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cDNA sequence of adrenodoxin reductase

Identification of NADP-binding sites in oxidoreductases

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Adrenodoxin reductase is an NADP dependent flavoenzyme which functions as the reductase of mitochondrial *P* 450 systems. We sequenced two adrenodoxin reductase cDNAs isolated from a bovine adrenal cortex cDNA library. The deduced amino acid sequence shows no similarity to the sequence of the microsomal *P* 450 systems or other known protein sequences. Nonetheless, by sequence analysis and comparisons with known sequences of dinucleotide-binding folds of two NADP-binding flavoenzymes, two regions of adrenodoxin reductase sequence were identified as the FAD- and NADP-binding sites. These analyses revealed a consensus sequence for the NADP-binding dinucleotide fold (GXGXXAXXXAXXXXXG, in one-letter amino acid code) that differs from FAD and NAD-binding dinucleotide-fold sequences. In the data base of protein sequences, the NADP-binding-site sequence appears solely in NADP-dependent enzymes, the binding sites of which were not known to date. Thus, this sequence may be used for identification of a certain type of NADP-binding site of enzymes that show no significant sequence similarity.

Adrenodoxin reductase is the first enzyme in the mitochondrial *P*-450 systems which catalyze several critical steps in the biosynthesis of steroid hormones in steroidogenic tissues [1 – 3], and the biosynthesis of bile acids [4, 5] and vitamin D [6] in the liver and kidney, respectively. These *P* 450 systems are composed of three enzymes which constitute an electron-transfer chain located on the matrix side of the inner mitochondrial membrane [7, 8]. The function of adrenodoxin reductase is to transfer electrons from NADPH to a specific ferredoxin (adrenodoxin), which in turn transfers them one at a time to the *P* 450 [1, 9, 10]. Adrenodoxin reductase cDNA has been recently isolated from both bovine and human steroidogenic tissue cDNA libraries [11 – 14] and used to demonstrate that adrenodoxin reductase is encoded by a single gene in both genomes [11, 14]. Thus, the same adrenodoxin reductase serves the different mitochondrial *P* 450 systems in steroidogenic tissues.

As an NADP-dependent electron-transfer protein, adrenodoxin reductase belongs to a group of NAD- and NADP-dependent oxidoreductases which constitute a large collection of enzymes that vary widely in their sizes, substrate specificities, and sequences. The structures of a large number of NAD-binding proteins have been studied extensively. Comparisons of the NAD-binding domains of these enzymes indicate that their dinucleotide(ADP)-binding sites share a similar $\beta\alpha\beta$ fold which forms a pocket to accomodate the coenzyme [15 – 18]. The sequences of these binding sites reveal a nearly universally conserved Gly-Xaa-Gly-Xaa-Xaa-Gly sequence which appears at the border between the first β-sheet strand and the α-helix allowing the formation of a tight turn [17, 18]. The structures of only two NADP-binding proteins have been well characterized to date [19, 20]. Glutathione reductase, like adrenodoxin reductase, is an FAD-containing flavoenzyme. The ADP-binding sites in both its FAD and NADP domains form a $\beta\alpha\beta$ fold similar to that of the NAD-binding site [16, 17, 19]. (For convenience we shall use the terms FAD- and NADP-binding sites to refer to the binding site for the ADP portion of these molecules.) In contrast, the NADP-binding site of dihydrofolate reductase shows a different structure [20].

In the absence of sequence information on adrenodoxin reductase its structural relationship to other NAD(P)-dependent enzymes remained unknown. In this study we report the cDNA sequence of adrenodoxin reductase and examine its similarity to other oxidoreductases. By sequence comparison and analysis, one region of adrenodoxin reductase is identified as the NADP-binding site, and a modified dinucleotide-binding-site consensus sequence is used to uniquely identify additional NADP-binding enzymes in the protein sequence databases.

MATERIALS AND METHODS

The cDNA of the adrenodoxin reductase plasmid, pAR, was isolated from a λgt11 cDNA expression library constructed from bovine adrenal cortex mRNA [11]. We sequenced an additional cloned cDNA which was identified by rescreening of the cDNA library using the pAR cDNA as a probe.

The DNA sequencing was performed as previously described [21 – 23]. *Bam*HI, *Ban*I, *Bss*hII, *Eco*RI, *Sal*I, and *Xma*I sites and the first *Ava*I site from the 5′ end of clone pAR were used for end-labelling (Fig. 1). Secondary digestions

Dedication. This paper is dedicated to my pre- and post-doctoral advisors Dr Colin R. Jefcoate and Dr Elaine V. Fuchs.

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Abbreviation. pAR, adrenodoxin reductase plasmid.

Enzyme. Adrenodoxin reductase (EC 1.18.1.2).

were performed with *Bgl*I, *Hph*I, *Kpn*I, *Pst*I or one of the enzymes mentioned above.

The NBRF and GenBank sequence databases were searched with the programs Seqnce (Delaney Software, Vancouver, Canada) and MicroGenie [24] on an IBM personal computer, and with the University of Wisconsin-Madison Sequence Analysis Program on a Micro Vax II computer. Homology matrix comparisons and hydrophobicity and protein secondary-structure analyses, were performed as described in [23, 25].

RESULTS

The characteristics of the sequenced cDNA

The correct reading frame of the pAR cDNA insert was identified using the sequences of partial tryptic fragments of adrenodoxin reductase which we had determined previously [11] (Fig. 1). Our sequence is in near-perfect agreement with the sequence of Sagara et al. [12]. However, the sequences of these four independent clones show many differences from the cDNA sequence of adrenodoxin reductase recently reported by Nonaka et al. [13]. The differences include many insertions and deletions that alter the reading frame of translation for tens of residues. These discrepancies are probably a result of sequencing errors as their sequence was not cross-checked by sequencing an independent clone.

Adrenodoxin reductase sequence shows no similarity with other oxidoreductase sequences

The comparison of the sequence of adrenodoxin reductase with the NBRF protein sequence database revealed no significant matches with any sequence using the programs noted in Materials and Methods. To detect low but significant similarity between adrenodoxin reductase and other oxidoreductases we carried out homology matrix analyses at low identity cutoff values ($> 25\%$) for segments of 30 residues (the FAD- and NADP-binding sites are 30 residues in other oxidoreductases). These analyses also failed to reveal similarity between adrenodoxin reductase and other oxidoreductase sequences including microsomal *P*-450 reductase [26–28] and spinach ferredoxin reductase [29].

Identification of the FAD-binding site of adrenodoxin reductase

In adrenodoxin reductase the dinucleotide-binding site consensus sequence [30] is found only at the amino-terminus region (Fig. 2). Secondary-structure-prediction analysis of this region provided further support for the identification of this segment as an ADP-binding $\beta\alpha\beta$ fold. Sagara et al. [12] also noted that this region may be an FAD- or NADP-binding site. But, as the FAD-binding domains of many flavoenzymes appear to be located close to the amino terminus of the protein, this region is most probably the FAD-binding site of adrenodoxin reductase (Fig. 2).

Identification of the NADP-binding site of adrenodoxin reductase

The adrenodoxin reductase sequence lacks a second Gly-Xaa-Gly-Xaa-Xaa-Gly sequence that might indicate an NADP-binding site. Hence, we sought a putative NADP-binding site by two criteria: (a) a sequence similarity with the NADP-binding domain of the human glutathione reductase, the crystal structure of which is known [16, 19]; and (b) a secondary structure that is similar to a $\beta\alpha\beta$ fold. As noted below only one region was found to fulfil both of these criteria.

The sequences of only two NADP-binding enzymes that are similar to the human glutathione reductase are known [37, 42]. These three sequences contain a consensus sequence which distinguishes the NADP-binding site from the FAD-binding site by the first Ala residue (Fig. 2). This sequence is also found in adrenodoxin reductase with the substitution of Pro for the last Gly (Pro and Gly are both helix breakers and show a tendency to occur in turns) (Fig. 2).

To check the statistical significance of this match (Fig. 2) we searched the NBRF database (release II) to determine the frequency of the following consensus sequence in the complete population of sequences (in one letter amino acid code, wherein X represents any amino acid):

sequence 1: XXXXXGXGXXAXXXAXXXXXXGXXXXXXX.

structure: $\beta\beta\beta\beta\beta\beta\beta$ $\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha$ $\beta\beta\beta\beta\beta\beta\beta$.

This search yielded only five matches (from nearly 5000 sequences). Two of these were for human glutathione reductase and mercuric reductase (*Escherichia coli* glutathione reductase was not included in the database; Fig. 2). The third matching sequence included only Gly and Ala and was thus eliminated. The fourth match was an NADP-specific glutamate dehydrogenase from *Neurospora crassa* [34]. The homologous enzyme from *E. coli* contains one conservative change in the consensus sequence (Fig. 2). These two sequences show no similarity with other NADP-binding enzymes listed in Fig. 2 and there was no previous identification of their NADP-binding sites. The fifth match was a bacteriophage protein [47]. Since the function of this protein is not defined, the significance of the match is not known.

The five matches noted above were a subset of a total of 42 different sequences found when the search was carried by substituting X instead of the last G in consensus sequence 1. All but one of the remainder (37 sequences), were incompatible with the more detailed consensus sequence shown in Fig. 2. The majority of these were eliminated by a simple rule: if a Pro or a stretch of Gly appears in a position that corresponds to the β-sheet or α-helix portion of sequence 1 it would break the secondary structure [48] and thus would not be compatible with a $\beta\alpha\beta$-fold-forming sequence.

The one exception was the NADP-specific octopine-synthase sequence which showed perfect compatibility with the consensus sequence in Fig. 2 except that the third Gly was moved by one position. This sequence showed no significant similarity with the full sequence of adrenodoxin reductase or any of the other enzymes.

A search for the consensus sequence in recently published NADP-binding enzyme sequences which were not yet included in the NBRF database revealed that NADP-specific 'malic' enzyme also possesses the consensus sequence with the exception of the position of the last Gly (Fig. 2).

Negative control: NAD- and FAD-binding-site consensus sequences do not recognize any NADP-binding enzymes

If consensus sequence 1 indicates a distinction between the NADP-binding vs. NAD- and FAD-binding sites, then a

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                                    130                                     140                                     150
        A   R   A   F   V   G   W   Y   N   G   L   P   E   N   R   E   L   A   P   D   L   S   C   D   T   A   V   I
      G GCC CGG GCC TTT GTG GGC TGG TAC AAT GGG CTT CCT GAG AAC CGG GAG CTG GCC CCG GAC CTG AGC TGT GAC ACA GCC GTG ATT
                                    390                                     420                                     450

                                    160                                     170                                     180
L   G   Q   G   N   V   A   L   D   V   A   R   I   L   L   T   P   P   D   H   L   E   K   T   D   I   T   E   A   A
CTG GGG CAG GGG AAT GTG GCT CTG GAC GTG GCC CGG ATC CTG CTG ACC CCC CCC GAC CAC CTG GAG AAA ACG GAC ATC ACT GAG GCC GCC
                                    480                                     510                                     540

                                    190                                     200                                     210
L   G   A   L   R   Q   S   R   V   K   T   V   W   I   V   G   R   R   G   P   L   Q   V   A   F   T   I   K   E   L
CTG GGA GCC CTG AGA CAG AGT CGG GTG AAG ACG GTG TGG ATC GTG GGC CGA CGT GGA CCC CTA CAA GTG GCC TTC ACC ATA AAG GAG CTT
                                    570                                     600                                     630

                                    220                                     230                                     240
R   E   M   I   Q   L   P   G   T   R   P   M   L   D   P   A   D   F   L   G   L   Q   D   R   I   K   E   A   A   R
CGG GAG ATG ATT CAG TTA CCA GGA ACT CGG CCC ATG TTG GAT CCT GCG GAT TTC TTG GGT CTC CAG GAC AGA ATC AAG GAG GCC GCT CGC
                                    660                                     690                                     720

                                    250                                     260                                     270
P   R   K   R   L   M   E   L   L   L   R   T   A   T   E   K   P   G   V   E   E   A   A   R   R   A   S   A   S   R
CCG AGG AAG CGG CTG ATG GAA CTG CTG CTT CGA ACA GCC ACG GAG AAG CCA GGG GTG GAG GAG GCT GCC CGC CGG GCA TCA GCC TCC CGT
                                    750                                     780                                     810

                                    280                                     290                                     300
A   W   G   L   R   F   F   R   S   P   Q   Q   V   L   P   S   P   D   G   R   R   A   A   G   I   R   L   A   V   T
GCC TGG GGC CTC CGC TTC TTC CGA AGC CCG CAG CAG GTC·CTG CCC TCG CCA GAT GGG CGG CGG GCG GCA GGC ATC CGC CTG GCA GTC ACC
                                    840                                     870                                     900

                                    310                                     320                                     330
R   L   E   G   I   G   E   A   T   R   A   V   P   T   G   D   V   E   D   L   P   C   G   L   V   L   S   S   I   G
AGA CTG GAG GGC ATT GGA GAG GCC ACC CGG GCA GTG CCC ACT GGG GAT GTG GAG GAC CTC CCC TGT GGG CTG GTG CTG AGC AGC ATT GGG
                                    930                                     960                                     990

                                    340                                     350                                     360
Y   K   S   R   P   I   D   P   S   V   P   F   D   P   K   L   G   V   V   P   N   M   E   G   R   V   V   D   V   P
TAT AAG AGC CGC CCC ATC GAC CCC AGT GTG CCC TTT GAC CCC AAG CTC GGG GTT GTC CCC AAT ATG GAG GGC CGG GTT GTG GAT GTG CCA
                                   1020                                    1050                                    1080

                                    370                                     380                                     390
G   L   Y   C   S   G   W   V   K   R   G   P   T   G   V   I   T   T   T   M   T   D   S   F   L   T   G   Q   I   L
GGC CTC TAC TGC AGC GGC TGG GTG AAG CGG GGA CCC ACA GGT GTC ATC ACC ACC ACC ATG ACC GAC AGC TTC CTC ACC GGC CAG ATT CTG
                                   1110                                    1140                                    1170

                                    400                                     410                                     420
L   Q   D   L   K   A   G   H   L   P   S   G   P   R   P   G   S   A   F   I   K   A   L   L   D   S   R   G   V   W
CTA CAG GAC CTG AAG GCC GGG CAC CTG CCG TCT GGC CCC AGG CCG GGC TCT GCA TTC ATC AAG GCC CTG CTG GAC AGC CGA GGG GTC TGG
                                   1200                                    1230                                    1260

                                    430                                     440                                     450
P   V   S   F   S   D   W   E   K   L   D   A   E   E   V   S   R   G   Q   A   S   G   K   P   R   E   K   L   L   D
CCC GTG TCT TTC TCG GAC TGG GAG AAA CTG GAT GCT GAG GAG GTG TCC CGG GGC CAG GCC TCG GGG AAG CCC AGA GAG AAG CTG CTG GAT
                                   1290                                    1320                                    1350

                                    460
P   Q   E   M   L   R   L   L   G   H   .
CCT CAG GAG ATG CTG CGG CTG CTG GGG CAC TGA GCCTAGATCCCAGCCCCGCTGGGTGCAGAGAGAAGAGGAGGGTGAGCCCAGATCCCAGCCCAGCTCAGAGAAGAG
                                   1380                    1403                1423                1443

AGGAGGCGCGCTGGACAGCGGAGAGGCGTCGGGGTCAGCCTGAGCGGGACTCTGCACCCCAGCTGCGTCGTCTGCCCGTCCTGGCATACAGCCCTGGCTGCCTCTTCTCCAGGGGCGTG
 1463                1483                1503                1523                1543                1563

GGAGCACTTTCTGGAGCTAGGTCACTGCTGCCAGTGTGGGTACCTTTCAGCAAGGAGATAACCTTAGTTAGGGATGGAGGCAGGTACAGGCTGACCTCCGTCCCTCCTGTCTCTCTCCT
 1583                1603                1623                1643                1663                1683

GCTGGACTGTGGAGGGTCCCCAGGTCAGGAATATGCTGGAAATAAAGCACCTGCCACCTAG(A)83
 1703                1723                1743
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Fig. 1. *The sequence of the pAR cDNA insert and the deduced amino acid sequence of adrenodoxin reductase.* The numbering of the DNA starts with the first base of the codon for the first NH_2-terminal residue of the nature adrenotoxin reductase (starting with STQ...) [11, 13], based on the cDNA sequence [12]. The last six bases of the sequence and the poly(A) sequence are from clone pAR1. The sequence of pAR1 starts at about 1000 and shows three silent base pair differences (1044:C, 1387:C, 1692:A), and one (1114:T) which changes residue 372 of the resultant amino acid sequence from Pro to Ser. The sequence of clone 16 of Sagara et al. [12] shows only two silent base pair changes (1445:T, 1507:G) from the sequence of pAR, and the sequence of clone 12 shows two additional silent base pair changes (1044:C, 1047:T). The sequence shown here does not include the first 150 base pairs of the pAR insert as this is an addition of unknown origin. The polyadenylation sequence AATAAA appears 21 base pairs before the poly(A)

Enzyme	Source		Sequence
			FAD-binding site
Adrenodoxin R	Bovine	1	STQEQTPQICVVGSGPAGFYTAQHLLKHHSRAHVDIYEKQLVPFGL
Adrenodoxin R	Human	1	STQEKTPQICVVGSGPAGFYTAQHLLKHP QAHVDIYEKQPVPFGL
Putidaredoxin R	*P. putida*	1	MNANDNVIVGTGLAGVEVAFGLRASGWEGNIRLVGDATVIPHH
D-Amino acid Ox	Porcine	1	MRVVVIGAGVIGLSTALCIHERYHSVLQPLDVKVYADRFT
Fumarate R	*E. coli*	1	MQTFQADLAIVGAGGAGLRAAIAAAQANPNAKIALISKVYPMRSH
Glutathione R	*E. coli*	1	MTKHYDYIAIGGGSGGIASINRAAMYGQKCALIEAKE LGGTCV
Glutathione R	Human	12	PPAAGAVASYDYLVIGGGSGGLASARRAAELGARAAVVESHK LGGTCV
Lipoamide DH	*E. coli*	1	STEIKTQVVVLGAGPAGYSAAFRCADLGLETVIVERYNTLGGVCL
Lipoamide DH	Human	1	ADQPIDADVTVIGSGPGGYVAAIKAAQLGFKTVCIEKNETLGGTCL
Lipoamide DH	Porcine	1	ADQPIDADVTVIGSGPGGYVAAIKAAQLGFKRVCIEKNETLGGTCL
Mercuric R	*Pseudomonas*	89	EKHSGNEPPVQVAVIGSGGAAMAAALKAVEQGAQVTLIERG TIGGTCV
NADH DH	*E. coli*	1	MTTPLKKIVIVGGGAGGLEMATQLGHKLGRKKKAKITLVDRNHSH
p-OH benzoate H	*Pseudomonas*	1	MKTQVAIIGAGPSGLLLGQLLHKAGIDNVILERQTPDYVLGR
Consensus sequence			±●●●●G G ●G● A +●●± G ± ●●
Secondary structure			TββββββββTααααααααααααααααTTββββββββT
			NADP-binding site
*Adrenodoxin R	Bovine	137	RELAPDLSCDTAVILGQGNVALDVARILLTPPDHLEKTDITEAALGALR
*Adrenodoxin R	Human	136	QELEPDLSCDTAVILGQGNVALDVARILLTPPEHLERTDITKAALGVLR
*Malic enzyme	Rat	285	RITKNKLSDQTVLFQGAGEAALGIAHLIVMAMEKEGLSKEKARQKIWLV
*Octopine Syn	*Agrobacterium*	1	MAKVAILGAGNVALTLAGDLARRLGQVSSIWAPISNRNSFN
*Glutamate DH	*E. coli*	224	KRHGMGFEGMRVSVSGSGNVAQYAIEKAMEFGARVITASDSSGTVVDES
*Glutamate DH	*N. crassa*	210	YSGAGSYAGKRVALSGSGNVAQYAALKLIELGATVVSLSDSKGALVATG
Glutamate DH	Yeast	209	TNGKESFEGKRVTISGSGNVAQYAALKVIELGGTVVSLSDSKGCIILET
Glutathione R	*E. coli*	155	DSDGFFALPERVAVVGAGYIAVELAGVINGLGAKTHLFVRKHAPLRSFD
Glutathione R	Human	179	GFFQLEELPGRSVIVGAGYIAVEMAGILSALGSKTSLMIRHDKVLRSFD
Mercuric R	*Pseudomonas*	261	EALASDTIPERLAVIGSSVVALELAQAFARLGSKVTVLARNTLFFREDP
Consensus sequence			+●●●●G G ●A● ●A ●● G +● ●
Secondary structure			TββββββββTααααααααααααααααTTββββββββT

Fig. 2. *Alignment of the putative FAD- and NADP-binding sites of adrenodoxin reductase with those of other oxidoreductases.* The NADP-binding sites for the enzymes marked with an asterisk are identified here for the first time. The secondary structure indicated is based on the known crystal structures of FAD- and NADP-binding sites of glutathione reductase [16, 19] (α, α-helix; β, β-sheet; T, turn). The sequence shown includes 10 residues on both sides of this βαβ fold. The number of the first residue shown is indicated before the sequence (1 = the first residue of the amino terminus of the mature protein). In the consensus sequence (●) indicates a hydrophobic residue, (+, −) a charged residue and (±) a hydrophilic residue. The sequences listed are: adrenodoxin reductase (bovine, this paper and [12]; human [14]); D-amino acid oxidase [31], fumarate reductase [32], glutamate dehydrogenase (*E. coli* [33]; *N. crassa* [34]; yeast [35, 36]), glutathione reductase (*E. coli* [37], human [38]), lipoamide dehydrogenase (*E. coli* [39]; human [40]; porcine [16, 40]), 'malic' enzyme [41], mercuric reductase (*Pseudomonas*) [42], NADH dehydrogenase [43], octopine synthase (*Agrobacterium*) [44], *p*-hydroxybenzoate hydroxylase (*Pseudomonas*) [45] and putidaredoxin reductase (*Pseudomonas putida*) [46]. Abbreviations, DH, dehydrogenase; H, hydroxylase; R, reductase; Ox, oxidase

search of the sequence database using NAD- and FAD-binding consensus sequences should not identify any NADP-binding enzymes. A search of the NBRF library using FAD- and NAD-binding consensus sequence (substituting Gly for the first Ala in consensus sequence 1) indeed identified FAD- and NAD-binding proteins, but it did not identify a single NADP-binding protein. Two of the proteins identified in this search are hypothetical proteins derived from open reading frames [49, 50]. Since their sequences matched perfectly the detailed consensus sequence (Fig. 2), these two hypothetical proteins probably function as oxidoreductases. Yet, whether they bind a dinucleotide remains to be determined.

Predicted secondary structure of the putative NADP-binding site

The conformational propensities [48] of the NADP-binding sites of all but two of the enzymes listed in Fig. 2 showed a profile that is perfectly compatible with a βαβ-fold structure. However, the profiles for adrenodoxin reductase and 'malic' enzyme showed higher helix potential at the expected position of the second β-sheet strand. The sequence of adrenodoxin reductase shows no other region that fits a βαβ-fold structure better than this region, despite the difference noted. In an ADP-binding βαβ fold the two β-sheet strands form the same β-pleated sheet [19]. It is possible that the second strand was substituted by a different β-sheet-strand-forming region in the course of evolution.

Analysis of the hydrophobicity of adrenodoxin reductase

Adrenodoxin reductase associates with the inner mitochondrial membrane within which the *P*-450 system is located. The hydrophobicity profile using two scales of hydrophobicity [51, 52] did not show any segment that satisfies the criteria for prediction of a membrane-spanning region in the adrenodoxin reductase sequence.

DISCUSSION

Is the region identified by the consensus sequence indeed the NADP-binding site?

The hypothesis that the regions identified by consensus sequence 1 in adrenodoxin reductase and other NADP-binding enzymes are indeed NADP-binding sites is supported by the following lines of evidence. (a) Among nearly 5000 different protein sequences known to date, this sequence appears solely in specific NADP-binding enzymes. (b) The secondary structure of the region in which this sequence is located is unequivocally predicted to be an ADP-binding $\beta\alpha\beta$ fold for the newly identified NADP-binding sites (see above for reservation for adrenodoxin reductase). (c) If the NADP-binding flavoenzymes listed in Fig. 2 shared a common ancestor, then the FAD- and NADP-binding sites of these enzymes would be expected to be at similar distances from one another, and this is indeed the case for adrenodoxin reductase (Fig. 2).

At present we do not know the structural significance of the observed sequence differences between NAD- and NADP-binding sites (Fig. 2). Some of the conserved differences may play a role in determining NAD vs. NADP specificity of the binding site. With the availability of the cDNA probes the structural role of the sequences identified in this paper can be examined by the production of altered molecules from cDNA modified using site-directed-mutagenesis techniques.

What are the different types of NADP-binding sites?

The sequences of several NADP-dependent enzymes do not include the NADP consensus sequence (Fig. 2) i.e. cytochrome *P*-450 reductase [26–28], spinach ferredoxin reductase [29], quinone (menadione) reductase [53], 6-phosphogluconate dehydrogenase [54] and glucose-6-phosphate dehydrogenase [55, 56]. Yet, the presence of this consensus sequence in certain NADP-binding enzymes which share no significant overall sequence similarity indicates that its conservation may be mandated by the structural requirements of coenzyme and protein association. Hence, we expect that most if not all NADP-binding sites that are structurally similar to a $\beta\alpha\beta$ fold will have the conserved consensus sequence. Following the nomenclature of Wierenga et al. [17], we suggest that these be referred to as type I NADP- binding sites. The enzymes that do not have the NADP consensus sequence may represent additional structurally distinct classes.

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Note added in proof (received February 6, 1989). The sequence data will appear in the EMBL/GenBank/DDBJ Nucleotide Sequence Databases under the accession number X13736. These results were presented in a preliminary form at the annual meeting of the American Society for Biochemistry and Molecular Biology (*FASEB J. 2*, A356, 1988).