Supplementary information

The X-ray structure of the Primary Adducts formed in the Reaction between Cisplatin and Cytochrome c

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Materials

Milli-Q water (Millipore, Bedford, MA) was used in all the experiments. Cytochrome c from horse heart and cisplatin were purchased from Sigma-Aldrich (St. Louis, MO) and used directly without further purification.

Crystallization and data collection

A new crystal form of horse heart cytochrome c that co-crystallized with nitrate and sulfate ions has been obtained. Crystallization was prepared by using the hanging-drop vapour diffusion method in Linbro plates and a reservoir contained 3.5 M ammonium sulfate, 0.6 M sodium nitrate. The droplets consisted of 1 microliter protein (30 mg/mL in water) and 1 microliter of reservoir. They were equilibrated against a 500 microliters reservoir solution at 20 °C. These conditions produced well shaped red crystals after 1 month. These crystals have been soaked for 24 h in a solution consisting of 0.005 M cisplatin in 2.0 M ammonium sulfate and 0.4 M sodium nitrate. To prepare this solution, Cisplatin was first dissolved in 5 mM sodium acetate buffer at pH 5.0. The crystals have been fished with a loop and cryo-cooled without cryo-protectant [1], as done in many other cases of complexes between proteins and metallodrugs [2]. Data have been collected at the CNR Institute of Biostructure and Bioimages, Naples, Italy using a Saturn944 CCD detector equipped with CuKα X-ray radiation from a Rigaku Micromax 007 HF generator.

CrystalClear software package from Rigaku has been used to collect the X-ray diffraction data. The datasets have been processed and scaled by using the program HKL2000 [3]. Crystals of the native protein diffract at about 2 Å resolution. The soaking severely cracks the crystals and significantly decreases their X-ray diffraction power. Initially, X-ray diffraction data for the adduct have been collected at about 3 Å resolution. Successively, a crystal was found that is able to diffract the X-ray at 2.19 Å resolution. Data collection statistics are reported in Table S1.

Structure solution and refinement

The structure of the cytochrome c-cisplatin adduct has been solved by the molecular replacement technique using the structure of cytochrome c refined at 1.94 Å resolution as search model (PDB CODE:1HRC) [4] from which all the solvent molecules were omitted. The correct orientation and translation of the six protein molecules in the asymmetric unit were determined using the program Phaser [5]. Refinement has been carried out using REFMAC5 [6]. Inspection of the initial Fo-Fc electron density maps clearly reveals the presence of Pt atoms bound to five out of the six molecules in the asymmetric unit. The occupancy factor of cisplatin fragment in the structure, i.e the measure of the fraction of molecules in the crystal where cisplatin fragment atoms actually occupies the position specified in the model, has been evaluated applying many refinement steps for different fixed values of the occupancy, until no residual positive or negative peaks of electron density map were observed at the Pt site. Non crystallographic symmetry (NCS) restraints and TLS refinements have been also carried out. In between the refinement cycles the model was subjected to manual rebuilding using Wincoot [7]. The same program was used to model the cisplatin fragments and to place several nitrate ions and two sulfate ions on the surface of the protein molecules. Validation has been performed using Procheck [8].

References

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Table S1. Data collection and refinement statistics for the Cisplatin-Cytochrome c structure

PDB code	4RSZ
Data-collection	
Space group	P3
Unit cell parameters	
a=b,c (Å)	120.34,36.67
Molecules per asymmetric unit	6
Observed reflections	316305
Unique reflections	30538
Resolution (Å)	104.22-2.19 (2.23-2.19)
Completeness (%)	99.8 (97.2)
Rmerge	0.109 (0.692)
I/σ(I)	18.1 (3.1)
Multiplicity	10.4 (7.1)
Refinement	
Resolution (Å)	104.22-2.19
number of reflections in working set	28987
number of reflections in test set	1544
R factor/Rfree (%)	0.231 (0.282)
Number of non-H atoms used in the refinement	5429
Occupancy of Pt ion	0.50/0.50/0.40/0.40/0.50 (molecules A, B, D, E and F)
B-factor overall (Å^2)	48.5
B-factor of Pt ion (Å^2)	54.8/58.5/55.8/65.9/58.5 (molecules A, B, D, E and F)
Ramachandran values (%) (by Procheck [8])	
Most favoured/ Additional allowed	91.6/8.4
Generously allowed/ Disallowed	0/0
R.m.s.d. bonds (Å)	0.015
R.m.s.d. angles(Å)	1.85

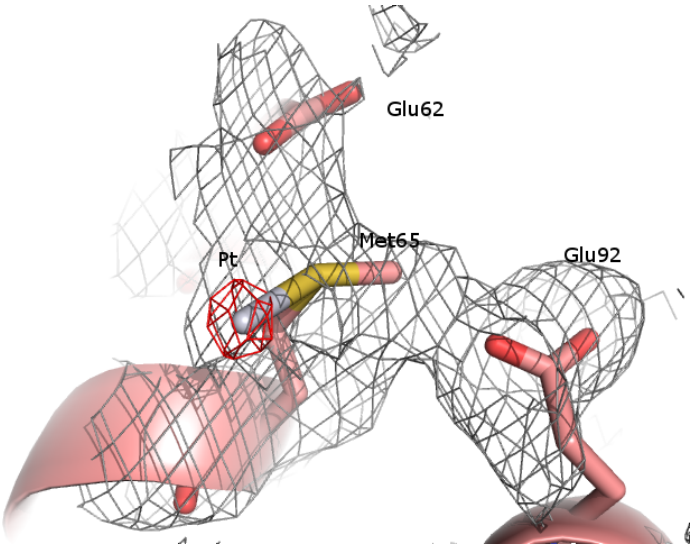

Figure S1. Details of the binding site of cisplatin in molecule E of the cytochrome c-cisplatin adduct. The Pt ion is coordinated to Met65. 2Fo-Fc electron density maps are contoured at 5σ (red) and 0.5σ (grey) level. The e.d. map is not well defined and thus it does not allow to obtain details of Pt coordination sphere. Structural refinements have suggested an occupancy value for Pt = 0.4.

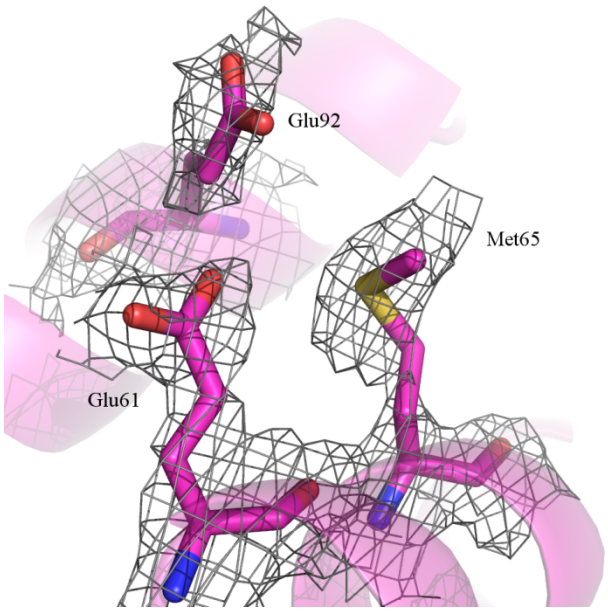

Figure S2. Met65, Glu61 and Glu92 side chains in the molecule C of the structure of cytochrome c-cisplatin adduct. No Pt ion is found coordinated to Met65. 2Fo-Fc electron density maps are contoured at 0.8σ (grey) level. At 5σ (red) no peaks are found in this region.