Atomic resolution X-ray crystal structure of cisplatin bound to hen egg white lysozyme stored for 5 years 'on the shelf'

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Synopsis

An X-ray crystal structure of cisplatin bound to hen egg white lysozyme stored for 5 years 'on the shelf' has been refined to a diffraction resolution of 1.0 Å and shows platinum binding to both His15 imidazole ND and NE nitrogens and an additional binding site on the NE side coordinated to the side chain of Arg14. The assignments of the ligands for the Pt bound to His15ND are [CINH₃NH₃His15]. The assignment of ligands for the Pt bound to His15NE and to Arg14 are uncertain.

Keywords

Cisplatin; hen egg white lysozyme; 5 years 'on the shelf'; chemical equilibrium 'final' state.

Abstract

An X-ray crystal structure of cisplatin bound to hen egg white lysozyme has been refined to a diffraction resolution of 1.0 Å; being better than 1.2 Å this is generally referred to as 'atomic resolution'. The crystals used had been stored for 5 years 'on the shelf'. Platinum binding to both His15 imidazole ND and NE nitrogens is seen as well as an additional binding site on the NE side coordinated to the side chain of Arg14. An omit electron density map allowed the placement of the cisplatin chlorine and ammine ligands, based on their respective peak heights, at the His15 ND. The Pt NE side of His 15 and the Arg14's Pt 20% weak binding site ligands could not be placed with certainty and have therefore not been made. Hydrogen atoms were not visible on the cisplatin ND side ammine ligands whereas some hydrogens are visible on the polypeptide at the stereochemically expected positions notably, for example, on the CE carbon atom on the His 15 imidazole side chain. The assignment of the ligands for the Pt bound to His15ND are then [CINH₃NH₃His15ND]. The side chains of His15 and Arg14 are well ordered. The multiple Pt binding sites around the His15 and Arg14 side chains are reminiscent of the triclinic atomic resolution cisplatin hen egg white lysozyme crystal structure (Tanley and Helliwell 2014, 2016 Structural Dynamics). The PDB code is 5LXW.

Introduction

An X-ray crystal structure of cisplatin bound to hen egg white lysozyme has been refined to a diffraction resolution of 1.0 Å; being better than 1.2 Å this is generally referred to as 'atomic resolution'. The crystals used had been stored for 5 years 'on the shelf' and therefore represents an excellent chance of being a chemical equilibrium 'final' state. This study will form a new crystal structure contribution within an overall Data Review article of our studies in this field (to be

submitted) and which take advantage of a comprehensive overview we have gained and exploration of diverse computational tools now available to us.

Methods

The crystallisation conditions are as described in Tanley et al 2012 namely:- Hen Egg White Lysozyme (49mg, ie 3.2mM), cisplatin (3mg, ie 10mM), 462.5 microlitres of 0.04 M sodium acetate, 462.5 microlitres of 10% sodium chloride, and with 7.5% DMSO (75 microlitres ie 1mM), pH 4.7, batch crystallisation method and room temperature (295K). The crystallisation pot was kept for five years and then the current crystal samples were extracted from the pot and frozen with paratone as cryoprotectant. The diffraction data were measured at 100K at the Diamond Light Source at an X-ray wavelength of 0.92819 Å and comprised measurements made on two separate crystals identically prepared and stored, as above. The diffraction data were processed using iMOSFLM (Battye et al 2011), then POINTLESS and SCALA of CCP4 (Evans 2011). The protein model was refined against the data from each crystal separately, and which yielded basically identical results, and then the diffraction data were merged realising a final improved data completeness. $\langle I/\sigma I \rangle$ crossed 1.0 at 1.1 Å diffraction resolution and $CC_{1/2}$ crossed 0.5 at 1.07 Å diffraction resolution; see Table 1 for a full summary. Both Phenix_Refine (Afonine et al 2012) and CCP4's Refmac (Murshudov et al 1997) were used for the model refinement, taking advantage of the advantages of each. COOT (Emsley and Cowtan 2004) was used to inspect the molecular model and the electron density maps.

Results and discussion

The cisplatin Pt ligand assignments (Figure 1) are [CINH₃NH₃His15ND] for Pt His15ND. The distances and angles for these ligands are shown in Table 2. All values lie within expected values for platinum to the respective chlorine or ammine ligands. The PT ND and its ligands are closely square planar (See Figure 2 for a side on view).

The diffraction data were measured at the Diamond Light Source at an X-ray wavelength of 0.92819 Å providing an enhanced f'' anomalous signal of 11.3 electrons, albeit not the maximum of 12.3 electrons for the platinum L I absorption edge but much increased over the Cu K α value of 7 electrons. This allowed the weak occupancy (20%) third platinum site bound to Arg14 to be definitely identified instead of for example a chloride, which has a very weak f'' at that X-ray wavelength (0.27 electrons) versus at Cu K α (0.7 electrons). The diffraction data comprised measurements made on two separate crystals identically prepared. The protein model was refined against these separately, and which yielded basically identical results, and then the diffraction data from these two crystals were then merged realising a final data completeness of 89.8% overall and 80.2% in the outer resolution shell. $CC_{1/2}$ crossed 0.5 at 1.07 Å diffraction resolution and $\langle I/\sigma I \rangle$ crossed 1.0 at 1.1 Å diffraction resolution. This study will form a new crystal structure contribution within an overall Data Review article of our studies in this field (to be submitted) and which takes advantage of (i) a comprehensive overview we have gained and (ii) exploration of diverse computational tools now available to us. We note that the third Pt "20% occupancy" site, is also logged in the 4gcb PDB file (at 22% occupancy), also coordinated by the Arg 14 side chain, this time one rather than both of its end side chain nitrogens. The assignment in the 4gcb case data is

interesting. For 4gcb it has a 9 sigma anomalous difference map peak which is identical in peak height to other Cl sites' anomalous difference map peaks. The 20% Pt site in the Diamond data case now has a 14 sigma anomalous difference map peak, consistent with the f'' increase between Cu Kalpha and an X-ray wavelength of 0.92819 Å. Whilst this anomalous difference map peak increased accordingly the other option of a fully occupied chlorine, as monitored by the Cls in 4gcb have lost their anomalous difference map peaks (with their much weaker f''). Whilst 4gcb's PDB file has this assigned as a 22% Pt the corresponding article has it labelled as 'Cl4' in a figure, obviously a preliminary assignment. This Data Review and the new diffraction data measured at DLS at the second X-ray wavelength allows an unambiguous assignment of a third Pt binding site at Arg14 close to the Pt NE site of the His15 imidazole.

Using the paired model refinement method of Diederichs and Karplus (2010) established that the resolution limit of the 1.0Å model was slightly better against the 1.05Å data than the final 1.05 Å model against the 1.05 Å data (R and Rfree values were respectively 16.88% versus 16.96% for Rwork and 19.72% versus 19.75% for Rfree). The diffraction data and the final protein model statistics are summarised in Table 1.

Several different attempts were made to assign the ligands to the PT on the His15NE side. A Cl ligand at 2.51 Å away from the Pt made no sense, i.e. was unlikely, and it was also well out of a square planar position. Also at 2Fo-Fc 1.0 rms there was a more plausible density for a ligand, and the Fo-Fc there was 4.0 sigma. But was it a chlorine or an ammine? If the latter it would make this a four nitrogens to Pt case ie Arg14 N, His NE, and two ammines. Firstly a chlorine there at 50% occupancy was tried along with a reassignment of the chlorine at 2.51 Å distance to be a 50% occupancy bound water. That led to a large negative peak (5.6 sigma in the Fo-Fc map) on the 50% Cl. Overall then it was not possible to assign ligands to the HisNE Pt. Similarly it was not possible to assign ligands to the Arg14 coordinated Pt.

The PDB code is 5LXW. This preprint and the raw diffraction data images have been placed in the Zenodo European Union Research Archive.

Conclusions

An X-ray crystal structure of cisplatin bound to hen egg white lysozyme has been refined to a diffraction resolution of 1.0 Å; being better than 1.2 Å this is generally referred to as 'atomic resolution'. The crystals used had been stored for 5 years 'on the shelf'. Platinum binding to both His15 imidazole ND and NE nitrogens is seen as well as an additional binding site on the NE side coordinated to the side chain of Arg14. An omit electron density map allowed the placement of the cisplatin chlorine and ammine ligands, based on their respective peak heights, at the His15 ND. The Pt NE side of His 15 and the Arg14's Pt 20% weak binding site ligands could not be placed with chemical certainty and have therefore not been made. Hydrogen atoms were not visible on the cisplatin ND side ammine ligands whereas some hydrogens are visible on the polypeptide at the stereochemically expected positions notably, for example, on the CE carbon atom on the His 15 imidazole side chain. The assignment of the ligands for the Pt bound to His15ND are then [CINH₃NH₃His15ND]. The assignments of the ligands for the Pt bound to His15NE and of the Pt bound to Arg14 are chemically uncertain and have therefore not been made. The side chains of His15 and Arg14 are well ordered. The multiple Pt binding sites around the His15 and Arg14 side chains are

reminiscent of the triclinic atomic resolution cisplatin hen egg white lysozyme crystal structure (Tanley and Helliwell 2014, 2016 Structural Dynamics).

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Figure 1 The His15 and Arg 14 cisplatin binding sites. 2Fo-Fc map in blue contoured at 1.2 rms, Fo-Fc map in green contoured at 4.0 sigma and anomalous difference map in orange contoured at 3.0 sigma.

Figure 2 The PT ND and its ligands are closely square planar. The 2Fo-Fc map in blue is contoured at 1.2 rms.

Table 1 Summary of data processing and model refinement statistics

Wavelength (Å)	0.92819		
Resolution range (Å)	27.39	- 1.0	(1.05 - 1.0)
Space group	P 43 21 2		

Unit cell	79.37 79.37 37.84 90 90 90
Total reflections	476495 (25140)
Unique reflections	57758 (4344)
Multiplicity	8.1 (3.3)
Completeness (%)	88.01 (80.2)
Mean I/sigma(I)	9.9 (0.5)
Wilson B-factor	11.90
R-merge	0.071 (1.869)
CC1/2	0.997 (0.076)
Reflections used for R-free	3011
R-work	0.1758 (0.3665)
R-free	0.2028 (0.3894)
Number of non-hydrogen atoms	1226
macromolecules	1103
ligands	15
water	108
Protein residues	154
RMS (bonds)	0.012
RMS (angles)	1.27
Ramachandran favored (%)	98
Ramachandran allowed (%)	2
Ramachandran outliers (%)	0
Clashscore	0.46
Average B-factor	19.40
macromolecules	18.60
ligands	26.00
solvent	26.70
Cruickshank DPI (Å)	0.027

Table 2 The platinum coordination geometry (a) ligand distances and (b) angles and their standard uncertainties in brackets derived from the Cruickshank dpi calculated using the webserver ‘calc dpi’ (Kumar et al 2015).

Ligand %	Distance Å
Pt ^δ to Cl	2.35 (0.05)
Pt ^δ to NH ₃ (end position) nitrogen atom	2.00 (0.05)
Pt ^δ to NH ₃ (trans to the Cl) nitrogen atom	1.93 (0.05)
Pt ^δ to His15ND	2.14(0.05)

Pt ^ε to Arg14N	2.01 (0.05)
Pt ^ε to His15 NE	2.43 (0.05)

Angle (degrees)	PDB code ???? angles
Cl to Pt ^δ to NH ₃ (end position) nitrogen atom	91
NH ₃ (trans to the Cl) nitrogen atom to Pt ^δ to NH ₃ (end position) nitrogen atom)	88
Cl to Pt ^δ to His15ND	91
NH ₃ (trans to the Cl) nitrogen atom to Pt ^δ to His15ND	89
His15 NE to Pt ^ε to Arg14N	93

Footnote

% The platinum atom and associated ligand occupancies were set equal.

& Using the standard uncertainties of the ligand distances in Table 2a, the uncertainty on these angles are then approximately 3 degrees.

% No platinum to ligand distance restraints were applied since this is an X-ray crystal structure at atomic resolution.

Supplementary

Comments to the PDB Validation Report:-

Comment from us:- We note that we have used the diffraction data to $\langle I/\sigma I \rangle$ of 0.5 using the paired model refinement method of Diederichs, K. & Karplus, P. A. (2013). Acta Cryst. D69, 1215–1222, as we have described in the text above. Thus the population of existing “similar resolution” protein crystal structures will likely be refinements to the more conventional $\langle I/\sigma I \rangle$ of approximately 2.0. We imagine therefore that our Rfree slider bar being ‘into the red’ is due to that i.e. our diffraction data is weaker in intensity. The RSRZ outliers is also into the red, albeit less so than the Rfree, but there are no further actions we can take as the fit to electron density of the amino acids we think is very good. As for the highest outliers these are:-

All (6) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	125	ARG	3.7
1	A	101	ASP	3.5
1	A	121[A]	GLN	3.2
1	A	129	LEU	3.1
1	A	6[A]	CYS	2.9
1	A	102[A]	GLY	2.0

The electron densities for the side chains of Arg125, Asp 101 and Gln 121A are indeed weak.

Re the ligands:-

Mol	Type	Chain	Res	Atoms	RSCC	RSR	LLDF	B-factors($\text{\AA}^2$)	Q<0.9
2	DMS	A	201	4/?	0.97	0.13	3.56	20,37,40,40	0
6	NH3	A	336	1/?	0.93	0.12	1.59	27,33,33,33	4
3	CL	A	332	1/?	0.97	0.11	0.51	25,25,25,25	1
3	CL	A	202	1/?	0.98	0.07	0.17	29,29,29,29	0
3	CL	A	334	1/?	1.00	0.02	-1.02	28,28,28,28	0
4	NA	A	204	1/?	1.00	0.04	-1.08	13,13,13,13	0
3	CL	A	339	1/?	1.00	0.02	-2.48	16,16,16,16	0
5	PT	A	330	1/?	1.00	0.02	-3.92	27,27,27,27	1
5	PT	A	333	1/?	1.00	0.03	-5.36	17,17,17,17	1
6	NH3	A	337	1/?	0.92	0.27	-	21,25,25,25	4
3	CL	A	340	1/?	0.99	0.17	-	33,33,33,33	0
5	PT	A	331	1/?	0.98	0.09	-	32,32,32,32	1

Comment from us on the one highlighted ligand:- the DMS 201 electron density looks fine to us:-

ie 2Fo-Fc (contoured in blue at 1.2 rms) looks fine and there is little to no Fo-Fc density (5.0 sigma, the COOT default cut off in green).