDTVKG RFIISRNNARNTLSLQMSSLRSEDTAIYYCTR YSSDPFYF-------DY WGQGTTLTVSS
                                                 * *    ** *   *           **                                  *      ***          *  * *
Yst9-1    EVKLVESGGGLVQPGGSLRLSCATSGFTFT DYYMS- WVRQPPGKALEWLG FIRNKADGYTTEYSASVKG RFTISRDNSQSILYLQMNTLRAEDSATYYCTR DPYGPA---------AY WGQGTLVTVSA
                                                 * *   * * *                                                   *     *  ****       ** ***
BV04-01   EVQPVETGGGLVQPKGSLKLSCAASGFSFN TNAMN- WVRQAPGKGLEWVA RIRSKSNNYATYYADSVKD RFTISRDDSQNMLYLQMNNLKTEDTAMYYCVR DQTGTAWF-------AY WGQGTLVTVSA
                                                 * *   * *** *           **                                    *    *   *****          *  ***
17/9      EVQLVESGGDLVKPGGSLKLSCAASGFSFS SYGMS- WVRQTPDKRLEWVA TISN--GGGYTYYPDSVKG RFTISRDNAKNTLYLQMSSLKSEDSAMYYCAR RERYDENGF------AY WGQGTLVTVSA
                                             *   * *    ** *    *          **                                  *    *   ***            *  **
8F5       EVQLQQSGAELVRPGASVKLSCTTSGFNIK DIYIH- WVKQRPEQGLEWIG RLDP--ANGYTKYDPKFQG KATITVDTSSNTAYLHLSSLTSEDTAVYYCDG YYSYYDM--------DY WGPGTSVTVSS
                                                   *    ****                                                   *        *****      ** ***
NC41      QIQLVQSGPELKKPGETVKISCKASGYTFT NYGMN- WVKQAPGKGLEWMG WINT--NTGEPTYGEEFKG RFAFSLETSASTANLQINNLKNEDKATFFCAR GEDNFGSLS------DY WGQGTTLTVSS

                                                 * *    ****               *                                   *      **   ****        **
Hil       AVKLVQAGGGVVQPGRSLRLSCIASGFTFS NYGMH- WVRQAPGKGLEWVA VIWY--NGSRTYYGDSVKG RFTISRDNSKRTLYMQMNSLRTEDTAVYYCAR DPDILTAFSF-----DY WGQGVLVTVSS
                                             *   * *    ** *    *          *                                   *            *** *****  **
Kol       EVQLVQSGGGVVQPGRSLRLSCSSSGFIFS SYAMY- WVRQAPGKGLEWVA IIWD--DGSDQHYADSVKG RFTISRNDSKNTLFLQMDSLRPEDTGVYFCAR DGGHGFCSSASCFGPDY WGQGTPVTVSS
                                           *       *    ** *    *          ** *                                *    * * ***          *  **
NEW       AVQLEQSGPGLVRPSQTLSLTCTVSGTSFD DYYWT- WVRQPPGRGLEWIG YVF---YTGTTLLDPSLRG RVTMLVNTSKNQFSLRLSSVTAADTAVYYCAR NLIAGGI--------DV WGQGSLVTVSS
                                             *   * *    ** *    *                                               *      * *      *****  ***
3D6       EVQLVESGGGLVQPGRSLRLSCAASGFTFN DYAMH- WVRQAPGKGLEWVS GISW--DSSSIGYADSVKG RFTISRDNAKNSLYLQMNSLRAEDMALYYCVK GRDYYDSGGYFTVAFDI WGQGTMVTVSS
                                                 * *    ** *               *                                   *      *  *****      *  **
POT       EVHLLESGGNLVQPGGSLRLSCAASGFTFN IFVMS- WVRQAPGKGLEWVS GVFG--SGGNTDYADAVKG RFTITRDNSKNTLYLQMNSLRAEDTAIYYCAK HRVSYVLTGF-----DS WGQGTLVTVSS
```

The residues which contact the opposite domain are indicated by (*). See footnote to Table 4 for the definition of contacts.

binding, in the manner of an "induced fit" {Edmundson *et al.*, 1974, 1987; Colman, 1988; Bhat *et al.*, 1990; Herron *et al.*, 1991; Rini *et al.*, 1992; [reviewed by Davies and Padlan (1992)]}. The conformation of the individual CDRs, especially the longer ones, could change, as could the mode of quarternary association of the variable domains (Colman *et al.*, 1987; Colman, 1988; Bhat *et al.*, 1990; Herron *et al.*, 1991; Rini *et al.*, 1992). These results suggest that the combining site structure is not rigid, rather, it is plastic and may assume different conformations depending on circumstance.

The C_L–C_H1 module

Representative C_L–C_H1 interactions are presented in Table 9 and Fig. 6. The contacts observed in Fab Kol (human IgG1,λ) [Table 9a and Fig. 6(a)] include two salt bridges: one between Glu123-L and Lys213-H and the other between Glu124-L and Lys147-H. In Fab 3D6 (human IgG1,κ) (Table 9b), no salt bridges are formed, despite the fact that a glutamic acid residue is present at position 123 also in the 3D6 light chain. Actually, in 3D6, there is a favorable electrostatic interaction between the charged moieties of Glu123-L and Lys213-H, which are within 4.0 Å of each other. This interaction would be only about half as strong as that in Kol where the corresponding distance is 2.8 Å. The second salt bridge in Kol is not possible in 3D6 because the Glu at position 124 in C_λ is replaced by a Gln in C_κ (Table 2). Despite the many differences in sequence, the patterns of C_L–C_H1 interaction in these human Fabs, one with a λ and the other with a κ chain, are similar.

The Fab of 17/9 (IgG2a,κ) (PDB Entry: 1HIL, first Fab in the entry) was chosen to represent the murine IgG subclasses in terms of the C_L–C_H1 interactions, since this structure has been determined to high resolution and refined to a high degree (Table 1). The C_L–C_H1 contact in 17/9 (Table 9c) shows a pattern similar to that of 3D6 (Table 9b), which is not surprising in view of the similarity in sequence of the homologous domains. The salt bridge between Glu123-L and Lys213-H is also found in 17/9 and in the C_L–C_H1 of the other murine IgG isotypes (data not shown). The inter-residue contacts in the C_L–C_H1 of the murine J539 (IgA,κ) Fab (PDB Entry: 2FBJ) are presented in Table 9d and Fig. 6(b) Again, the pattern of the contacts is similar to the others. However, the salt bridge found in the C_L–C_H1 interface in the murine and human IgGs is not possible in IgAs (murine or human), since the Lys at heavy-chain position 213 is not present in these molecules.

A cavity has been observed between C_L and C_H1 (Padlan *et al.*, 1986). It was proposed that the function of this cavity is to provide greater flexibility in the interaction between the domains, so that the variation in the interface residues in the different isotypes can be tolerated while still preserving the basic quaternary structure of the C_L–C_H1 module.

The Fab bend

The interactions between V_L and C_L and between V_H and C_H1 are not very strong and, in view of the variation in the Fab bend (Table 3), are variable. The contacts, computed for the most bent Fab, 8F5 [Fig. 3(a)], and for the most straight Fab, R19.9 (PDB Entry: 2F19) [Fig. 3(b)], are presented in Table 10. Included in Table 10 are the contacts for an Fab with an intermediate bend angle (153.0°), B1312 (PDB Entry: 1IGF, first Fab in the entry).

In the case where the Fab is most bent [Fig. 3(a), Table 10a], the residues from V_H, which are involved in the contact, are from the N- and C-terminal segments of the domain; those from C_H1 are from the N-terminal segment and from two bends: one at around position 151 and the other at around position 205; those from V_L come from the bend at around position 81 and from the C-terminal segment of the domain; those from C_L come from two bends: one at around position 140 and the other at around position 168. As the Fab becomes straighter (Table 10b), fewer contacts are formed; the V_H and C_L segments involved in the interaction remain in contact, but interactions involving the N- and C-terminal segments of C_H1 become less and less and are ultimately lost, as well as the interactions involving the bend at around position 81 of V_L. Not surprisingly, as the Fab becomes almost straight [Fig. 3(b), Table 10c], the N-terminal segment of V_L starts to be involved in the contact, so that the V_L–C_L and V_H–C_H1 interactions become more analogous.

The biological function of the C_L–C_H1 module is not obvious although the association of C_L and C_H1 necessarily increases the probability of a proper V_L–V_H quaternary interaction. In addition, the presence of additional domains in the Fab arms extends the reach of the antibody and the loose connection between the Fv and the C_L–C_H1 module contributes to the flexibility of the molecule (see below). It is also conceivable that the C_L and C_H1 may help conceal other ligand binding sites that are uncovered only when antigen is bound. A more definite function for C_L–C_H1 may be revealed in time.

The Fc

The structure of this fragment has been reviewed elsewhere (Padlan, 1990*b*) and some parts of that review are reproduced here. Crystal structures are available for the Fc of human IgG1 (Deisenhofer, 1981) and for that of rabbit IgG (Sutton and Phillips, 1983), and they are very similar. However, atomic coordinates are available for only the human Fc (PDB Entries: 1FC1 and 1FC2). The structure of the pFc′ of guinea pig IgG has also been analysed (Bryant *et al.*, 1985) (PDB Entry: 1PFC) and it is found to be similar to that of the Cγ3–Cγ3 module of human Fcγ. We will confine our discussion to the structure of human Fcγ.

The structure that is available for human Fcγ is comprised of residues 238-443 (Eu numbering), which includes most of the Cγ2 and Cγ3 domains. In some crystal forms of Fc or of intact antibody, the two halves of the fragment are related by a crystallographic, i.e. exact, two-fold axis. In the crystal form of human Fcγ that was analysed crystallographically, the two chains are related by a pseudo-dyad axis. In that structure

Table 9a. C_L–C_H1 contacts in the human (IgG1,λ) Kol (PDB Entry: 2FB4)

	V125	F126	P127	L128	A129	K133	A141	L142	L145	K147	H168	F170	P171	V173	Q175	S176	L182	S183	V185	K213	K218	S219	C220	Totals
T116							1																	1
F118				9	2		5	2											2					20
S121		2	3																					5
S122																					1			1
E123	1	4	1																	6[s]				12
E124		13								4[s]														17
K129										2														2
T131									2	2														4
V133									2									1						3
L135												7						2	1					10
I136												2												2
S137											1	2												3
E160														2	4	3								9
T162														3										3
S165													1											1
Q167											3													3
A174												2												2
A175												2												2
S176												2												2
Y178									1					3			2	5						11
T208						2																		2
E213																					4		2	6
C214																						4		4
Totals	1	19	4	9	2	2	6	2	5	8	4	17	1	8	4	3	2	8	3	6	5	4	2	125

Table 9b. C_L–C_H1 contacts in the human (IgG1,κ) 3D6 (PDB Entry: 1DFB)

	F126	P127	L128	A129	S131	S132	S136	T139	A141	L145	K147	H168	F170	P171	V173	Q175	V185	T187	S219	Totals
F116						6	5	2	6									2		21
I117						6														6
F118			7	1	1				5											14
S121		3																		3
E123		2																		2
Q124	13										1									14
T129											1									1
S131										1	3									4
V133			3																	3
L135									1				1				3			5
N137												2						1		3
N138												1								1
Q160															1	3				4
S162													1	8	1					10
V163														3						3
T164												1	4							5
S174												1	4							5
L175													3							3
S176													7							7
T180											1									1
C214																			1	1
Totals	13	5	10	1	1	12	5	2	12	1	6	5	20	11	2	3	3	3	1	116

Table 9c. C_L–C_H1 contacts in the murine (IgG2a,κ) 17/9 (PDB Entry: 1HIL, first Fab in the entry)

	Y126	P127	L128	A129	P130	V131	T135	G137	L145	K147	H168	T169	F170	P171	V173	Q175	T181	S183	S184	S185	K213	Totals
S116							4															4
F118			12	4	2		4															22
P119						1																1
S121	3	4																				7
E123	2	2																			2[s]	6
Q124	19																					19
S131									2	3												5
V133			4						1													5
F135			2				1	2					4					2	2	8		21
N137													1							3		4
N138											5											5
L160															4	2	2					8
N161															1							1
S162													4	8	1							13
W163														4								4
T164												1	2									3
D167											2											2
S174											6		4									10
M175													8									8
S176													9					3				12
S180										3												3
Totals	24	6	18	4	2	1	9	2	3	6	13	1	32	12	6	2	2	5	2	11	2	163

Table 9d. C_L–C_H1 contacts in the murine (IgA,κ) J539 (PDB Entry: 2FBJ)

	Y126	P127	L128	T129	L130	L134	I141	L145	H147	T166	V168	F170	P171	A173	L174	A175	T181	S183	Q185	Totals
S116							1													1
I117					1	2														3
F118			7	6	6		6													25
S121		6																		6
E123	4	3																		7
Q124	18																			18
S127	2																			2
S131								2	1											3
V133			2					1												3
F135			2									6						3	10	21
N137																			8	8
L160														4	4	4	2			14
N161														1						1
S162													2	1						3
W163													3							3
T164											1	2								3
K169										1										1
S174											1	3								4
M175												7								7
S176												10						2		12
T178								1												1
T180									1											1
Totals	24	9	11	6	7	2	7	4	2	1	2	28	5	6	4	4	2	5	18	147

The residues on the horizontal lines are from C_L and those along the vertical are from C_H1. In these matrices of contacts, the element $c(i,j)$ represents the number of interacting atom pairs, one from residue i and the other from residue j. The superscript [s] that is found after some matrix elements indicates that the contact includes a favorable electrostatic interaction; the superscript [h] signifies that the contact involves at least one hydrogen bond. In 3D6 and in Kol, a disulfide bond exists between Cys214-L and Cys220-H. See footnote to Table 4 for the definition of contacts.

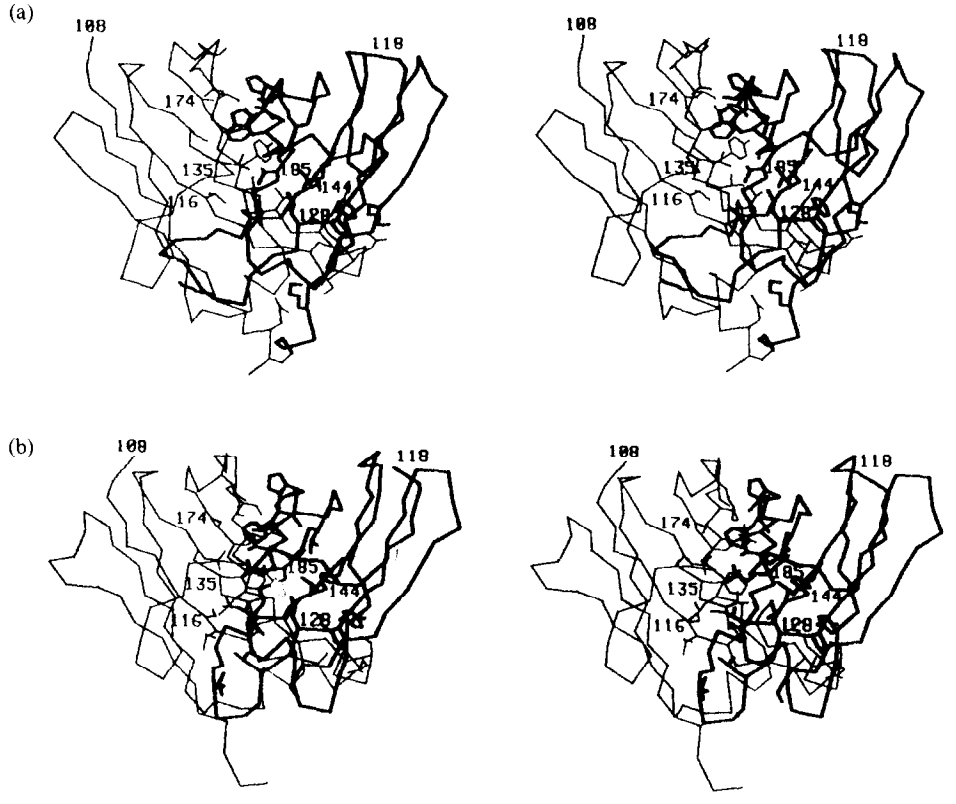


Fig. 6. Stereodrawings of the α-carbon trace of the C_L–C_H1 modules of Kol (a) and of J539 (b) showing the residues which are in the C_L–C_H1 contact. The C_Ls are drawn with thinner lines on the left. The side chains of the residues involved in the contact are shown in full.

(Fig. 7), the two Cγ3 domains are related by a rotation angle of 179.3° while the Cγ2 domains are related by a rotation of 174.3°. Carbohydrate moieties, that are linked to the asparagines at position 297 (Eu numbering) in both chains, lie between the two Cγ2 domains. The Cγ3 domains are in close association and form a compact globule, while the Cγ2 domains are farther apart.

The Cγ2 and Cγ3 domains both resemble closely the constant domains of the Fabs. The residues which form the bilayer β-pleated sheet structure are indicated in Table 2. The interior of both domains is filled with mainly hydrophobic side chains. Side chains emanating from one β-sheet in each bilayer form the contact between the Cγ3 (Fig. 8). The interaction is strong and involves more than 20 residues from each domain (Table 11). Approximately 2000 Å^2 of surface area are buried in the Cγ3–Cγ3 interface, where several hydrophobic residues, including some with large, aromatic side chains, are found. In addition, there are a number of hydrogen-bond interactions and three salt bridges.

In contrast, the analogous sheets in the Cγ2 domains are covered by the carbohydrates linked to the asparagines at 297 (Fig. 7), so that a mode of association, like the one in the Cγ3 domains, is not possible. Instead, the Cγ2 domains interact through their carbohydrate moieties, and only weakly. Even without the

Table 10a. Atomic contacts between the variable and constant domains of the same chain in 8F5 Fab (PDB Entry: 1BBD)

				C_H1				
V_H	A118	K119	T120	F150	P151	P153	P205	A206
A9						1	2	1
L11	1	1	4	1	1			
S108						1		
T110				1	3			
S112				2				

			C_L		
V_L	Y140	Q166	S168	K169	S171
A80			5	2	
E81			2		
L83		4			
E105		2			
L106	2	11			1
K107	1				

Table 10b. Atomic contacts between the variable and constant domains of the same chain in B13I2 Fab (PDB Entry: 1IGF)

		C_H1		
V_H	A118	T120	F150	P151
L11	1	1	3	3
T110			1	
S112			2	

			C_L		
V_L	Y140	Q166	S168	S171	Y173
L83		2	1		
E105	6	5			5
I106	1	6		4	
K107	5				

Table 10c. Atomic contacts between the variable and constant domains of the same chain in R19.9 Fab (PDB Entry: 2F19)

	C_H1	
V_H	F150	P151
L11	4	1
S112	4	

	C_L			
V_L	Y140	Q166	S171	Y173
S12	1			
E105	4	3		4
I106	4	11	2	
K107	10			

The residues on the horizontal lines are from the constant domains and those along the vertical are from the variable domains of the Fabs. See footnote to Table 9.

carbohydrates, the Cγ2 domains may not be able to associate in the manner of the Cγ3; the N-termini of the Cγ2 domains are probably too close, in view of the interchain disulfide bridge(s) in the hinge region, while the N-termini of the Cγ3 domains are far (more than 40 Å) apart.

The longitudinal contact between the Cγ2 and Cγ3 domains is substantial, with approximately 780 Å^2 of surface area buried by the interaction. Seventeen residues, 8 from Cγ2 and 9 from Cγ3, are involved in this contact, which includes two salt-bridges (Table 12).

The amino acid sequence of human IgG1 Cγ2 and Cγ3 and those of the homologous domains of the other human heavy chains can be compared in Table 2. The various IgG isotypes are similar to a very high degree and there is little doubt that the structures of their Fc will be essentially the same. The sequences of the other heavy chains are not as similar but they share enough structural features with IgG1 to justify the assertion that the homologous structures in their Fc will have the same general architecture.

No obvious structural function can be attributed to the carbohydrate attached to the Asn at 297 in IgG1 (Fig. 7). Interestingly, all the other heavy chains are probably glycosylated at the same (homologous) position, except the IgAs. In the latter, a probable site of carbohydrate attachment is the Asn at position 258 (Table 2). It is interesting that the residue at position 258 in IgG1 is close to the end of the carbohydrate moiety (Fig. 7) and contacts the last sugar residue. One wonders whether, in the IgAs, a carbohydrate attached to residue 258 might follow a course reversed in relation to that found in IgG1, but nevertheless covering one face of C_H2 in much the same way as that observed in IgG1 (Deisenhofer, 1981). It should be pointed out that the

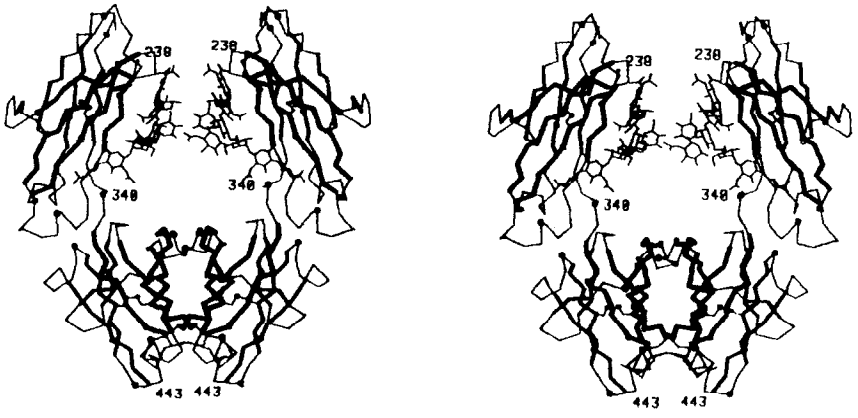


Fig. 7. Stereodrawing of the α-carbon trace of human IgG1 Fc. The C_H2 domains are on top and the C_H3 domains are at the bottom. The first (number 238) and last (number 443) residues that were visible in the crystal structure (Deisenhofer, 1981), and the C-terminus of the C_H2 domain (number 340) are labeled in each chain. Every tenth residue, starting from number 240, is indicated by a larger, open circle. The intradomain disulfide bonds are drawn with thicker bonds and filled circles in the middle of each domain. The β-pleated sheets are drawn with thick bonds, thicker for the first sheets.

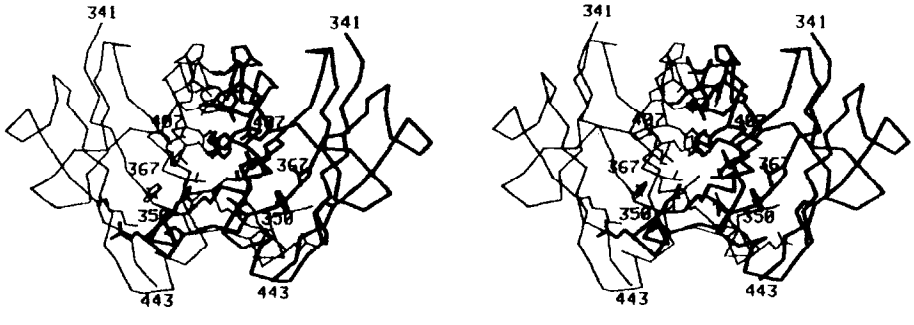


Fig. 8. Stereodrawing of the α-carbon trace of C_H3–C_H3 module of human IgG1 Fc. The side chains of the residues involved in the interdomain contact are shown in full.

Table 11. C_H3–C_H3 contacts between the first and second chains in human IgG1 Fc (PDB Entry: 1FC1)

1st	2nd Q347	Y349	L351	P352	S354	E356	E357	T366	L368	K370	N390	K392	T394	P395	V397	L398	D399	S400	F405	Y407	K409	K439	Totals
Y349					1	5	12																18
L351			2	1	1																		4
P352			1																				1
S354		1	3																				4
E356		4																				5[s]	9
E357		10								3[s]													13
K360	2	1																					3
S364									1	1													2
T366			1																	7			10
L368																					2		2
K370							2																2
N390																		1					1
K392																4	1		5				10
T394													5		3				1				9
P395														1	1								2
V397													3	1									4
L398												3											3
D399												5									3		8
S400											1												1
F405												5	2								2		9
Y407								7					1							16	7		31
K409									1								2		3	6			12
K439					1	10[s]																	11
Totals	2	16	7	1	3	15	14	7	2	4	1	13	11	2	4	4	3	1	9	31	14	5	169

The residues on the horizontal line are from the second chain and those along the vertical are from the first chain of the human IgG1 Fc (PDB Entry: 1FC1). See footnote to Table 9.

Table 12. C_H2–C_H3 contacts in human IgG1 Fc (PDB Entry: 1FC1, first chain in the entry)

	C_H3									
C_H2	Y373	P374	E376	I377	E380	M428	H429	E430	H435	Totals
P247			1	1						2
K248					3[s]	5				8
L251						2	4	3	7	16
M252						3				3
L314								4	1	5
K338		1						7[s]		8
A339	2	1								3
K340	2									2
Totals	4	2	1	1	3	10	4	14	8	47

The residues on the horizontal line are from the C_H3 domain and those along the vertical are from the C_H2 domain of the first chain of the human IgG1 Fc (PDB Entry: 1FC1). See footnote to Table 9.

carbohydrate moieties attached to the asparagines at 297 in rabbit IgG were found to be asymmetrically disposed and to be in greater contact than those in human Fcγ (Sutton and Phillips, 1983). A detailed comparison of these two Fcγ structures, when atomic coordinates for the rabbit Fc become available, may reveal other differences.

The hinge

Antibodies exhibit segmental flexibility, which is made possible by the presence of the hinge region between the Fabs and the Fc and of the switch regions within each fragment [see, for example, Burton (1985, 1990*b*) and Tan *et al.* (1990)]. The hinge permits the fragments to rotate or to wag, while the switch regions permit the fragments to flex. By and large, the hinge can be viewed as consisting of three parts: a flexible upper region which permits the Fabs to rotate and wag and which also determines the separation between the Fab arms, a stiff middle which acts as a spacer between the Fabs and the Fc, and a flexible lower region which allows the Fc to wag. The rigidity of the middle part is almost certainly provided by the inter-heavy chain cystines and by the many proline residues that are often found in the hinge. The lower part of the hinge would be the segment between the last inter-heavy chain cystine and the residue which marks the beginning of the compact Fc (Pro238 in human IgG1 or homologous residue in the other antibody classes). The residues which constitute the flexible upper part of the hinge are not easily identified in the absence of three-dimensional structure. The size of the three hinge parts varies among the different antibody classes, so that the middle part, for example, ranges in size from the single cystine in human IgD to more than 40 residues in IgG3 (Table 2).

Very little three-dimensional information is available for the hinge, mainly because of segmental flexibility. The crystal structure of the intact human IgG1, Kol, for example, did not reveal the Fc, which ostensibly assumed several different positions and orientations and, thus, was "averaged out" in the electron-density map (Marquart *et al.*, 1980). The portion of Kol that was visible extended only to Cys229 (Eu numbering). Consequently, the octapeptide, PAPELLGG, between Cys229 (the last residue visible in Kol) and Pro238 [the first residue visible in the Fc structure (Deisenhofer, 1981)], has not been visualized and probably represents the most flexible part of human IgG1. The crystal structure of a murine IgG2a monoclonal antibody showing a complete hinge has been reported (Harris *et al.*, 1992), but full details of the structure have not been presented.

In the picture of the human IgG1 hinge that is available (Fig. 9), the segment, DKTHTCPPC, which represents the upper and middle portions of the hinge, contains strong secondary structural elements; the KTHT region is α-helical and the CPPC segments from the two chains form a poly-L-proline double helix, cross-linked by the two pairs of cysteines (Marquart *et al.*, 1980). This part of the IgG1 can be expected to be rigid. Indeed, a visual examination of this region in the crystal structure (Fig. 9) strongly suggests that at least a partial unraveling of the α-helical structure may

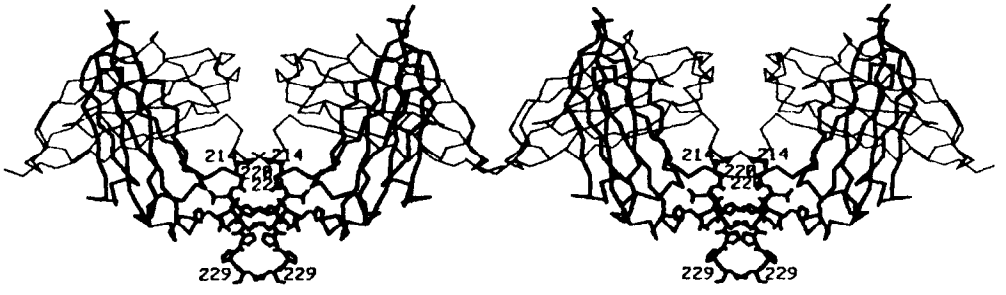


Fig. 9. Stereodrawing of the part of the hinge that is visible in the crystal structure of Kol (Marquart *et al.*, 1980). The orientation is the same as in Fig. 1.

be required to give the Fabs the freedom to rotate. In this antibody, the Fabs are separated by only 10 Å (the distance between the C-termini of the Fabs).

We can only speculate on the nature of the hinge in the other antibody classes and isotypes. The hinge of human IgD is probably the most floppy, having 46 amino acids between the putative end of the Fab and the inter-heavy chain cystine (Table 2); of course, the possible occurrence of helical structures in this stretch cannot be ruled out. The human IgD hinge also may provide the greatest possible separation between the Fabs [each of the two 46-a.a. segments could be as long as 160 Å (3.5 Å per residue) if fully extended]. The hinge of human IgG3, on the other hand, has been proposed to separate the Fabs from the Fc with a stiff rod 140 Å long in the form of a polyproline double helix stabilized by many disulfide bonds (Marquart *et al.*, 1980). The hinge regions of human IgG4 and of the two IgAs are probably also stiff in view of the presence of many prolines (Table 2).

The C_H2 domains of IgE and IgM could also be viewed as rigid Fab separators and thus function in part as "hinges". Modeling of the Fc of IgE (Padlan and Davies, 1986; Pumphrey, 1986; Helm *et al.*, 1991) positions the ends of the Fabs approximately 33 Å apart. A flexible segment following the (last) inter-heavy chain disulfide bridge has been proposed for IgE (Helm *et al.*, 1991) and for IgM (Perkins *et al.*, 1991).

Nature seems to have made provision for coping with different varieties of antigens by the use of the hinge. The variation in hinge length and amino acid sequence in the different heavy chain classes and isotypes results in antibodies with different reach and rotational adaptability, giving the immune system the means to cope with the possibility of different spacings and orientations of antigenic determinants (e.g. Beale and Feinstein, 1976; Burton, 1990*b*). One wonders also whether the variation in the structure of the hinge may have a bearing on the respective roles that the different antibody types play in the overall immune response.

The demonstrated flexibility of the lower portion of the hinge poses an intriguing question since a concerted conformational change in the two disulfide-linked chains would be required to produce an overall deformation in this part of the antibody. In view of the fact that alterations in the conformation of a polypeptide backbone are accomplished by changes in the peptide dihedral angles, which are restricted (Sasisekharan, 1962), this part of the hinge may not be dynamically, i.e. continuously, deformable. Indeed, flexed forms of antibodies, e.g. states in which the Fc and the Fabs are not co-planar, may persist for sufficiently long times to be observable (Zheng *et al.*, 1991, 1992).

STRUCTURAL ASPECTS OF ANTIBODY FUNCTION

Antigen binding

The antibody function for which we have the most information is antigen binding. It is well known that the binding of antibody to its antigen is exquisitely specific. Yet, it is also known that many antibodies are polyreactive, i.e. they are capable of binding to several different antigens, although with low affinity [see, for example, Burastero *et al.* (1988)]. Polyreactivity is usually associated with natural or pre-immune antibodies which frequently display reactivity towards self-antigens and which are usually encoded by unmutated or essentially unmutated germline genes [see, for example, Baccala *et al.* (1989) and Logtenberg (1990)]. The structural basis for polyreactivity is not yet clear. What is better understood is the basis for high-affinity antibody–antigen interactions.

(i) *Structural correlates of antigen-binding specificity.* The high specificity of the binding of antibody to its antigen is due to the complementarity in the structures of the antibody combining site and the antigenic determinant (the epitope). This is illustrated by the complexes between the antibodies D1.3, HyHEL-5 and HyHEL-10 and their antigen, hen egg white lysozyme (Table 13,

Table 13a. Contacts between antibody and ligand in the D1.3–lysozyme complex (PDB Entry: 1FDL)

	Antibody															
	Light Chain							Heavy Chain								
Lysozyme	[Y32	Y49	Y50	T53	F91	W92	S93]	[G31	Y32	W52	G53	D54	D97	Y98	R99]	Totals
D18			5													5
N19		1	4	2												7
G22		1											1		3	5
Y23													2			2
S24													9	2		11
N27													3			3
K116								2	1							3
G117								3		1	6	1				11
T118										1		4				5
D119										12				4		16
V120														4		4
Q121	9				4	8	1							7		29
I124	1					2										3
R125						6										6
Totals	10	2	9	2	4	16	1	5	1	14	6	5	15	17	3	110

Table 13b. Contacts between antibody and ligand in the HyHEL-5–Lysozyme complex (PDB Entry: 2HFL)

	Antibody																					
	Light Chain								Heavy Chain													
Lysozyme	[N31	Y32	Y34	D50	W91	G92	R93	P95]	[W33	E35	W47	E50	L52	S54	G55	S56	T57	N58	G95	N96	Y97]	Totals
Q41														2	1	3						6
T43									2							6	2	7				17
N44																		2				2
R45					11	4	12	5			3	4[s]										39
N46						2	3															5
T47						1	2															3
D48	2																					2
G49					3																	3
T51									1													1
Y53									5													5
G67																					5	5
R68					7				9	1[s]		2[s]							4	1	6	30
T69																					5	5
P70		5	1	3																	7	16
S81													1									1
L84													1	3		1						5
Totals	2	5	1	3	21	7	17	5	17	1	3	6	2	5	1	10	2	9	4	1	23	145

Table 13c. Contacts between antibody and ligand in the HyHEL-10–lysozyme complex (PDB Entry: 3HFM)

	Antibody																				
	Light Chain									Heavy Chain											
Lysozyme	[G30	N31	N32	Y50	Q53	S91	N92	W94	Y96]	[T30	S31	D32	Y33	Y50	S52	Y53	S54	S56	Y58	W95]	Totals
R14		1																			1
H15		8																			8
G16	3	6	1																		10
Y20			3			5	4														12
R21							4	2	3					6					6		21
W63													1			5					6
R73										12	4										16
L75											2	1				1					4
T89					3																3
N93				4	7																11
K96		4	1	13																	18
K97												1	8							1	10
I98													3								3
S100													1	5					1	4	11
D101													3		7	19	2	5	3		39
G102																		2	5		7
Totals	3	19	5	17	10	5	8	2	3	12	6	2	16	11	7	25	2	7	15	5	180

Table 13d. Contacts between antibody and ligand in the B13I2–peptide complex (PDB Entry: 2IGF)

	Antibody																		
	Light Chain						Heavy Chain												
Peptide	[D28	D30	Y32	G91	V94	P95]	[R31	A33	I51	S52	S52a	G53	S55	Y56	F58	Y95	P99	F100]	Totals
E1										2	3	5	7	3					20
V2							4			3	12						2		21
V3										1				3	2				6
P4								1	4	3					2	5	1		16
H5				4	6	5										8	2	6	31
K6					1										4				5
K7	6[s]	2[s]	4															2	14
Totals	6	2	4	4	7	5	4	1	4	9	15	5	7	6	8	13	5	8	113

The residues on the horizontal line are from the antibody and those along the vertical are from the ligand. See footnote to Table 9.

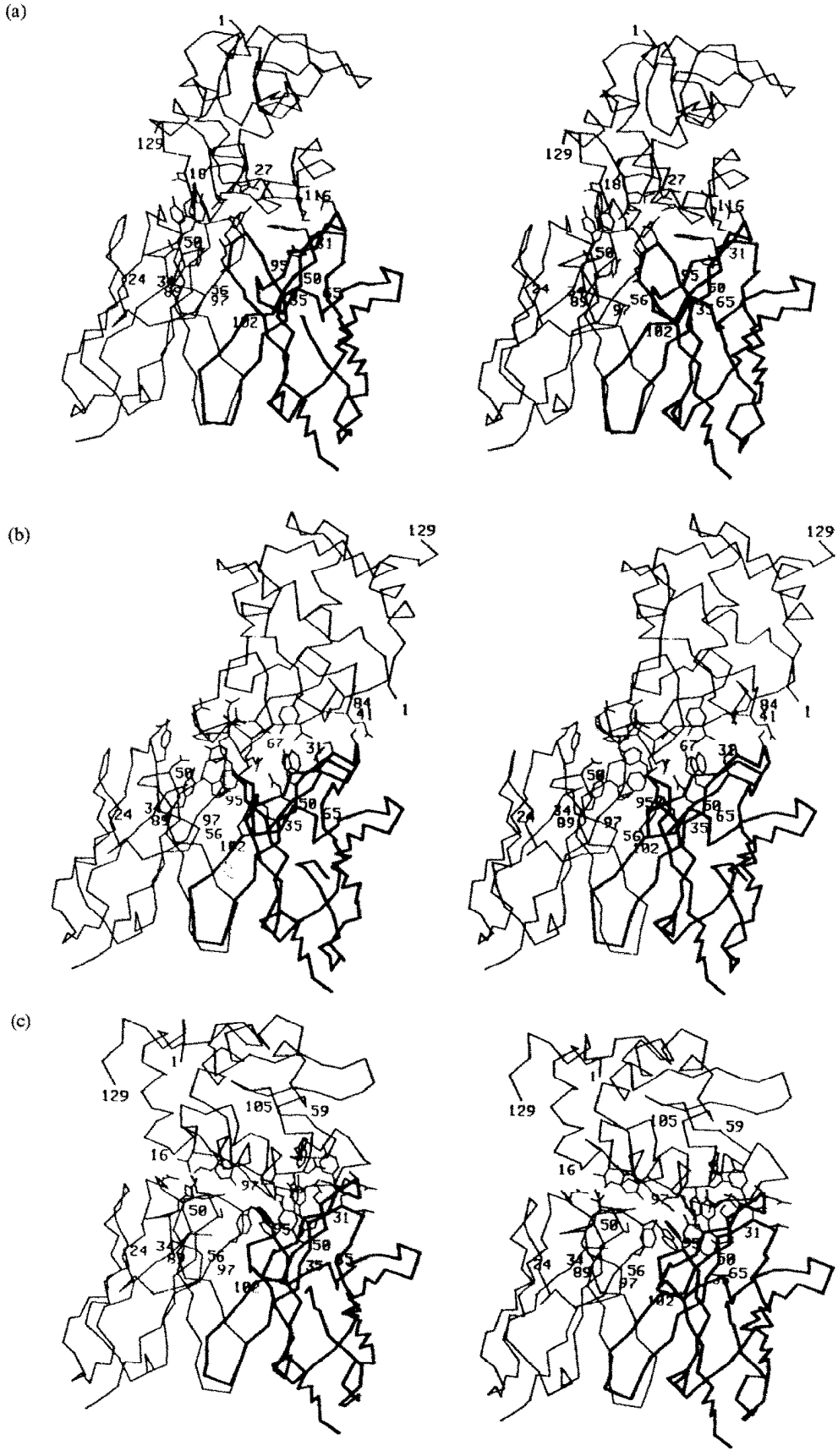


Fig. 10. Stereodrawing of the interaction between the antigen, hen egg white lysozyme, and the antibodies D1.3 (a), HyHEL-5 (b) and HyHEL-10 (c). Only the α-carbons are traced, except for the residues involved in the interaction, which are drawn in full. In these drawings, the lysozyme is on top and the Fv of the antibody is at the bottom; the V_Ls are drawn with thinner lines on the left. The figures are oriented such that the Fvs are maximally superposed.

Fig. 10). There is complementarity in the antibody and antigen surfaces which are in contact, so that depressions in one are by and large filled by protrusions from the other [in D1.3 and HyHEL-5, solvent molecules are found trapped in the interface between the two macromolecules (Sheriff *et al.*, 1987; Fischmann *et al.*, 1991)]. There is also complementarity in the physical and chemical properties of the interacting surfaces, so that hydrogen bonds are formed whenever possible and, in the case of HyHEL-5, oppositely-charged side chains form ion pairs (Table 13b). Many aromatic residues figure prominently in these antibody–antigen interactions.

The interactions between antibody and specific ligand in various complexes are summarized in Table 14. The contacts involve van der Waals' interactions, hydrogen bonds between polar groups and ion pairs. Interestingly, the hydrogen bonds mostly involve side chain atoms; very few main-chain–main-chain hydrogen bonds are observed in these antibody–ligand complexes.

The interaction between antibody and larger ligands, e.g. peptides and trinucleotides, is comparable to that between antibody and whole antigen (Table 14). It is seen in Table 14, that antibody–peptide interactions approximate those between antibody and whole protein antigens, in terms of the surface areas buried upon complexation, the number of van der Waals' contacts and the number of hydrogen bonds formed. Details of the contact between B13I2 and a myohemerythrin-peptide homolog are included in Table 13 for comparison.

In the three anti-lysozymes, HyHEL-10, HyHEL-5 and D1.3, the surface that is used in the binding to antigen represents only 28.4, 26.5 and 21.4%, respectively, of the total surface formed by the CDRs; the CDR residues which contact the antigen represent only 35.8, 37.0 and 25.0% of the total number of CDR residues. The antibody–antigen contact utilizes primarily the central portion of the CDR surface in the three anti-lysozyme cases, and in all of the antibody–ligand structures determined so far.

The CDR residues involved in the contact with ligand in various antibody–ligand complexes of known structure are shown in Table 15. Many aromatic residues are seen to be involved in the contacts, whereas the involvement of small, apolar aliphatics is rare, a trend that was noted previously [Padlan (1990*a*); see also Mian *et al.* (1991)]. The ligand-contacting residues are seen to originate mostly from the C-terminal part of CDR1-L, the first and sometimes also the middle position in CDR2-L, from the whole of CDR3-L, CDR1-H and CDR3-H, and from the N-terminal part and the middle of CDR2-H. Thus, it is the central portion of the CDR surface (Fig. 4) that contacts the ligand in these, and possibly in all, antibody–antigen complexes.

The CDR3-L and CDR3-H are seen to play a prominent role, not only in ligand binding (Table 15), but also in the contact with the opposite domain (Table 8) and in the contact with the other CDRs (Table 6). It is probably no coincidence that these two CDR3s also usually display the greatest variability (Kabat *et al.*, 1991) and, in CDR3-H, the greatest variation in length and conformation (e.g. Wu *et al.*, 1993).

Although antigen binding primarily involves the CDRs, framework residues have been found on occasion to be involved also in the interaction with ligand. Thus the framework residue, Tyr49-L in D1.3 (Amit *et al.*, 1986; Fischmann *et al.*, 1991), and the Tyr49-L also in NC41 (Tulip *et al.*, 1992*a*), were found to be in contact with the antigen, as were the framework residues, Trp47-H in HyHEL-5 (Sheriff *et al.*, 1987) and Thr30-H in HyHEL-10 (Padlan *et al.*, 1989).

(ii) *The diversity of antigen-binding specificities.* The antigen-binding specificity of an antibody is defined by the physical and chemical properties of its CDR surface;

Table 14. Various antibody–ligand interactions

Antibody	Surf. buried	Ligand	Surf. buried	vdW	m.c.vdW	H.b.	m.c.H.b.	I.p.
McPC603 Fab	151	Phosphocholine	138	54	4	3		2
AN02 Fab	363	DNP-spin-label	230	129	5	2		0
4-4-20 Fab	338	Fluorescein	247	107	36	3		0
BV04-01 Fab	523	$d(pT)_3$	443	184	48	12		1
B13I2 Fab	503	Myohemerythrin peptide	439	113	27	13	2	2
17/9 Fab[a1]	488	Hemagglutinin peptide	418	143	36	14	1	1
Fab[a2]	536	Hemagglutinin peptide	459	154	34	13	1	1
Fab[b]	488	Hemagglutinin peptide	417	144	41	12	1	0
D1.3 Fab	537	Lysozyme	541	110	18	14	2	0
HyHEL-5 Fab	744	Lysozyme	741	145	25	13	0	3
HyHEL-10 Fab	716	Lysozyme	759	180	21	19	1	0
NC41 Fab	882	Neuraminidase	838	154	20	13	1	0

[a]From PDB Entry: 1HIM ([1]first Fab in the entry; [2]second Fab in the entry).

[b]From PDB Entry: 1HIN.

The contacts involving main chain atoms (m.c.) are listed separately. See footnote to Table 4 for the definition of contacts.

Table 15. CDR residues in contact with ligand in murine and human antigen-binding regions of known three-dimensional structure

	CDR1-L	CDR2-L	CDR3-L	CDR1-H	CDR2-H	CDR3-H
	*** * *	** * *	*** * *	*** * *	** * *	*
HyHEL-10	RASQ------SIGNNLH	YASQSIS	QQSNSWP-YT	SDYWS-	YVS---YSGSTYYNPSLKS	WDG------DY
	** * *	*** *	* *	* *	*****	***
HyHEL-5	SASS-------SVNYMY	DTSKLAS	QQWGRN--PT	DYWIE-	EILP--GSGSTNYHERFKG	GNYDF----DG
	* * *	***	**	* **		***
D1.3	RASG------NIHNYLA	YTTTLAD	QHFWSTP-RT	GYGVN-	MIW---GDGNTDYNSALKS	ERDYRL---DY
	** * *	*	*		* *	
McPC603	KSSQSLLNSGNQKNFLA	GASTRES	QNDHSYP-LT	DFYME-	ASRNKGNKYTTEYSASVKG	NYYGSTWYFDV
	* * *	* *	*		***	
4-4-20	RSSQSLVHS-QGNTYLR	KVSNRFS	SQSTHVP-WT	DYWMN-	QIRNKPYNYETYYSDSVKG	SYYGM----DY
	* * *	* *			**	
AN02	SASS-------SVYYMY	DTSNLAS	QQWSSYPPIT	SDYAWN	YMS---YSGSTRYNPSLRS	GWPL-----AY
	* * *	* * *	* *	*** * ** *	* **	
B13I2[@]	RSNQTILLS-DGDTYLE	KVSNRFS	FQGSHVP-PT	RCAMS-	GISS--GGSYTFYPDTVKG	YSSDPFWF-DY
	** * *	****	***	* ** *	*******	
BV04-01	RSSQSLVHS-NGNTYLH	KVSNRFS	SQSTHVP-LT	TNAMN-	RIRSKSNNYATYYADSVKD	DQTGTAWF-AY
	*	**** *		* ** **** *	*** *	
17/9[#]	TSSQSLFNSGKQKNYLT	WASTRES	QNDYSNP-LT	SYGMS-	TISN--GGGYTYYPDSVKG	RERYDENGFAY
	* * **	** * *	**	* *	**** **	
NC41	KASQ------DVSTAVV	WASTRHI	QQHYSPP-WT	NYGMN-	WINT--NTGEPTYGEEFKG	GEDNFGSL-DY

[@] From PDB Entry: 2IGF.
[#] From PDB Entry: 1HIM (first Fab in the entry).
See footnote to Table 4 for the definition of contacts.

these in turn are determined by the conformation of the individual CDRs, by the relative disposition of the CDRs, and by the nature and disposition of the side chains of the amino acids in the CDRs. Thus, the pronounced structural variability in the CDRs, which is due not only to sequence variability, but also to the insertions and deletions frequently found in these segments, and the possible pairing of different V_L and V_H provide a ready explanation for the wide diversity of antigen-binding specificities. Within each CDR, there are residue positions that are more hypervariable than others; these positions are presumably more involved in the determination of antigen-binding specificity and in the diversification of specificities. The other residues in the CDRs may play an ancillary role and simply provide additional "stickiness" (see below). In addition, the more conserved residues in the CDRs may play a structural role and serve to stabilize the combining-site structure (Kabat *et al.*, 1977; Padlan, 1977*b*).

The possibility of "induced fit" provides an additional means of generating other specificities, but in this case without introducing any new ingredients into the picture. "Induced fit" can be achieved by small movements of side chains, or by more substantial structural modifications like the deformation of the CDR loops, or by a change in the relative disposition of the variable domains. The flexibility of the combining site, which allows the occurrence of "induced fit" necessarily results in the entropic loss upon complexation, but the greater interaction due to a more precise fit may result in an overall increase in binding energy.

Evidence is accumulating which suggests that any macromolecule, if presented properly, can be antigenic and, further, that all accessible areas of a macromolecule can potentially be bound by antibody [see Benjamin *et al.* (1984) for a review]. Moreover, there appear to be no special structural requirements for antigenicity (Padlan, 1992). If antibody combining sites are to participate in strong interactions with many possible antigenic structures, they must possess structural features which make them especially suited for interacting with ligands.

A survey (Padlan, 1990*a*) of the then available primary and three-dimensional data on the CDRs revealed that Tyr, His and Asn have a propensity for being in the CDRs. Moreover, although the exposure patterns of the various amino acid types in immunoglobulins are comparable to those in other water-soluble proteins, those with aromatic side chains are more exposed when in CDRs than when in the framework regions. Those results are confirmed when the now larger structural database on combining-site structures is analysed. The solvent accessibilities of the side chains in various Fv fragments of known three-dimensional structure are presented in Table 16a; the solvent exposures of the CDR and framework residues are presented separately in Tables 16b and 16c, respectively. For comparison, the solvent accessibilities of the side chains in 50 highly-refined structures of water-soluble proteins (Padlan, 1990*a*) are provided in Table 17.

Again, Trp and Tyr are found to be more buried when in the framework and more exposed when in the CDRs than in water-soluble proteins in general (Fig. 11); phenylalanines also seem to be following the trend. The Trp, Tyr and Phe in the framework regions of antibody variable domains are usually found in the interdomain interface (Fig. 5) and in the domain interiors [see also Poljak *et al.* (1975*a*), Davies *et al.* (1975*b*) and Novotny *et al.* (1983)]. When in the CDRs, these aromatic residues are frequently found to be involved in the interaction with ligand (see above and Fig. 10).

Amino acids with aromatic side chains can contribute significantly to ligand binding because of their large size (for greater hydrophobic effect), their large polarizabilities (for greater contribution to the van der Waals'

Table 16a. Exposure of residues in murine and human Fvs of known three-dimensional structure

	Bu	mB	pB	mE	Ex	Total
ALA	111	21	17	16	75	240
ARG	37	40	31	45	32	185
ASN	23	13	30	50	30	146
ASP	45	17	32	49	61	204
CYS	82	0	1	0	0	83
GLN	67	48	16	63	67	261
GLU	19	3	21	59	56	158
GLY	117	0	0	0	329	446
HIS	9	1	12	5	6	33
ILE	122	19	17	7	12	177
LEU	227	34	37	27	26	351
LYS	4	7	24	87	84	206
MET	59	5	8	5	0	77
PHE	118	14	12	7	3	154
PRO	25	10	30	76	43	184
SER	32	21	49	167	334	603
THR	46	36	82	132	91	387
TRP	85	10	9	5	0	109
TYR	114	54	55	35	15	273
VAL	162	53	21	16	28	280
Total	1504	406	504	851	1292	4557

interaction), and their ability to hydrogen bond [through their aromatic rings (Levitt and Perutz, 1988) or through polar atoms in their side chain], and, very importantly, because of their relative rigidity since they have few degrees of freedom (for lesser loss of conformational entropy upon complexation).

On the other hand, the apolar, aliphatic side chains of Val, Ile and Leu are incapable of participating in ionic interactions. They can contribute to ligand binding only through van der Waals' interactions and the hydrophobic effect. Moreover, in view of their rotational degrees of freedom which could be frozen upon complex formation, they can have a significant negative contribution to ligand binding (Novotny *et al.*, 1989; Padlan, 1990*a*).

It should also be noted that asparagines are more buried when in the CDRs than when in the framework. In the CDRs, the asparagines are found to be often involved in hydrogen bonds, many to main chain atoms. It was argued (Padlan, 1990*a*) that these asparagines serve to help stabilize the CDR loops (the main stabilizing factor would be the strong architecture of the domain framework) and permit the exposure of the hydrophobic aromatics, which otherwise would tend to be buried in the domain interior.

Thus the high incidence of exposed aromatic residues, as well as the paucity of apolar, aliphatic side chains, on part of its surface would give an antibody a "sticky patch" and give the molecule the capacity to bind diverse ligands. Specificity for a particular antigen would arise from the precise complementarity of the interacting surfaces and the correct positioning of complementary polar, esp. charged, groups on those surfaces (Levy *et al.*, 1989).

(iii) *Possible structural correlates of polyreactivity.* The affinity of polyreactive antibodies for the antigens to which they bind is rather low, with affinity constants ranging from 10^{-4} to 10^{-7} M (to be contrasted with monoreactive antibodies which show affinities for their specific ligands of 10^{-7} M or better) [see, for example, Burastero *et al.* (1988)]. Binding to many different ligands with low affinity may simply reflect the intrinsic stickiness of the antibody combining site. Indeed, it may be possible that an antibody that has been designated as being monospecific is actually capable of interacting with many different ligands, but that we are aware of only that one ligand for which the affinity is significantly higher than for the rest.

It should be pointed out that a 10-fold increase in affinity requires an improvement in the binding interaction of only 1.37 kcal/mole at room temperature which corresponds roughly to the energy of a hydrogen bond; a 1000-fold increase in affinity corresponds to an energy difference of 4.12 kcal/mole, or roughly the energy associated with a hydrogen bond that involves a charged group (Fersht *et al.*, 1985); the energies associated with salt bridges are difficult to estimate, but these would normally be higher and lead to greater increases in affinity than hydrogen bonds. This means that the difference in the reactivity patterns of polyreactive and monospecific antibodies may simply be a reflection of a single amino-acid difference.

Nevertheless, the concept of polyreactivity is intriguing and the existence of polyreactive natural antibodies clearly provides an organism the ability to cope, albeit weakly, with a deleterious antigen that it had not previously encountered (a better defense against subsequent antigenic challenge is then achieved by

Table 16b. Exposure of CDR residues in murine and human antigen-binding regions of known three-dimensional structure

	Bu	mB	pB	mE	Ex	Total
ALA	31	12	10	3	12	68
ARG	6	6	12	18	7	49
ASN	22	10	19	22	18	91
ASP	9	10	17	22	21	79
CYS	2	0	1	0	0	3
GLN	28	4	3	9	12	56
GLU	6	3	7	4	5	25
GLY	22	0	0	0	76	98
HIS	8	1	12	3	1	25
ILE	29	3	6	2	2	42
LEU	30	5	20	4	3	62
LYS	0	2	7	16	19	44
MET	16	1	0	2	0	19
PHE	16	8	8	5	1	38
PRO	7	10	5	14	6	42
SER	27	12	20	59	71	189
THR	11	15	31	20	4	81
TRP	6	8	9	5	0	28
TYR	13	36	39	35	15	138
VAL	24	9	2	3	4	42
Total	313	155	228	246	277	1219

Table 16c. Exposure of framework residues in murine and human antigen-binding regions of known three-dimensional structure

	Bu	mB	pB	mE	Ex	Total
ALA	80	9	7	13	63	172
ARG	31	34	19	27	25	136
ASN	1	3	11	28	12	55
ASP	36	7	15	27	40	125
CYS	80	0	0	0	0	80
GLN	39	44	13	54	55	205
GLU	13	0	14	55	51	133
GLY	95	0	0	0	253	348
HIS	1	0	0	2	5	8
ILE	93	16	11	5	10	135
LEU	197	29	17	23	23	289
LYS	4	5	17	71	65	162
MET	43	4	8	3	0	58
PHE	102	6	4	2	2	116
PRO	18	0	25	62	37	142
SER	5	9	29	108	263	414
THR	35	21	51	112	87	306
TRP	79	2	0	0	0	81
TYR	101	18	16	0	0	135
VAL	138	44	19	13	24	238
Total	1191	251	276	605	1015	3338

The fractional solvent accessibility values for the individual residues were computed as described by Padlan (1990*a*); residues, the sidechains of which have fractional accessibility values between 0.00 and 0.20, are designated as being completely buried (Bu), between 0.20 and 0.40 as mostly buried (mB), between 0.40 and 0.60 as partly buried/partly exposed (pB), between 0.60 and 0.80 as mostly exposed (mE), and at least 0.80 as completely exposed (Ex). In the special case of glycine, the residue is considered completely exposed if its α-carbon atom is accessible to solvent, otherwise it is considered completely buried. Here, residue exposures are defined in the context of an isolated Fv. The unliganded forms of McPC603, BV04-01, B13I2 (PDB Entry: 1IGF, first Fab in the entry) and 17/9 (PDB Entry: 1HIL, first Fab in the entry) were used in the computations.

affinity maturation). Several factors may contribute to polyreactivity and these are discussed below. Which, if any, can account for polyreactivity in antibodies may become obvious when sufficient structural data on these molecules become available.

It is possible, for example, that different areas of the antibody CDR surface are used for different antigens. In the case of the three anti-lysozymes examined above, less than a third of the CDR surface is utilized in the binding to the antigen so that many other antibody–ligand interactions can be envisioned. In addition, more of the framework residues could be recruited for involvement in the binding interaction.

A close fit between antibody and antigen is a feature of tight binding, and this may not be the case for polyreactivity. Departures from a close fit could lead to the presence of cavities in the interface, which is energetically expensive and will result in lower affinity. An imprecise fit between antibody combining site and antigenic determinant could be smoothed out by solvent molecules, but the immobilization of solvent molecules will result in a decrease in the entropy of the system and subtract from the total binding energy, again leading to lower affinity.

It may be that the combining site structure of a polyreactive antibody is unusually plastic so that the site could adopt many different conformations enabling it to accommodate many different antigenic structures. The plasticity of the combining site structure is mainly dependent on the deformability of the CDR loop structures and on the ability of V_L and V_H to assume different quaternary modes of association. The deformability of the CDRs will depend on their length and on the presence or absence of stabilizing interactions with neighboring structures, as well as on the presence or absence in these regions of certain amino acid types like glycine (which usually confers flexibility), proline (which usually confers rigidity), or asparagine (which, by hydrogen bonding to main chain atoms, contributes to the stability of the local structure). Greater plasticity, therefore, may be reflected in a more frequent occurrence of glycines and/or a reduced presence of prolines or asparagines. In this regard, it is interesting that, of the three possible reading frames, the one used to transcribe the D-gene segment is usually that which results in the presence of glycines in the CDR3-H (Abergel and Claverie, 1991).

It is also possible that a polyreactive combining site is unusually sticky, as a consequence of the presence or absence of certain amino acid types which give the site a greater capacity for binding ligands. Greater stickiness

Table 17. Exposure of amino acid residues in 50 highly-refined water-soluble protein structures

	Bu	mB	pB	mE	Ex	Total
ALA	309	79	97	117	199	801
ARG	21	42	83	80	50	276
ASN	49	52	99	123	145	468
ASP	69	53	79	113	151	465
CYS	174	46	26	12	1	259
GLN	39	47	61	106	91	344
GLU	30	33	72	132	127	394
GLY	291	0	0	0	635	926
HIS	63	39	41	23	25	191
ILE	273	85	62	22	8	450
LEU	372	121	70	43	22	628
LYS	10	29	91	194	187	511
MET	88	20	17	13	6	144
PHE	177	70	42	8	8	305
PRO	81	32	54	86	109	362
SER	156	74	79	145	293	747
THR	140	68	123	145	135	611
TRP	68	44	14	7	3	136
TYR	106	113	74	48	20	361
VAL	385	101	84	61	36	667
Total	2915	1157	1268	1478	2251	9069

Here, a structure is considered to be a highly-refined structure if it has been determined to a resolution of 1.8 Å or better and refined to a crystallographic R-value of 0.200 or better. See footnote to Table 16.

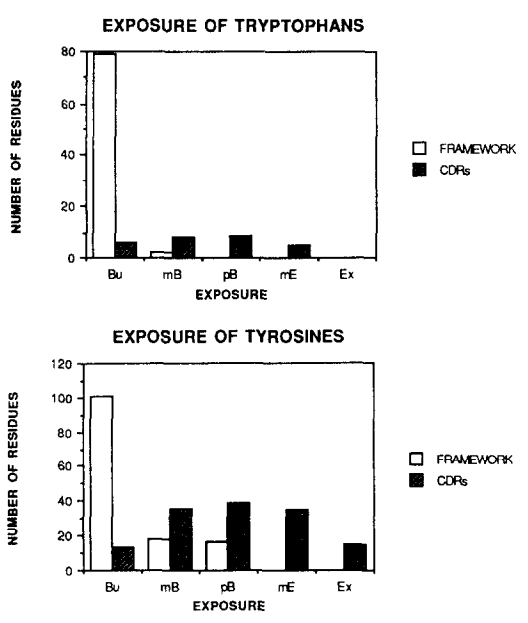


Fig. 11. Histograms showing the exposure patterns of the Trp (a) and Tyr (b) residues in antigen-binding regions of known three-dimensional structure. Shown are the numbers of these residues which are completely buried (Bu), mostly buried (mB), partly buried (pB), mostly exposed (mE) and completely exposed (Ex), as enumerated in Table 16.

may be reflected in an increased presence of aromatics vs aliphatic residues, for example. The observation that some polyreactive antibodies have many charged residues in and around their CDRs (Gonzalez-Quintial *et al.*, 1990) is intriguing.

Fc–ligand interactions

A number of molecules are known to bind to Fc, including low- and high-affinity Fc receptors, C1q and Protein A. For these, the location of their binding sites on the Fc has been deduced by sequence correlations, chemical modifications and site-directed mutagenesis, and, in the case of Protein A, by direct crystallographic analysis.

Duncan *et al.* (1988), by site-directed mutagenesis, have shown that Leu235 (Eu numbering) is a major determinant in the binding of murine Fc to the high-affinity receptor on monocytes. Unfortunately, the corresponding residue is part of the stretch that is disordered in the crystal structure of human IgG1 Fc and, therefore, cannot be located in that structure. The recent crystal structure analysis of the intact murine IgG2a antibody (Harris *et al.*, 1992), in which the full hinge is visible, should help in delineating the receptor-binding site on IgG.

It is known that C1q binds to the C_H2 domain and numerous attempts have been made to localize the site of binding [reviewed by Burton (1985)]. Recently, by systematically altering residues that are expected to lie on the surface, Duncan and Winter (1988) have been able to propose the location of the minimal residues that are probably involved in the binding of C1q; these are residues 318, 320 and 322 (Eu numbering). In human IgG1, these residues lie on the outside face of $C\gamma2$ (Fig. 12) and their disposition will allow a direct contact with C1q.

The crystallographic analysis of the binding of Fragment B of Protein A to human IgG1 Fc (Deisenhofer, 1981) demonstrated that the Protein A binding site is at the junction of the $C\gamma2$ and $C\gamma3$ domains (Fig. 12). Both domains are involved in the contact and approximately 1200 Å of accessible surface area on the Fc and the ligand are buried by the interaction. The contact is mainly hydrophobic with a few hydrogen bonds.

STRUCTURAL ASPECTS OF ANTIBODY APPLICATIONS

Antibodies of predefined specificity have many potential uses in industry and in medicine [see, for example, Steinman (1990), Schlom (1991), Waldmann (1991) and Co and Queen (1991)] and the advent of hybridoma technology (Koehler and Milstein, 1975) has made possible the generation of virtually limitless amounts of such antibodies. Monoclonal antibodies are being used for

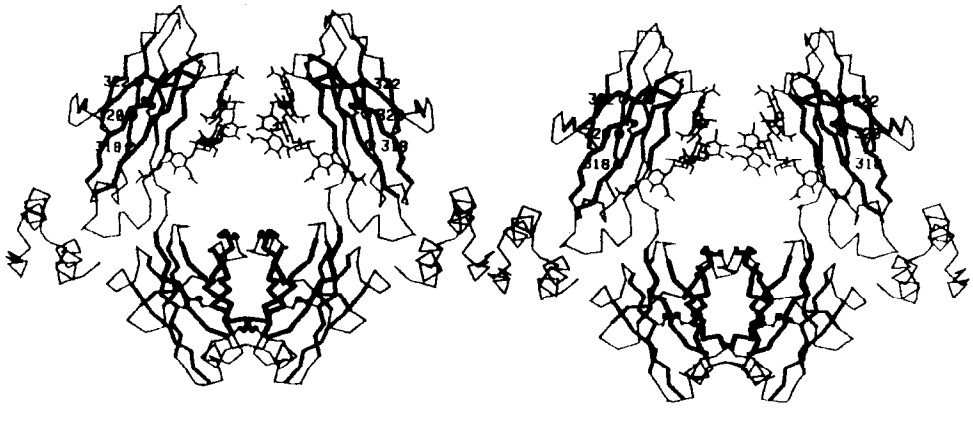


Fig. 12. Stereodrawing of the α-carbon trace of human IgG1 Fc showing various binding sites. The orientation is as in Fig. 7. Fragment B of Protein A is shown as the tri-helical structure near the junction of the C_H2 and C_H3 domains. Residues 318, 320 and 322, which have been identified as being involved in C1q binding (Duncan and Winter, 1988), are indicated by large, filled circles on C_H2.

imaging purposes in diagnosis [see, for example, Colcher *et al.* (1988), Schlom *et al.*, (1991)], and in therapy by targeting specific tissues or cells and by delivering toxins or radioactivity to predetermined locations [see, for example, Chaudary *et al.* (1988), and Schlom (1991)].

Furthermore, in view of the similarity in structure of antibodies belonging to the different classes and isotypes, the structure of a particular antibody could be manipulated to generate new functions or to endow it with a desired reactivity. For example, it may be possible, through the construction of chimeric IgE molecules of pre-selected specificity, to harness the cytotoxic potential of the IgE-isotypic response mediated by the low-affinity receptor for IgE and effect the killing of tumor cells and parasites by inflammatory cells (Helm, 1989).

Another important application is the use of antibodies as enzymes. It was proposed (Jencks, 1969) that catalytic antibodies could be generated by the use of transition-state analogs as immunogens, and a number of such antibodies have been produced [see, for example, Lerner and Tramontano (1988), Schultz (1988) and Benkovic (1992)]. This opens the possibility of generating enzymes for chemical reactions for which no natural catalysts exist.

Monoclonal antibodies are still more easily obtained from nonhuman sources, usually rodent, and the use of those antibodies in human subjects will be hindered by the patients' immune system. Since the reduction of the immunogenicity in humans of xenogeneic antibodies will make those molecules more efficacious reagents in therapy and diagnosis, various procedures for "humanizing" nonhuman antibodies are being developed.

Structural considerations in the "humanization" of antibodies

Ideally, "humanization" should result in an antibody that is nonimmunogenic, with complete retention of the antigen-binding properties of the original molecule. However, in order to retain all the antigen-binding properties of the original antibody, the structure of its combining-site has to be faithfully reproduced in the "humanized" version. This could be achieved by transplanting the combining site of the nonhuman antibody onto a human framework, either (a) by grafting the entire nonhuman variable domains onto human constant regions (Morrison *et al.*, 1984; Morrison and Oi, 1988) (which preserves the ligand-binding properties, but which also retains the immunogenicity of the nonhuman variable domains); (b) by grafting only the nonhuman CDRs onto human framework and constant regions (Jones *et al.*, 1986; Verhoeyen *et al.*, 1988); or (c) by transplanting the entire nonhuman variable domains (to preserve ligand-binding properties) but also "cloaking" them with a human-like surface through judicious replacement of exposed residues (to reduce antigenicity) (Padlan, 1991).

(i) *"Humanization" by CDR-grafting.* "Humanization" by transplanting only the CDRs (Jones *et al.*, 1986; Verhoeyen *et al.*, 1988) should result in almost complete elimination of immunogenicity (except if allotypic or idiotypic differences exist). However, it is found that in order to recover the ligand-binding properties of the original molecule, some framework residues from the original antibody, those which influence the structure of the combining site, also need to be preserved [see, for example, Riechmann *et al.* (1988), and Queen *et al.* (1989)].

In theory, all the framework residues which could influence the structure of the combining site should be kept. These include those which are in contact with the CDRs, since they provide the primary support for the combining site structure, and those which are involved in the V_L–V_H contact, since they influence the relative disposition of the CDRs. It may be necessary to keep also those framework residues which are buried in the domain interior, since they could influence the overall domain structure and, thereby, the structure of the combining site. Further, if the interaction with antigen involves "induced fit", the structural elements which permit the conformational change(s) must also be preserved in the "humanized" molecule.

The important framework residues could be identified if the three-dimensional structure of the antibody is known, preferably in a complex with antigen. In the absence of three-dimensional structure, the identification of the important framework residues could be attempted by other means, for example, by modeling the combining site structure (e.g. Queen *et al.*, 1989; Carter *et al.*, 1992), or by studying the effect of framework mutations on the ligand-binding properties of the molecule (e.g. Tempest *et al.*, 1991; Foote and Winter, 1992). Useful hints can be obtained from an examination of the known antibody structures.

The framework residues, which probably play a role in maintaining the combining site structure are presented in Tables 18–21 for the murine antibodies of known structure. The framework residues in the V_L domains, the side chains of which contact CDR residues, are listed in Table 18; those in the V_H are listed in Table 19. The framework residues, which contact framework residues in the opposite domain and which influence the quaternary structure of the Fv, have already been identified (Table 8). The buried, inward-pointing, framework residues in the V_L, i.e. those which are located in the domain interior, are listed in Table 20; those in the V_H are listed in Table 21. These results are collected in Table 22 for V_L and in Table 23 for V_H.

It is seen that there are many framework residues that contact the CDRs, that contact the opposite domain and that are found in the domain interior. These framework residues, which could influence the structure of the combining site and thus the antigen-binding characteristics of an antibody, are different from antibody to antibody, although many are common to all.

If one of the murine antibodies listed in the tables were to be "humanized" by CDR-grafting with the view to preserving their ligand-binding properties, it would probably be wise to retain all of its framework residues that are listed in Tables 22 and 23. At first glance, it would appear that there would be too many nonhuman

Table 18. V_L framework residues that contact CDR residues in murine Fabs of known three-dimensional structure

Position	Antibody														
	J539	McPC603	HyHEL-10	HyHEL-5	R19.9	4-4-20	BV04-01	36-71	B13I2	D1.3	Yst9-1	AN02	17/9	8F5	NC41
1	E (2)	D (5)	D (10)	D (3)	D (5)	D (8)		D (4)	D (5)	D (11)	D (5)	Q (5)	D (1)	D (5)	D (5)
2	I (11)	I (15)	I (18)	I (18)	I (20)	V (12)	V (9)	I (21)	V (7)	I (12)	I (25)	I (17)	I (15)	I (13)	I (16)
3		V (3)	V (2)	V (3)	Q (20)	V (2)		Q (2)	L (1)		Q (2)	V (2)	V (2)	V (1)	V (3)
4	L (13)	M (11)	L (12)	L (17)	M (14)	M (18)	M (12)	M (9)	M (6)	M (12)	M (9)	L (14)	M (11)	M (7)	M (8)
5		T (3)	T (1)		T (1)	T (3)	T (2)			T (1)	T (1)	T (2)	T (4)	T (4)	T (2)
7					T (3)				T (1)						
22														T (4)	
23	C (3)	C (3)	C (4)	C (4)	C (3)	C (3)	C (2)	C (3)	C (3)	C (1)	C (3)	C (5)	C (5)	C (3)	
35	W (8)	W (6)	W (7)	W (8)	W (8)	W (5)	W (6)	W (10)	W (7)	W (10)	W (9)	W (6)	W (7)	W (7)	W (3)
36	Y (17)	Y (21)	Y (12)	Y (10)	Y (19)	Y (13)	Y (10)	Y (19)	Y (14)	Y (18)	Y (8)	Y (17)	Y (21)	Y (10)	Y (11)
45					K (5)	K (5)									
46	P (8)	L (9)	L (4)	R (21)	L (8)	V (15)	L (12)	L (8)	L (12)	L (9)	L (7)	L (9)	V (5)	L (14)	L (8)
48	I (10)	I (11)	I (11)	I (12)	I (11)	I (9)	I (10)	I (13)	I (10)	V (9)	I (13)	I (13)	I (9)	I (11)	I (2)
49	Y (36)	Y (33)	K (17)	Y (18)	Y (31)	Y (29)	Y (31)	Y (30)	Y (28)	Y (29)	Y (32)	Y (27)	Y (37)	Y (34)	Y (14)
58	V (8)	V (6)	I (6)	V (11)	V (7)	V (9)	V (12)	V (4)	V (8)	V (6)	V (9)	V (9)	V (8)	V (10)	V (4)
60		D (1)				D (2)			D (1)			V (1)	D (2)	D (4)	D (2)
62				F (1)	F (1)	F (1)	F (1)	F (1)				F (1)			
63									S (1)					T (1)	
67		S (3)	S (2)							S (1)			S (2)	S (4)	
69	T (5)	T (9)	T (8)	T (2)	T (8)	T (14)	T (7)	T (8)	T (4)	T (9)	T (9)	T (10)	T (4)	T (6)	
70		D (2)			D (3)		D (2)	D (6)	D (6)		D (9)		D (1)		
71	Y (14)	F (23)	F (23)	Y (23)	Y (21)	F (17)	F (28)	Y (21)	F (21)	Y (25)	Y (25)	Y (12)	F (16)	F (14)	Y (13)
88	C (4)	C (3)	C (3)	C (2)	C (3)	C (3)	C (2)	C (2)	C (2)	C (5)	C (4)	C (4)	C (6)	C (2)	C (1)
98	F (11)	F (15)	F (13)	F (7)	F (14)	F (12)	F (14)	F (12)	F (17)	F (17)	F (14)	F (17)	F (10)	F (16)	F (6)

The number in parentheses after each residue name corresponds to the number of atomic contacts in which the amino acid is involved. See footnote to Table 4 for the definition of contacts. The liganded forms of McPC603, BV04-01, B13I2 and 17/9 (PDB Entry: 1HIM, first Fab in the entry) were used in the computations.

Table 19. V_H framework residues that contact CDR residues in murine Fabs of known three-dimensional structure

Position	Antibody														
	J539	McPC603	HyHEL-10	HyHEL-5	R19.9	4-4-20	BV04-01	36-71	B13I2	D1.3	YST9-1	AN02	17/9	8F5	NC41
1							E (3)	E (3)			E (2)				
2	V (11)	V (3)	V (8)		V (3)			V (7)	V (13)	V (12)	V (3)		V (11)	V (10)	I (6)
4	L (3)	L (6)	L (5)	L (2)	L (1)	L (3)		L (3)	L (3)	L (4)	L (7)	L (5)	L (4)	L (7)	L (2)
22															C (2)
24		T (2)	V (6)			A (1)	A (1)				T (1)	V (5)			A (1)
27	F (3)	F (2)		Y (14)	Y (7)	F (26)	F (2)	Y (4)	F (2)	F (4)	F (8)	Y (16)	F (8)	F (15)	Y (4)
28	D (9)	T (6)		T (3)	T (4)	T (6)	S (1)	T (2)	T (7)		T (8)	S (1)	S (3)		
29	F (4)	F (4)		F (10)	F (6)	F (13)	F (4)	F (6)	F (5)	L (1)	F (4)	I (7)	F (2)	I (5)	F (6)
30	S (2)	S (1)	T (4)	S (3)	T (9)	S (9)	N (1)	T (4)	S (1)	T (2)	T (10)	T (2)	S (1)	K (1)	T (1)
36	W (4)	W (3)	W (6)	W (5)	W (6)	W (7)	W (4)	W (8)	W (4)	W (6)	W (4)	W (3)	W (3)	W (8)	
37		V (1)			V (1)		V (1)		V (4)	V (1)		I (4)	V (2)		
38	R (1)	R (2)	R (4)	K (2)	K (2)	R (4)	R (1)	K (2)	R (1)			R (1)			K (4)
40				R (1)											
46	E (3)	E (4)	E (1)	E (27)	E (7)	E (4)	E (2)	E (9)		E (1)		E (2)		E (4)	E (7)
47	W (34)	W (46)	Y (22)	W (22)	W (36)	W (31)	W (34)	W (36)	W (25)	W (36)	W (28)	W (37)	W (42)	W (37)	W (19)
48	I (1)	I (1)	M (6)	I (12)	I (8)	V (1)	V (4)	I (9)	V (2)	L (1)	L (1)	M (3)		I (1)	M (10)
49		A (4)				A (3)	A (5)		A (3)				A (4)		
66	K (2)	R (3)	R (16)	K (1)	K (2)	R (8)	R (8)	K (1)	R (5)	R (3)	R (2)	R (3)	R (8)	K (3)	
67	F (6)	F (10)	I (9)	A (1)	T (7)	F (13)	F (5)	T (8)	F (10)	L (10)	F (9)	I (10)	F (6)	A (5)	F (9)
68		I (1)				T (11)					T (1)	S (1)	T (3)		
69	I (9)	V (7)	I (12)	F (16)	L (12)	I (25)	I (19)	L (9)	I (12)	I (9)	I (9)	I (10)	I (19)	I (12)	F (16)
71	R (27)	R (28)	R (7)	A (4)	V (2)	R (13)	R (28)	V (7)	R (21)	K (9)	R (23)	R (23)	R (21)	V (4)	L (6)
73	N (3)	T (3)			R (3)	D (3)	D (1)	K (2)	N (1)		N (7)		N (1)	T (1)	
78	L (5)	L (7)	Y (15)	A (1)	A (1)	V (2)	L (3)	A (1)	L (5)	V (4)	L (3)	F (11)	L (6)	A (2)	A (2)
80						L (1)									
82			L (2)							M (1)					
82a				N (1)	R (2)							K (1)			
86						D (2)									
92	C (1)	C (1)	C (1)	C (2)	C (1)			C (2)		C (2)		C (1)	C (1)	C (1)	C (1)
93	A (5)	A (6)	A (1)	L (6)	A (4)	T (6)	V (8)	A (2)	T (6)	A (5)	T (3)	A (2)	A (5)	D (7)	A (1)
94	R (39)	R (26)	N (18)	H (9)	R (35)		R (14)	R (29)	R (27)	R (34)	R (36)	R (39)	R (36)		R (16)
103	W (13)	W (14)	W (15)	W (3)	W (16)	W (7)	W (10)	W (14)	W (7)	W (7)	W (2)	W (12)	W (11)	W (7)	W (3)

See footnote to Table 18.

residues to keep; however, searching through the tabulation of immunoglobulin sequences (Kabat *et al.*, 1991), one finds that there are human variable domain sequences which have most of the framework residues that need to be preserved. For example, the "humanization" of the murine antibody, HyHEL-5, would require keeping nine murine framework residues in the V_L, using the human V_κ sequence, BI (or REI and a few others), as template, and 13 framework residues in the V_H using the human V_H sequence, AND (or 21/28′CL, and a few others). On the other hand, the "humanization" of the murine antibody, B13I2, would require the retention of only three murine framework residues in the V_L, using the human V_κ sequence, CUM (or NIM), as template, and only two framework residues in the V_H, using the human V_H sequence, M72 (or M74 and a few others). It is possible that there exist other human sequences that are even more similar to these murine domains, that are known but were not included in the compilation of Kabat *et al.* (1991).

The results presented in Tables 22 and 23 could be used in the design of a "humanization" protocol for an antibody of unknown structure. If a high degree of sequence similarity to an antibody of known three-dimensional structure exists, the results for the latter could be used. If no obvious sequence similarity exists, the results which are common to all the antibodies analysed could be used as a first guess and the protocol later refined, if needed, by site-directed mutagenesis studies.

It is seen from Tables 22 and 23 that many of the important framework residues flank the CDRs. Among these flanking positions are most of the framework residues that are involved in the contact with the opposite domain (Table 8) and many of those which are in contact with the CDRs (Tables 18 and 19). Moreover, all of the framework residues which have been observed to participate in the binding to antigen (Amit *et al.*, 1986; Sheriff *et al.*, 1987; Padlan *et al.*, 1989; Bentley *et al.*, 1990, Fischmann *et al.*, 1991; Tulip *et al.*, 1992*a*), are in these flanking regions. These results suggest that, if during "humanization", not just the CDRs are transplanted, but also some of the residues immediately adjacent to the CDRs, there would be a better chance of retaining the ligand-binding properties of the original antibody. The likelihood will be even greater if the first few amino acids in the N-termini of both chains are transplanted also, since some of them are found to be in contact with CDRs. In fact, the N-termini are contiguous with the CDR surface and are in a position to be involved in ligand binding (Fig. 4). It should be noted that the N-termini of the light and heavy chains are not equivalent in this regard, with the N-terminus of the light chain being more involved in the contact with the CDRs (this finding may have important implications for the properties of single-chain antibodies where either the end of the heavy chain component is linked to the beginning of the light chain (Huston *et al.*, 1988), or *vice versa* (Bird *et al.*, 1988), especially when residues in the N-terminus are excised).

(ii) "*Humanization*" *by replacing surface residues to make an antibody more* "*human-like*". It may be possible to reduce the antigenicity of a nonhuman Fv, while preserving its antigen-binding properties, by simply replacing those exposed residues in its framework regions which differ from those usually found in human antibodies (Padlan, 1991). This would "humanize" the surface of the xenogeneic antibody while retaining the interior and contacting residues which influence its antigen-binding characteristics. The judicious replacement of exterior residues should have little, or no, effect on the interior of the domains, or on the interdomain contacts.

The solvent accessibility patterns of the Fv of Kol (PDB Entry: 2FB4) and of J539 (PDB Entry: 2FBJ) have been analysed (Padlan, 1991) and are reproduced here. The fractional accessibility values for the framework residues in the Kol and J539 V_L are compared in Table 24 and those for the framework residues in the Kol and J539 V_H are presented in Table 25. Examination of these tables reveals a very close similarity in the exposure patterns of the Fvs of Kol (a human IgG1,λ) and J539 (a murine IgA,κ). Indeed, in almost all the positions in which the two patterns differ significantly, one or both antibodies have glycine. Here, glycine is designated as being completely exposed if its α-carbon is accessible to the solvent probe and completely buried otherwise (see footnote to Table 16), so that the slightest difference in structure could result in a different exposure designation for this amino acid.

The very close similarity of the exposure patterns for the variable domains of Kol and J539 points to the close correspondence of the tertiary structures of the homologous domains and of the dispositions of the individual residues. This is particularly remarkable since (a) these antibodies are from different species, (b) their light chains are of different types (J539 has a κ light chain, while Kol has a λ light chain), (c) half of their CDRs, specifically CDR1-L, CDR3-L and CDR3-H, have very different lengths and backbone conformations (Table 8, Fig. 5), and (d) Kol and J539 have only 44 identical residues out of the 79 corresponding positions in the V_L framework and 60 out of the 87 in the V_H framework. An even closer similarity in overall structure and in the exposure patterns can be expected for two molecules that are more similar in sequence than this pair. These results suggest that the solvent exposure of a residue can be more easily predicted than perhaps its involvement in the maintenance of the combining site structure.

The procedure that was proposed (Padlan, 1991) for reducing the antigenicity of a xenogeneic variable domain, while preserving its ligand-binding properties, would replace only the exposed framework residues which differ from those of the host with the corresponding residues in the most similar host sequence. Thus, the framework residues, which are at least partly exposed in the corresponding domains of Kol or J539 (those with pB, mE, or Ex designations in Tables 24 and 25), would be replaced, while the framework residues, corresponding to those which in Kol and J539 are completely or mostly buried (mB and Bu designations in Tables 24 and

Table 20. Inward-pointing, buried framework residues in the V_L of murine Fabs of known three-dimensional structure

Position	Antibody J539	McPC603	HyHEL-10	HyHEL-5	R19.9	4-4-20	BV04-01	36-71	B13I2	D1.3	Yst9-1	AN02	17/9	8F5	NC41
2	I	I	I	I	I	V	V	I	V	I	I	I	I	I	I
4	L	M	L	L	M	M	M	M	M	M	M	L	M	M	M
6	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q
11	T	L	L	M	L	L	L	L	L	L	L	M	L	L	M
13	A	V	V	A	A	V	V	A	V	A	A	A	V	V	T
19	V	V	V	V	V	A	A	V	A	V	V	V	V	V	V
21	I	M	L	M	I	I	I	I	I	I	I	M	M	M	I
23	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
35	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
37	Q	Q	Q	Q	Q	L	L	Q	L	Q	Q	Q	Q	Q	Q
47	W	L	L	W	L	L	L	L	L	L	L	L	L	L	L
48	I	I	I	I	I	I	I	I	I	V	I	I	I	I	I
58	V	V	I	V	V	V	V	V	V	V	V	V	V	V	V
61	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
62	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
71	Y	F	F	Y	Y	F	F	Y	F	Y	Y	Y	F	F	Y
73	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
75	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
78	M	V	V	M	L	V	V	L	V	L	L	M	V	V	V
82	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
83										F					
84	A	A		A	A			A			A	A	A	A	A
86	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
88	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
102	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
104	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
106	L	I	I						I		I	L		L	I

An inward-pointing residue is designated as buried if at least 50% of its side chain is inaccessible to solvent. Solvent accessibilities were computed as described previously (Padlan, 1990*b*); residue exposure is defined in the context of an isolated domain. See footnote to Table 16 for the definition of solvent accessibility. The coordinates used were those considered in Table 18.

Table 21. Inward-pointing, buried framework residues in the V_H of murine Fabs of known three-dimensional structure

Position	Antibody														
	J539	McPC603	HyHEL-10	HyHEL-5	R19.9	4-4-20	BV04-01	36-71	B13I2	D1.3	Yst9-1	AN02	17/9	8F5	NC41
2	V	V	V		V			V	V	V	V	V	V	V	I
4	L	L	L	L	L	L	P	L	L	L	L	L	L	L	L
6	E	E	E	Q	Q	E	E	Q	E	E	E	E	E	Q	Q
12	V	V	V	M	V	V	V	V	V	V	V	V	V	V	K
18	L	L	L	V	V	M	L	V	L	L	L	Q	L	V	V
20	L	L	L	I	M	L	L	M	L	I	L	L	L	L	I
22	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
24	A	T	V	A	A	A	A	A	A	V	T	V	A	T	A
27	F	F		Y	Y	F		Y	F	F	F	Y	F	F	Y
29	F	F	I	F	F	F	F	F	F	L	F	I	F	I	F
36	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
38	R	R	R	K	K	R	R	K	R	R	R	R	R	K	K
40						S							T	R	
46			E	E								E			
48	I	I	M	I	I	V	V	I	V	L	L	M	V	I	M
49		A				A	A		A				A		
66		R	R			R	R		R	R	R	R	R		R
67	F	F	I	A	T	F	F	T	F	L	F	I	F	A	F
69	I	V	I	F	L	I	I	L	I	I	I	I	I	I	F
71	R	R	R	A	V	R	R	V	R	K	R	R	R	V	L
73							D				N		N		
76						S									
78	L	L	Y	A	A	V	L	A	L	V	L	F	L	A	A
80	L	L	L	M	M	L	L	M	L	L	L	L	L	L	L
82	M	M	L	L	L	M	M	L	M	M	M	L	M	L	I
82c	V	L	V	L	L	L	L	L	L	L	L	V	L	L	L
86	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
88	A	A	A		A		A	A	A	A	A	A	A	A	A
90	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	F
92	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
94	R	R	N	H	R		R	R	R	R	R	R	R		R
107	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
109	V	V	V	L	L	V	V	L	L	L	V	V	V	V	L
111	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V

See footnote to Table 20.

25), would be retained. With this procedure also, the number of framework residues in xenogeneic domains, that would be needed to be replaced by human residues, was found to be not very large in the cases examined (Padlan, 1991).

Structural aspects which relate to catalysis by antibodies

Antibodies can be elicited against essentially any structure (if presented properly) so that it may be possible to generate catalytic antibodies of any desired specificity. However, antibodies capable of high enzymatic rates may not be so readily obtainable.

It is difficult to compare the interaction between antibody and antigen and that between enzyme and substrate. While we can analyse the interatomic contacts in an antibody–antigen complex (in the crystal), a comparable study cannot be performed on an enzyme–substrate complex, which is usually of fleeting existence. However, a comparison could be attempted using the more stable enzyme–inhibitor complexes, of which a number have been elucidated by X-ray diffraction.

The interactions of various enzymes with protein and peptide inhibitors are presented in Table 26. By and large, the interactions shown in Table 26 are comparable to those presented in Table 14 between antibodies and their specific ligands, except in one respect: more main chain atoms are found to be involved in the enzyme–inhibitor interactions than in the antibody–ligand complexes. This is especially noticeable in the case of hydrogen-bond formation (see also Janin and Chothia, 1990). In the three anti-lysozyme–lysozyme complexes, on average only 15% of the atomic contacts involve main chain atoms, whereas in the enzyme–protein inhibitor complexes the main-chain involvement is almost three times as great; in regard to the hydrogen bonds, in the anti-lysozyme–lysozyme complexes, only about 7% involve main chain atoms, whereas in the enzyme–protein inhibitor complexes, almost one-half of the hydrogen bonds involve main chain atoms. The number of interatomic contacts between antibody and peptide ligand (Table 14) and between enzyme and peptide inhibitor (Table 26) are roughly the same, but here again there are more hydrogen bonds involving main chain atoms in the enzyme–peptide inhibitor than in antibody–peptide complexes.

The polypeptide backbone is more restricted in its movements than are side chains. Furthermore, hydrogen bonds involving main chain atoms are more "directed" and will be stronger (Jeffrey and Saenger, 1991). Compared to antibody combining sites, the active sites of enzymes are more rigid, being constructed with main-chain and side-chain components that are held in place by strong interactions with surrounding structural elements. A ligand would therefore be interacting with a much less deformable site in an enzyme than in an antibody.

The plasticity of the combining site could have serious implications for the enzymatic activity of catalytic antibodies. A flexible active site cannot produce as much "strain" (Jencks, 1969) on the substrate as could a rigid site and this may result in the diminution of enzymatic efficiency. The observation that most of the binding to antigen involves side chain atoms is perhaps an omen that antibodies with catalytic efficiencies comparable to those of enzymes will be a rarity.

CONCLUDING REMARKS

Antibodies are remarkable molecules and serve as paradigms for macromolecular recognition. They have a binding site that is designed to accommodate ligands of variable structure (the combining site) and sites that bind to ligands of constant structure (e.g. the C1q binding site). The available crystallographic data allow us to assess only the first of these sites (the combining site), but nature's use of structure to achieve a particular function is already evident from this example.

The basic element used in the construction of an antibody molecule is the anti-parallel β-pleated sheet, which is strong because the hydrogen-bonding pattern involves not just one stretch of polypeptide chain, as in an α-helix, but several in a synergistic arrangement. The individual strands in a β-sheet would be resistant to proteolytic digestion and the sheet might not unravel even if some of the loops connecting the strands were cleaved. Indeed, the connecting loops could be varied in size and sequence and still preserve the basic structure of the β-sheet (as is seen in the variable domains).

If one were to construct a binding site that could better withstand the action of digestive enzymes, one is well served to use the surface of a β-sheet. Moreover, the chances of preserving that site during evolution would be good, since such a site can better survive mutational events. In view of the stability of the sheet structure, about the only mutation that can significantly affect the binding site structure is one that alters a ligand-contacting residue; replacements of even the residues immediately adjacent to the ligand-contacting residues, unless drastic, may have little effect on the topography of the exposed surface.

On the other hand, if one were to construct a binding site that is deformable and that is easily varied (by mutation), one would choose to build it with loops. The structure of a loop is very sensitive to changes in length and sequence. Moreover, the residues in a loop are more likely to be exposed to solvent, and, thereby, are more available for ligand binding, than residues in a β-sheet. A survey of the exposures of the residues in the J539 and Kol V_L and V_H (computed in the context of isolated domains) reveals that the ratio of exposed (mE and Ex designations as defined in the footnote to Table 16) to buried (mB and Bu designations) residues is 0.70 when in the β-sheets (theoretically, this should be close to 1.0 since in an infinite sheet every other residue would be buried) and 1.72 when in the CDRs. Since some CDR residues are in fact in the sheets, the difference would have been even

Table 22. The framework residues in V_L which are in contact with CDRs or with V_H and those which are inward-pointing in murine Fabs of known three-dimensional structure

	10 20		35 40 49		60 70 80 88		98 107
	|		| | |||| |		| |		|||
J539	EI.L.Q....T.A.....V.I.C		WYQQ....SP.PWIY		.V..RF......T.Y.L.I..M...D.A.YYC		F...T.L.L.
McPC603	DIVMTQ....L.V.....V.M.C		WYQQ....PP.LLIY		.V.DRF....S.TDF.L.I..V...D.A.YYC		FGA.T.L.I.
HyHEL-10	DIVLTQ....L.V.....V.L.C	C	WYQQ....SP.LLIK	C	.I. RF....S.T.F.L.I..V...D..MYFC	C	F...T.L.I.
HyHEL-5	DIVL.Q....M.A.....V.M.C		WYQQ....SP.RWIY		.V..RF......T.Y.L.I..M...D.A.YYC		FG..T.L...
R19.9	DIQMTQT...L.A.....V.I.C	D	WYQQ....TVKLLIY	D	.V..RF......TDY.L.I..L...D.A.YFC	D	F.G.T.L...
4-4-20	DVVMTQ....L.V.....A.I.C		WYLQ....SPKVLIY		.V.DRF......T.F.L.I..V...D...Y.C		F...T.L...
Yst9-1	DIQMTQ....L.A.....V.I.C	R	WYQQ...G.V.LLIY	R	.V..RF......TDY.L.I..L...D.A.YIC	R	FG..T.L.I.
36-71	DIQM.Q....L.A.....V.I.C		WYQQ...G.I.LLIY		.V..RF......TDY.L.I..L...D.A.YFC		F...T.L...
B13I2	DVLM.QT...L.V.....A.I.C		WYLQ....SP.LLIY		.V.DRFS.....TDF.L.I..V...D...YYC		F...T.L.I.
D1.3	DI.MTQ....L.A.....V.I.C	1	WYQQ....SP.LLVY	2	.V..RF....S.T.Y.L.I..L...DF..YYC	3	F.G.T.L...
BV04-01	.V.MTQ....L.V.....A.I.C		WYLQ...QSP.LLIY		.V..RF......TDF.L.I..V...D...YFC		F.A.T.L...
AN02	QIVLTQ....M.A.....V.M.C		WYQQ..GSSP.LLIY		.V.VRF......T.Y.L.I..M...D.A.YYC		F.V.T.L.L.
17/9	DIVMTQ....L.V.....V.M.C		WYQQ..G.PP.VLIY		.V.DRF....S.TDF.L.I..V...D.A.YYC		F...T.L...
8F5	DIVMTQ....L.V.....V.MTC		WYQQ....SP.LLIY		.V.DRFT...S.T.F.L.I..V...D.A.YYC		F...T.L.L.
NC41	DIVMTQ....M.T.....V.I.C		WYQQ....SP.LLIY		.V.DRF........Y.L.I..V...D.A.YYC		F...T.L.I.
	^^^^^ ^ ^^		^^ ^^ ^^		^ ^ ^^ ^ ^^^ ^		^
			*				

Summary of the results presented in Tables 8, and 18–21.
| Residues which contact the opposite domain.
^ Residues which contact CDRs.
*Residues which have been found to be involved in antigen binding.

Table 23. The framework residues in V_H which are in contact with CDRs or with V_L and those which are inward-pointing in murine Fabs of known three-dimensional structure

	10 20 30		40 49		70 82abc 90		102 110
J539	.V.L.E.....V.....L.L.C.A..FDFS		WVRQ...KGLEWI.		KF.I.R.N....L.L.M..V...D.A.YYCAR		WGQ.T.V.V..
McPC603	.V.L.E.....V.....L.L.C.T..FTFS		WVRQ....RLEWIA		RFIV.R.T....L.L.M..L...D.A.YYCAR		WG..T.V.V..
HyHEL-10	.V.L.E.....V.....L.L.C.V....IT	C	WIRK...N.LEYMG	C	RI.I.R......Y.L.L..V...D.A.YYCAN	C	WG..T.V V..
HyHEL-5	...L.Q.....M.....V.I.C.A..YTFS		WVKQR...GLEWI.		KA.F.A......A.M.LN.L...D...YYCLH		WG..T.L.V..
R19.9	.V.L.Q.....V.....V.M.C.A..YTFT	D	WVKQ....GLEWI.	D	KT.L.V.R....A.M.LR.L...D.A.YFCAR	D	WGQ.T.L.V..
4-4-20	...L.E.....V.....M.L.C.A..FTFS		WVRQS....LEWVA		RFTI.R.D..S.V.L.M..L...D...YYCT.		WGQ.T.V.V..
Yst9-1	EV.L.E.....V.....L.L.C.T..FTFT	R	WVRQ....AL.WL.	R	RFTI.R.N....L.L.M..L...D.A.YYCTR	R	W.Q.T.V.V..
36-71	EV.L.Q.....V.....V.M.C.A..YTFT		WVKQ...Q.LEWI.		KT.L.V.K....A.M.L..L...D.A.YFCAR		W.Q.T.L.V..
B13I2	.V.L.E.....V.....L.L.C.A..FTFS		WVRQ...K.L.WVA		RF.I.R.N....L.L.M..L...D.A.YYCTR		WG..T.L.V..
D1.3	.V.L.E.....V.....L.I.C.V..F.LT	1	WVRQ....GLEWL.	2	RL.I.K......V.L.M..L...D.A.YYCAR	3	WGQ.T.L.V..
BV04-01	E..P.E.....V.....L.L.C.A..FSFN		WVRQ...K.LEWVA		RF.I.R.D....L.L.M..L...D.A.YYCVR		WGQ.T.V.V..
AN02	.V.L.E.....V.....Q.L.C.V..YSIT		WIRQ...NKLEWM.		RISI.R......F.L.LK.V...D.A.YFCAR		WGQ.T.V.V..
17/9	.V.L.E.....V.....L.L.C.A..FSFS		WVRQT..K.LEWVA		RFTI.R.N....L.L.M..L...D.A.YYCAR		WGQ.T.V.V..
8F5	.V.L.Q.....V.....V.L.C.T..F.IK		WVKQR...GLEWI.		KA.I.V.T....A.L.L..L...D.A.YYCD.		WG..T.V.V..
NC41	.I.L.Q.....K.....V.I.C.A..Y.FT		W.KQ....GLEWM.		RF.F.L......A.L.I..L...D.A.FFCAR		WGQ.T.L.V..
	^^ ^ ^ *		^^^ ^ ^^^^ *		^^^^ ^ ^ ^ ^ ^^ ^ ^^^		^

See footnote for Table 22.

Table 24. Solvent exposures of side chains of framework residues in Kol and J539 V_L

Position	Kol Residue	Fractional accessibility Kol Exposure		J539 Residue	Fractional accessibility J539 Exposure	
1	Q	1.00	Ex	E	0.99	Ex
2	S	1.00	Ex	I	0.16	Bu
3	V	0.77	mE	V	0.87	Ex
4	L	0.00	Bu	L	0.00	Bu
5	T	0.92	Ex	T	0.80	mE
6	Q	0.00	Bu	Q	0.00	Bu
7	P	0.62	mE	S	0.89	Ex
8	P	1.00	Ex	P	0.67	mE
9	—	—	—	A	1.00	Ex
10	S	1.00	Ex	I	0.94	Ex
11	A	0.34	mB	T	0.30	mB
12	S	0.71	mE	A	0.59	pB
13	G	1.00	Ex	A	0.00	Bu
14	T	0.73	mE	S	0.78	mE
15	P	0.75	mE	L	0.79	mE
16	G	1.00	Ex	G	1.00	Ex
17	Q	0.69	mE	Q	0.64	mE
18	R	0.79	mE	K	0.74	mE
19	V	0.21	mB	V	0.22	mB
20	T	0.62	mE	T	0.65	mE
21	I	0.00	Bu	I	0.00	Bu
22	S	0.92	Ex	T	0.69	mE
23	C	0.00	Bu	C	0.00	Bu
35	W	0.00	Bu	W	0.00	Bu
36	Y	0.00	Bu	Y	0.00	Bu
37	Q	0.46	pB	Q	0.14	Bu
38	Q	0.00	Bu	Q	0.24	mB
39	L	0.75	mE	K	0.69	mE
40	P	0.91	Ex	S	1.00	Ex
41	G	1.00	Ex	G	1.00	Ex
42	M	0.74	mE	T	0.90	Ex
43	A	0.62	mE	S	0.30	mB
44	P	0.00	Bu	P	0.00	Bu
45	K	0.95	Ex	K	0.90	Ex
46	L	0.23	mB	P	0.43	pB
47	L	0.15	Bu	W	0.16	Bu
48	I	0.00	Bu	I	0.00	Bu
49	Y	0.39	mB	Y	0.42	pB
57	G	1.00	Ex	G	1.00	Ex
58	V	0.14	Bu	V	0.13	Bu
59	P	0.70	mE	P	0.61	mE
60	D	0.95	Ex	A	1.00	Ex
61	R	0.31	mB	R	0.36	mB
62	F	0.12	Bu	F	0.00	Bu
63	S	0.85	Ex	S	0.94	Ex
64	G	0.00	Bu	G	0.00	Bu
65	S	1.00	Ex	S	1.00	Ex
66	K	0.41	pB	G	1.00	Ex
67	S	1.00	Ex	S	1.00	Ex
68	G	1.00	Ex	G	1.00	Ex
69	A	0.71	mE	T	0.75	mE
70	S	1.00	Ex	S	0.98	Ex
71	A	0.00	Bu	Y	0.09	Bu
72	S	1.00	Ex	S	0.70	mE
73	L	0.00	Bu	L	0.00	Bu
74	A	0.74	mE	T	0.43	pB
75	I	0.00	Bu	I	0.00	Bu
76	G	1.00	Ex	N	0.83	Ex
77	G	1.00	Ex	T	0.83	Ex
78	L	0.00	Bu	M	0.00	Bu
79	Q	0.76	mE	E	0.63	mE
80	S	1.00	Ex	A	0.96	Ex
81	E	0.78	mE	E	0.91	Ex
82	D	0.09	Bu	D	0.13	Bu
83	E	0.64	mE	A	0.55	pB
84	T	0.34	mB	A	0.00	Bu
85	D	0.30	mB	I	0.58	pB
86	Y	0.00	Bu	Y	0.00	Bu
87	Y	0.16	Bu	Y	0.11	Bu
88	C	0.00	Bu	C	0.00	Bu
98	F	0.04	Bu	F	0.00	Bu
99	G	0.00	Bu	G	1.00	Ex
100	T	0.59	pB	A	1.00	Ex
101	G	1.00	Ex	G	0.00	Bu
102	T	0.00	Bu	T	0.00	Bu
103	K	0.82	Ex	K	0.79	mE
104	V	0.00	Bu	L	0.00	Bu
105	T	0.86	Ex	E	0.89	Ex
106	V	0.19	Bu	L	0.44	pB
106a	L	0.70	mE	—	—	—
107	G	1.00	Ex	K	0.77	mE

See footnote to Table 16 for the definition of solvent accessibility.

more pronounced if only the CDR residues which are in loop regions were used in the comparison. Thus the mutation of a loop residue could have a profound effect on ligand binding, either directly, since by being more accessible the residue is more likely to be involved in the contact, or indirectly, since a change in its character could more easily affect the conformation of the local structure or that of the whole loop.

It comes as no surprise, therefore, that C1q binding, which is a desirable trait in all antibody types, (probably) involves residues in a sheet, while antigen binding involves loops.

One wonders whether deformability is built into (some) CDR structures. Deformability of the combining site structure may permit "induced fit", which could result in better binding to ligand. Deformability may also result in the capacity to bind to many different ligands (polyreactivity). The ability to react to a greater variety of antigens using fewer antibodies, albeit with low affinity, affords an organism a distinct survival advantage. The observation that the occurrence of glycines in CDR3-H is a natural consequence of the preferred mode of transcription of D-gene segments (Abergel and Claverie, 1991) is of relevance. In view of the fact that glycines can confer flexibility to a polypeptide segment and that CDR3-H is often observed to play a central role in ligand binding, a deformable combining site may be the norm. It would be interesting to compare pre-immune and mature antibodies in this respect. Are pre-immune antibodies more deformable than mature ones? Is the rigidification of the combining site structure a factor in affinity maturation?

An increased rigidity of the combining site can be achieved by reducing the inherent flexibility of the individual CDRs (by reducing the number of glycines,

Table 25. Solvent exposures of side chains of framework residues in Kol and J539 V_H

Position	Kol Residue	Fractional accessibility Exposure		J539 Residue	Fractional accessibility Exposure	
1	E	1.00	Ex	E	1.00	Ex
2	V	0.23	mB	V	0.37	mB
3	Q	0.82	Ex	K	0.82	Ex
4	L	0.00	Bu	L	0.10	Bu
5	V	0.87	Ex	L	1.00	Ex
6	Q	0.00	Bu	E	0.09	Bu
7	S	0.94	Ex	S	0.94	Ex
8	G	1.00	Ex	G	1.00	Ex
9	G	0.00	Bu	G	0.00	Bu
10	G	1.00	Ex	G	1.00	Ex
11	V	0.90	Ex	L	0.81	Ex
12	V	0.25	mB	V	0.25	mB
13	Q	0.71	mE	Q	0.87	Ex
14	P	0.59	pB	P	0.64	mE
15	G	1.00	Ex	G	1.00	Ex
16	R	0.73	mE	G	1.00	Ex
17	S	0.66	mE	S	0.75	mE
18	L	0.28	mB	L	0.26	mB
19	R	0.66	mE	K	0.75	mE
20	L	0.00	Bu	L	0.00	Bu
21	S	0.71	mE	S	0.82	Ex
22	C	0.00	Bu	C	0.00	Bu
23	S	1.00	Ex	A	1.00	Ex
24	S	0.00	Bu	A	0.00	Bu
25	S	0.87	Ex	S	1.00	Ex
26	G	1.00	Ex	G	1.00	Ex
27	F	0.10	Bu	F	0.10	Bu
28	I	0.85	Ex	D	0.72	mE
29	F	0.00	Bu	F	0.00	Bu
30	S	0.74	mE	S	0.83	Ex
36	W	0.00	Bu	W	0.00	Bu
37	V	0.00	Bu	V	0.00	Bu
38	R	0.10	Bu	R	0.31	mB
39	Q	0.15	Bu	Q	0.28	mB
40	A	0.95	Ex	A	0.75	mE
41	P	0.90	Ex	P	0.73	mE
42	G	1.00	Ex	G	1.00	Ex
43	K	0.86	Ex	K	0.86	Ex
44	G	1.00	Ex	G	1.00	Ex
45	L	0.00	Bu	L	0.00	Bu
46	E	0.75	mE	E	0.73	mE
47	W	0.10	Bu	W	0.04	Bu
48	V	0.00	Bu	I	0.00	Bu
49	A	0.00	Bu	G	0.00	Bu
66	R	0.36	mB	K	0.51	pB
67	F	0.00	Bu	F	0.00	Bu
68	T	0.87	Ex	I	0.88	Ex
69	I	0.00	Bu	I	0.00	Bu
70	S	0.78	mE	S	0.79	mE
71	R	0.11	Bu	R	0.00	Bu
72	N	0.61	mE	D	0.55	pB
73	D	0.44	pB	N	0.43	pB
74	S	0.85	Ex	A	0.97	Ex
75	K	0.88	Ex	K	0.77	mE
76	N	0.69	mE	N	0.68	mE
77	T	0.41	pB	S	0.33	mB
78	L	0.00	Bu	L	0.00	Bu
79	F	0.45	pB	Y	0.35	mB
80	L	0.00	Bu	L	0.00	Bu
81	Q	0.53	pB	Q	0.69	mE
82	M	0.00	Bu	M	0.00	Bu
82a	D	0.73	mE	S	0.58	pB
82b	S	0.98	Ex	K	0.96	Ex
82c	L	0.00	Bu	V	0.00	Bu
83	R	0.73	mE	R	0.83	Ex
84	P	0.75	mE	S	0.90	Ex
85	E	0.82	Ex	E	0.90	Ex
86	D	0.00	Bu	D	0.11	Bu
87	T	0.54	pB	T	0.47	pB
88	G	1.00	Ex	A	0.00	Bu
89	V	0.58	pB	L	0.63	mE
90	Y	0.00	Bu	Y	0.00	Bu
91	F	0.00	Bu	Y	0.08	Bu
92	C	0.00	Bu	C	0.00	Bu
93	A	0.00	Bu	A	0.00	Bu
94	R	0.17	Bu	R	0.15	Bu
103	W	0.09	Bu	W	0.07	Bu
104	G	0.00	Bu	G	1.00	Ex
105	Q	0.93	Ex	Q	0.99	Ex
106	G	0.00	Bu	G	0.00	Bu
107	T	0.22	mB	T	0.26	mB
108	P	0.99	Ex	L	0.67	mE
109	V	0.00	Bu	V	0.00	Bu
110	T	0.76	mE	T	0.69	mE
111	V	0.00	Bu	V	0.00	Bu
112	S	0.98	Ex	S	0.74	mE
113	S	0.94	Ex	A	0.84	Ex

See footnote to Table 16 for the definition of solvent accessibility.

increasing the number of prolines or asparagines, by shortening the CDR, etc), by increasing the interaction of the CDRs or of the individual CDR residues with surrounding structures (framework and other CDRs), by replacing critical CDR residues with amino acids having fewer degrees of freedom, and/or by strengthening the V_L–V_H interaction. In this regard, it is interesting to note that the residues which have been replaced during the maturation of the anti-2-phenyloxazolone response, the ligand-contacting residues at positions 34 and 36 of the light chain (Alzari *et al.*, 1990), are also frequently found to be involved in the V_L–V_H contact (Table 8a).

Another contact that deserves mention is that between framework and CDRs within each domain. The observation that a framework residue can influence the conformation of a CDR implies that differences in framework structure, albeit small, could have a significant effect on the specificity of the combining site. This could explain in part why particular variable region families or subgroups are found to be associated with certain antigen-binding specificities. Conversely, particular CDR–framework combinations may not be favorable and may not be formed (see also Kirkham *et al.*, 1992).

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Table 26. Various enzyme–polypeptide inhibitor interactions

PDB Code	Enzyme	Surf. buried	Ligand	Surf. buried	vdW	m.c.vdW	H.b.	m.c. H.b.	I.p.
1CSE	Subtilisin Carlsberg	667	Eglin C	667	162	75	16	7	0
3TEC	Thermitase	674	Eglin C	670	158	64	16	7	0
2PTC	β-trypsin	714	BPTI	588	170	78	16	5	0
2SNI	Subtilisin novo	776	Chyt. inhib. (barley)	742	180	70	13	9	0
1CHO	α-Chyt.	701	Ovomucoid (turkey)	628	138	60	12	8	0
2KAI	Kallikrein A	694	BPTI	606	179	77	14	6	0
3SGB	Proteinase B (S. Griseus)	571	Ovomucoid (turkey)	545	144	67	11	7	0
4SGB	Proteinase B (S. Griseus)	578	Chyt. inhib. (potato)	517	126	62	9	5	0
1TAB	Trypsin	662	Prot. inhib. (Bowman-Birk)	584	134	69	13	5	1
4CPA	Carboxypep. A	598	Carb. inhib. (potato)	483	96	21	9	1	1
3APR	Rhizopuspep.	709	Peptide inhibitor	551	145	36	9	5	0
4ER4	Endothiapep.	814	Peptide inhibitor	696	168	54	10	5	1

See footnote to Table 4 for the definition of contacts.

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