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Crystal Structures of Cisplatin Bound to a Human Copper Chaperone

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Cisplatin (*cis*-diamminedichloroplatinum(II)) is a highly effective anticancer therapeutic in wide clinical use. The use of cisplatin and related drugs is limited by intrinsic and acquired cellular resistance, however.[1] Although many different systems are implicated in cisplatin trafficking within the cell,[1] mounting evidence suggests a linkage between cisplatin resistance and the human copper homeostatic proteins Atox1 and ATP7A or ATP7B (Menkes and Wilson disease proteins).[2] The copper chaperone Atox1 binds Cu(I) with a conserved CXXC motif and delivers it to the N-terminal metal binding domains (MBDs) of ATP7B and ATP7A, which are Cu(I) specific P_{1B}-type ATPases.[3] Each human Cu(I) ATPase has six MBDs, which also bind Cu(I) with CXXC motifs and resemble Atox1 in overall structure.[4] The relationship between cisplatin and Cu(I) transporters is underscored by recent observations that cisplatin therapy combined with siRNA-mediated ATP7B silencing significantly reduces tumor growth in a mouse ovarian cancer model.[5] This striking effect is not understood on the molecular level, but recent *in vitro* data suggest that there is a direct interaction between cisplatin and the ATP7B MBDs.[6] As a model for cisplatin interaction with these domains, we have investigated cisplatin binding to the human copper chaperone Atox1. The crystal structures reported here establish that cisplatin binds specifically to the Cu(I) binding site in Atox1.

The structure of a stoichiometric cisplatin-Atox1 adduct (Pt-Atox1) was determined to 1.6 Å resolution (Table S1, PDB accession code 3IWL). Inductively coupled plasma atomic emission spectroscopy (ICP-AES) analysis shows 1.0 ± 0.03 Pt ions per Atox1 (Supporting Information). There is one monomer in the asymmetric unit, in contrast to previous metal-bridged dimeric structures solved in the presence of Cu(I), Cd(II), and Hg(II).[7] The overall fold is similar to that of Atox1 in the dimeric structures (Figure S1) with an average root-mean-square deviation (rmsd) of 0.391 Å for 65 Cα coordinates. The Pt(II) ion is coordinated by Cys12 and Cys15 from the CXXC motif with Cys(S)−Pt distances of 2.30 and 2.35 Å, respectively (Figure 1B and Figure S1). These distances are similar to those reported for cisplatin bound to metallothionein.[8] Broad spectral features indicative of Pt(II)−S ligand-to-metal charge transfer (LMCT) transitions are consistent with cysteine ligation (Figure S2).[9] The orientations of the coordinating cysteines are quite similar to those observed in the other metal-loaded Atox1 structures, with Cys15 occupying the identical position and the sulfur and amide nitrogen atoms of Cys12 deviating slightly (Figure 1B). The geometry is square planar with the two cysteine ligands oriented *trans* to one another. The remaining ligands are provided by the backbone amide nitrogen of Cys12 at 2.27 Å and an exogenous donor best modeled as a (2-carboxyethyl)phosphine (TCEP) molecule with a TCEP(P)−Pt distance of 2.48 Å. This distance is long compared to small molecule compounds in the Cambridge Structural Database but may be due to steric limitations of the protein binding site. The TCEP is likely adopting several conformations as well. The TCEP molecule forms a hydrogen bond with the side chain of Lys60, a conserved residue proposed to play a key role in metal transfer from Atox1 to target domains.[7] The crystallographic model is consistent with electrospray ionization mass spectrometry (ESI-MS) data showing a species corresponding to Atox1 plus a single Pt(II) ion and a TCEP molecule (Figure S3). Thus, the chloride and ammine ligands have been displaced from cisplatin.

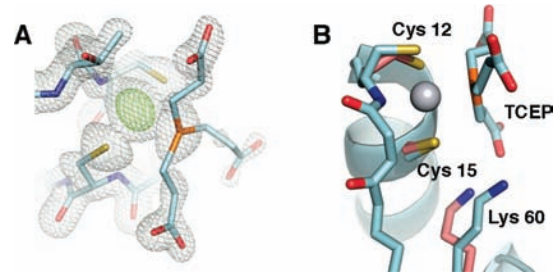

Figure 1. Crystal structure of cisplatin bound to an Atox1 monomer (Pt-Atox1). (A) $2F_o-F_c$ electron density map (gray, 1.35σ) of Pt-Atox1 metal center with anomalous difference Fourier density showing the Pt(II) ion superimposed (green, 9σ). (B) Superposition of the metal binding sites in Pt-Atox1 (light blue) and Cu(I)-Atox1 (salmon).

The structure of a dimeric cisplatin adduct Pt-$(Atox1)_2$ was determined to 2.14 Å resolution (Table S1, PDB accession code 3IWX). This complex contains 0.63 ± 0.09 Pt ions per Atox1. The two molecules in the asymmetric unit are linked by the Pt(II) ion (Figure S4) as was observed for the previous metal-loaded Atox1 structures, also solved in space group $P6_5$.[7] The overall fold is similar to that of Pt-Atox1 with an average rmsd of 0.285 Å for 65 Cα coordinates. In the Pt-$(Atox1)_2$ structure, the two Cys15 residues are coordinated to the Pt(II) ion with Cys(S)−Pt distances of 2.31 and 2.10 Å (Figure 2). Interestingly, the two Cys12 residues are too far away for direct coordination with Cys(S)−Pt distances of 2.48 and 2.46 Å. The two additional coordination sites are instead occupied by the ammine groups, which were modeled into two persistent positive peaks in the difference Fourier map, resulting in square planar geometry around the Pt(II) ion (Figure 2A, Figure S5). The positions of the two noncoordinating cysteine residues shift minimally to accommodate the ammine ligands, probably because each Cys12 side chain maintains a hydrogen bond with the Thr11 side chain on the opposite monomer, as observed previously.[7] The orientations of the Cys12 side chains relative to the Pt(II) atom may constitute secondary interactions with the nonbonding face of the cisplatin adduct.[10] As a result, the Pt(II)−N distances of 1.99 and 1.80 Å, as modeled, are slightly shorter than those observed in other cisplatin crystal structures.[11] The adduct is further stabilized by hydrogen bonding between the ammine groups and the side chains of Thr11 and the noncoordinating Cys12. The retention of the ammine ligands was verified by ESI-MS analysis (Figure S3).

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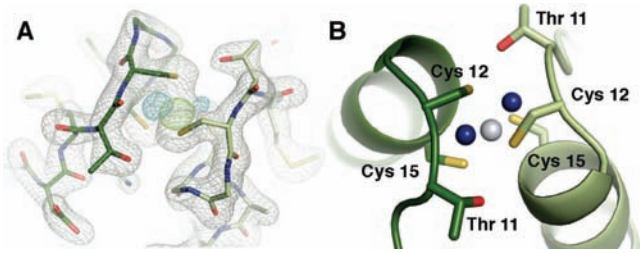

Figure 2. Crystal structure of cisplatin bound to an Atox1 dimer. (A) $2F_o-F_c$ electron density map (gray, 1.35σ) of the Pt-$(Atox1)_2$ metal center with anomalous difference Fourier density (green, 12σ) showing the Pt(II) ion and F_o-F_c difference density (blue, 4σ) showing the ammine ligands superimposed. The two monomers are shown in dark green and light green. (B) Close-up view of the metal center. The Pt(II) ion is shown as a gray sphere, and the ammine nitrogens are shown as blue spheres.

These two structures show definitively that cisplatin binds to the CXXC motif in Atox1. Interaction of cisplatin with these sites is not surprising given the known affinity of Pt(II) for highly polarizable, oxidizing ligands.[12] Although both structures reveal a square planar Pt(II) complex, the differences between them illustrate that cisplatin interaction with a biological CXXC metal binding site can be quite variable. In the Pt-Atox1 structure, cisplatin loses all of its original ligands, consistent with the strong nucleophilicity of S/P donors in Pt(II) ligand exchange reactions.[12] The presence of a bulky exogenous ligand may dictate the chemical composition of the Pt(II) binding site while preventing interaction with a second protein molecule. Coordination by exogenous ligands in general could thus affect the equilibrium between Pt-Atox1 and Pt-$(Atox1)_2$. In the Pt-$(Atox1)_2$ structure, the chloride ligands are replaced by the sulfur atoms from the two Cys15 side chains whereas the ammine ligands are retained. Given that thiolates are predicted to be stronger nucleophiles for Pt(II) and have a greater *trans* effect than the ammine ligands,[12] the failure of the Cys12 side chains to displace the ammines is surprising. The structural and dynamic requirements of the protein environment may prevent dissociation of the original ammine ligands. If loss of the ammine ligands is very slow, the dimer structure may represent an intermediate in the cisplatin-Atox1 reaction in which the final product is a Pt(II) ion coordinated by four Cys-S ligands.

These findings are potentially relevant to the connection between cisplatin resistance and copper homeostasis since cisplatin likely interacts with the ATP7B MBDs in a similar fashion. Moreover, Atox1 has been found in the nucleus and is proposed to act as a transcription factor.[13] Cisplatin binding to the MBD CXXC motifs would be consistent with data demonstrating that mutation of the cysteine residues abolishes the ability of the first four ATP7B MBDs to prevent cisplatin-induced filamentation in *E. coli*.[6] The existence of additional cisplatin binding sites within ATP7B is possible given that cisplatin stimulated phosphorylation of ATP7B is still observed upon mutation of the first five CXXC motifs.[6] The structures also support the idea that cisplatin interaction with Cu(I) binding motifs leads to unfavorable therapeutic outcomes not only due to unproductive cisplatin trafficking but also perhaps as a result of aberrant Cu(I) transport in cisplatin resistant tumors.[5] The presence of TCEP in the Pt-Atox1 structure suggests that biologically relevant exogenous ligands such as glutathione could play a role in cisplatin−protein interactions *in vivo*, though the different affinities of these reducing agents for Pt(II) must be considered.[14] Finally, the observation of a Pt(II) bridged dimer is significant since metal transfer between Atox1 and its target MBDs is believed to involve complexation and ligand exchange reactions between structurally homologous domains.[4] Cisplatin trafficking inside the cell may be accomplished in part by similar mechanisms.

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Supporting Information Available: Supporting methods, optical spectra, ESI-MS data, crystallographic data table, and stereo images (complete ref 5, Table S1, Figures S1−S5) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Wang, D. L.; Lippard, S. J. *Nat. Rev. Drug Discovery* **2005**, *4*, 307–320.
(2) (a) Kalayda, G. V.; Wagner, C. H.; Buss, I.; Reedijk, J.; Jaehde, U. *BMC Cancer* **2008**, *8*, 175. (b) Katano, K.; Kondo, A.; Safaei, R.; Holzer, A.; Samimi, G.; Mishima, M.; Kuo, Y. M.; Rochdi, M.; Howell, S. B. *Cancer Res.* **2002**, *62*, 6559–6565. (c) Nakagawa, T.; Inoue, Y.; Kodama, H.; Yamazaki, H.; Kawai, K.; Suemizu, H.; Masuda, R.; Iwazaki, M.; Yamada, S.; Ueyama, Y.; Inoue, H.; Nakamura, M. *Oncol. Rep.* **2008**, *20*, 265–270. (d) Safaei, R.; Maktabi, M. H.; Blair, B. G.; Larson, C. A.; Howell, S. B. *J. Inorg. Biochem.* **2009**, *103*, 333–341.
(3) Lutsenko, S.; Gupta, A.; Burkhead, J. L.; Zuzel, V. *Arch. Biochem. Biophys.* **2008**, *476*, 22–32.
(4) Boal, A. K.; Rosenzweig, A. C. *Chem. Rev.* **2009**, ASAP DOI: 10.1021/cr900104z.
(5) Mangala, L. S.; et al. *Clin. Cancer Res.* **2009**, *15*, 3770–3780.
(6) (a) Dolgova, N. V.; Olson, D.; Lutsenko, S.; Dmitriev, O. Y. *Biochem. J.* **2009**, *419*, 51–56. (b) Leonhardt, K.; Gebhardt, R.; Mossner, J.; Lutsenko, S.; Huster, D. *J. Biol. Chem.* **2009**, *284*, 7793–7802.
(7) Wernimont, A. K.; Huffman, D. L.; Lamb, A. L.; O'Halloran, T. V.; Rosenzweig, A. C. *Nat. Struct. Biol.* **2000**, *7*, 766–771.
(8) Pattanaik, A.; Bachowski, G.; Laib, J.; Lemkuil, D.; Shaw, C. F.; Petering, D. H.; Hitchcock, A.; Saryan, L. *J. Biol. Chem.* **1992**, *267*, 16121–16128.
(9) Knipp, M.; Karotki, A. V.; Chesnov, S.; Natile, G.; Sadler, P. J.; Brabec, V.; Vasak, M. *J. Med. Chem.* **2007**, *50*, 4075–4086.
(10) Nakamoto, K.; McCarthy, P. J.; Fujita, J.; Condrate, R. A.; Behnke, G. T. *Inorg. Chem.* **1965**, *4*, 36.
(11) (a) Milburn, G. H. W.; Truter, M. R. *J. Chem. Soc. A* **1966**, 1609–1616. (b) Sherman, S. E.; Gibson, D.; Wang, A. H. J.; Lippard, S. J. *Science* **1985**, *230*, 412–417.
(12) (a) Appleton, T. G.; Connor, J. W.; Hall, J. R. *Inorg. Chem.* **1988**, *27*, 130–137. (b) Lempers, E. L. M.; Reedijk, J. *Adv. Inorg. Chem.* **1991**, *37*, 175–217.
(13) Itoh, S.; Kim, H. W.; Nakagawa, O.; Ozumi, K.; Lessner, S. M.; Aoki, H.; Akram, K.; McKinney, R. D.; Ushio-Fukai, M.; Fukai, T. *J. Biol. Chem.* **2008**, *283*, 9157–9167.
(14) Ralle, M.; Lutsenko, S.; Blackburn, N. J. *J. Biol. Chem.* **2003**, *278*, 23163–23170.

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