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Cisplatin binding to human serum albumin: a structural study

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Experimental Section

Materials

Milli-Q water (Millipore, Bedford, MA) has been used in all the experiments. Human serum albumin has been purchased from Sigma-Aldrich and further purified by gel-filtration chromatography to be dimer-free as previously described [1,2].

Crystallization and data collection

Single crystals of HSA suitable for X-ray diffraction analysis were obtained at 20 °C by using the sitting-drop vapour diffusion method in Linbro plates. Crystals were grown after four weeks in 4, 6 or 8 microliter drops formed by mixing equal volumes of purified protein (30 mg/mL) in water and of a reservoir solution containing 30% w/v PEG 3350 and 50 mM potassium phosphate at pH 7.4. These crystals were soaked for 24 h in a solution consisting of 0.005 M cisplatin in the same reservoir and, before freezing, for a few seconds in a cryoprotectant solution containing 20% glycerol and 25% w/v PEG 3350 and 50 mM potassium phosphate at pH 7.4. Soaking experiments have been already used to obtain crystals between model proteins and cisplatin.[37-38] X-ray diffraction measurements were performed at the CNR Institute of Biostructures and Bioimages, Naples, Italy, using a Saturn944 CCD detector equipped with CuKα X-ray radiation from a Rigaku Micromax 007 HF generator. Data were processed and scaled by using the program HKL2000 [3]. X-ray diffraction data for the adduct were then collected at 3.16 Å resolution. Crystals belong to the space group $P2_1$ with one molecule in the asymmetric unit. Details of data collection statistics are listed in Table S1. Further X-ray diffraction data were collected at 3.29 Å resolution (using a new crystal soaked for 24 h in a solution consisting of 0.005 M cisplatin) and at 3.98 Å resolution (using a crystal soaked for 3 months in a solution consisting of 0.005 M cisplatin) (Table S1) .

Structure solution and refinement

The structure of the adduct has been solved by the molecular replacement method using the program Phaser [4] of the CCP4 package [5] and the structure of HSA in complex with thyroxine (refined at 2.65 Å resolution in the space group $P2_1$, PDB CODE:1HK1) [6] stripped of all its ligands, as the search model. Rigid body, positional and isotropic B-factor refinements have been carried out using REFMAC5 [7]. Following initial rigid body refinement, calculation of Fo-Fc and 2Fo-Fc electron density maps gave a clear indication of the presence of heavy atoms bound to the protein. In particular, residual electron density peaks were found close to His105, His288, Met298, Met329 and Met548 side chains. Refinements have been carried out using secondary structure restraints constructed by Prosmart [8]. In between the refinement cycles the model was subjected to manual rebuilding using Wincoot [9]. A pair of short helices (residues 77–88), part of the N-

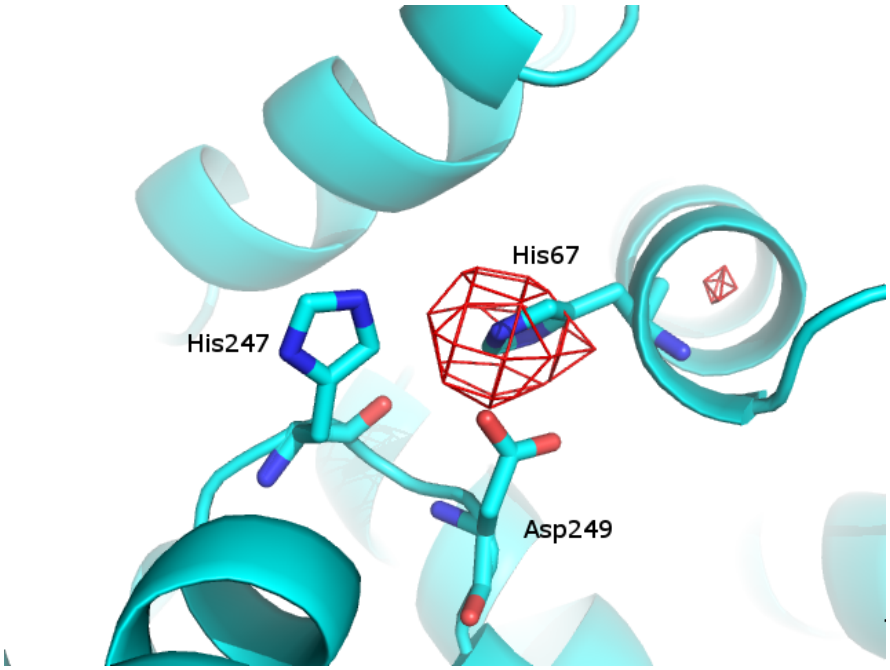
terminal region (residues 1-4) in subdomain IA, residues 502-512 and part of the C-terminal tail (residues 571–585) in subdomain IIIB are disordered and are not included in the final model. These regions lie close to symmetry-related molecules in the crystal. In particular, residues 502-512 lie close to the cisplatin binding site Met298 of a symmetry-related molecule. The electron density maps corresponding to residues 1-4 and to part of the C-terminal tail are not observed also in high resolution X-ray structures of HSA and are considered to be regions of the polypeptide chain with high conformational flexibility [10]. The model also misses several residue side chains that have been truncated to alanine-like residues, as done in many other medium-low resolution structure of HSA, including the starting model [6]. Electron density map corresponding to Cys34 side chain suggests that in our sample this residue is not covalently modified as suggested by other authors.[11] As typically observed for HSA structures [49], B-factors significantly differ residues by residues, with buried residues which present B-factors between 20 and 30 $Å^2$ and solvent accessible surface residues that present values as high as 100 $Å^2$. The average B-factor for non-H atoms in the cisplatin/HSA adduct (49.0 $Å^2$) is in line with that obtained using X-ray diffraction data collected on other HSA isomorphous crystals (average B-factors are in the range 52.4-73.5 Å2; average B-factor= 62.4 $Å^2$ in the starting model) [6]. Pt centre occupancy factor has been evaluated refining the structure with different occupancy factor starting values and evaluating the presence of positive and negative peaks in the Fo-Fc electron density map in correspondence to the Pt centre. Analysis with PROCHECK [12] indicates that 99.0% of the residues are in core or favourable regions of the Ramachandran plot, 0.6 % of the residues are in generously allowed regions and, as in the starting model, two residues (0.4%) are outliers. Atomic coordinates have been deposited in the Protein Data Bank. The entry identification code is 4S1Y. Figures were prepared by using PYMOL [13]. The results of the structural analysis have been further validated using the 3.29 Å resolution dataset (Table S1). Due to the low resolution of the data collected using the crystal soaked for 3 months in a solution of cisplatin (3.98 Å), no structural refinement was performed and detailed interactions at the binding sites cannot be deduced. However, as also done by other authors for adduct of cisplatin with Na^+/K^+ ATPase [14], we have used these data to solve the structure and calculate a Fo-Fc electron density map, which provides information on the location of Pt on HSA structure. Interestingly, we found additional binding sites, beyond those characterized in the structure reported in the manuscript (Figure S1).

Table S1. Data collection and refinement statistics for the HSA-cisplatin adducts

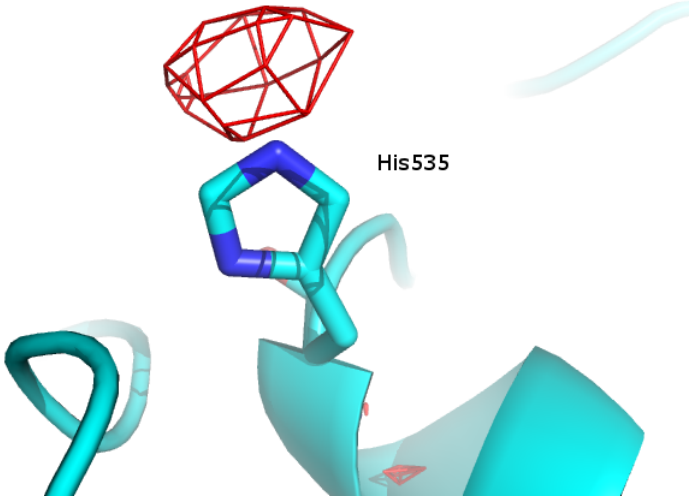
PDB code	4S1Y	not deposited	not refined
Data-collection			
Space group	$P2_1$	$P2_1$	$P2_1$
Unit cell parameters			
a,	57.79	57.54	57.65
b,	86.47	86.32	85.99
c (Å),	59.14	59.17	59.39
β (°)	103.7	103.3	103.3
Molecules per asymmetric unit	1	1	1
Observed reflections	23736	26891	12032
Unique reflections	9393	8628	5161
Resolution (Å)	57.46-3.16 (3.23-3.16)	57.59-3.29 (3.36-3.29)	50.00-3.89 (3.96-3.89)
Completeness (%)	96.5 (96.4)	99.5 (99.8)	95.9 (98.8)
Rmerge	0.149 (0.503)	0.127 (0.586)	0.090 (0.459)
I/σ(I)	4.8 (2.0)	6.0 (2.1)	8.1 (2.5)
Multiplicity	2.5 (2.6)	3.1 (3.1)	2.3 (2.1)

Refinement

PDB code	4S1Y	not deposited
Resolution (Å)	57.46-3.16	57.59-3.29
number of reflections in working set	8934	8208
number of reflections in test set	449	409
R factor/R free (%)	0.223 (0.327)	0.212 (0.297)
Number of non-H atoms used in the refinement	4318	4217
Estimated occupancy of Pt ion	0.80/0.80/0.40/0.35/0.30 (cisplatin binding site close to H105, M329, M548, M298, H288)	0.70/0.70/0.30/0.30/0.25
B-factor overall (Å^2)	49.0	73.6
B-factor of Pt ion (Å^2)	62.2/56.7/60.9/73.4/41.7 (cisplatin binding site close to H105, M329, M548, M298, H288)	78.6/66.8/89.5/74.2/75.6
Ramachandran values (%)		
Most favoured/ Additional allowed	90.5/8.5	87.9/10.9
Generously allowed/ Disallowed	0.6/0.4	1.0/0.2
R.m.s. deviation from ideality		
R.m.s.d. bonds (Å)	0.011	0.011
R.m.s.d. angles (°)	1.38	1.43



A



B

Figure 1. Residues surrounding the additional cisplatin binding sites in the cisplatin/HSA adduct solve at 3.98 Å resolution. The Fo-Fc electron density map is shown in red at a contour level of 3.0 σ.

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