Inorganic Chemistry

Communication

pubs.acs.org/IC

Cisplatin Binding to Proteins: Molecular Structure of the Ribonuclease A Adduct

Luigi Messori[†] and Antonello Merlino*[,‡]

[†]Department of Chemistry, University of Florence, Via della Lastruccia 3, 50019 Sesto Fiorentino, Italy

[‡]Department of Chemical Sciences, University of Naples Federico II, Complesso Universitario di Monte Sant' Angelo, Via Cintia, I-80126 Napoli, Italy

Supporting Information

ABSTRACT: The crystal structure of the main adduct formed in the reaction between cisplatin and bovine pancreatic ribonuclease is reported here. Notably, in both of the protein molecules present in the asymmetric unit, platinum(II) binding takes place exclusively at the level of Met29. In one of the two molecules, the Gln28 side chain completes the platinum coordination sphere, anchoring the cisplatin fragment to the protein in a bidentate fashion. These results contain interesting implications for understanding the biological chemistry of this important drug.

Cisplatin [*cis*-diamminedichloroplatinum(II)] is one of the most potent anticancer drugs in current clinical use for the treatment of several types of solid tumors.[1] The cytotoxic effect of cisplatin is thought to arise primarily from the formation of platinum−DNA adducts involving two adjacent nucleobases;[2] this kind of DNA damage eventually triggers apoptosis in cancer cells and produces the therapeutic effects.[2,3] However, DNA is not the only biological target for cisplatin. The effectiveness of cisplatin is also linked to resistance mechanisms, including reduced cell uptake and rapid detoxification, in which a number of extra- and intracellular proteins could be involved.[4]

The interaction between cisplatin and proteins is underscored by a number of biochemical and biophysical studies that have characterized the formation of complexes between this important molecule and hen egg white lysozyme (HEWL),[5] transferrin,[6] the copper chaperone Atox-1,[7] hemoglobin,[8] cytochrome *c*,[9] ubiquitin,[10] DNA polymerase,[11] metallothionein,[12] and bovine erythrocyte copper, zinc superoxide dismutase (beSOD).[13]

From the structural point of view, the first crystallographic model of a cisplatin−protein adduct, i.e., a complex with beSOD, was reported in 2006 by Calderone et al.[13a] This structure documented unambiguously selective platination of the His19 side chain in the two subunits of the enzyme. Later on, His platination has been observed in the cisplatin complex with HEWL.[5] In this case, cisplatin binds the protein by coordinating the ND1 atom of the imidazole ring of His15[5a] or both the ND1 and NE2 atoms of the same His,[5b,c] after release of one chlorine ligand. Similar results were obtained with cisplatin derivatives.[14]

More recently, the crystal structures of the cisplatin−Atox1 monomeric and dimeric adducts (Pt-Atox1) have been solved.[7a] In these structures, a platinum(II) ion is coordinated by Cys12 and Cys15 from the copper(I) binding CXXC motif.[15] Thus, up to now, only cisplatin binding sites containing His residues or previously recognized metal binding sites have been described at atomic details. However, it is known that HEWL His15 is a reactive residue[5,14,16] and that the accessibility of Cys6 and Met115, the only sulfur-donor-containing residues in beSOD, is quite limited.[13a] Furthermore, it has been shown that, in other proteins, methionines are (alternative) reactive sites to which cisplatin can efficiently coordinate.[17] The lack of structural information on cisplatin binding sites distinct from His (or from a CXXC metal binding motif) led us to perform new crystallographic investigations on additional cisplatin−protein adducts to give a detailed structural description of new types of metal−protein interactions.

Here we report the crystal structure of the adduct formed upon reacting cisplatin with bovine pancreatic ribonuclease (RNase A). RNase A has often been used as a model for the interaction of metallodrugs with enzymes.[18] The reaction of cisplatin with RNase A had already been described in the literature. Early experiments performed by Neault et al. in 1999 suggested the direct binding of platinum(II) to sulfur donor atoms and indirect interactions with the polypeptide chain.[19]

Crystals of the RNase A−cisplatin adduct have been obtained by soaking experiments, where pregrown monoclinic crystals were incubated for 3 h with an excess of the platinum drug (at a protein-to-platinum ratio of 1:10). This procedure has already been used to obtain productive crystals of the complexes between RNase A and many other ligands.[20] X-ray diffraction data have been collected on these crystals at 1.85 Å resolution. Details of crystallization, data collection, and structure refinement are given in the Supporting Information (SI). The model of the cisplatin−RNase A adduct (Figure 1), which presents two molecules in the asymmetric unit and consists of 2059 non-hydrogen atoms, refines to R_{factor} = 18.9 (R_{free} = 24.7). The electron density maps are well-defined, with the exception of residues 16−22 of the two chains. The overall structure of the protein in the adduct is very similar to that of the ligand-free enzyme (PDB code 1JVT):[20b] the root-mean-square deviation in positions of CA atoms are in the range 0.40−0.67 Å. In other words, the binding of cisplatin does not elicit any major structural change in the protein conformation.

Although RNase A possesses four His residues, two of which are metal-reactive—in fact they are located in the enzyme active site and were already shown to bind metallodrugs[18]—RNase A platination in both molecules present in the asymmetric unit

Received: February 20, 2014
Published: April 3, 2014

   dx.doi.org/10.1021/ic500360f | *Inorg. Chem.* 2014, 53, 3929−3931

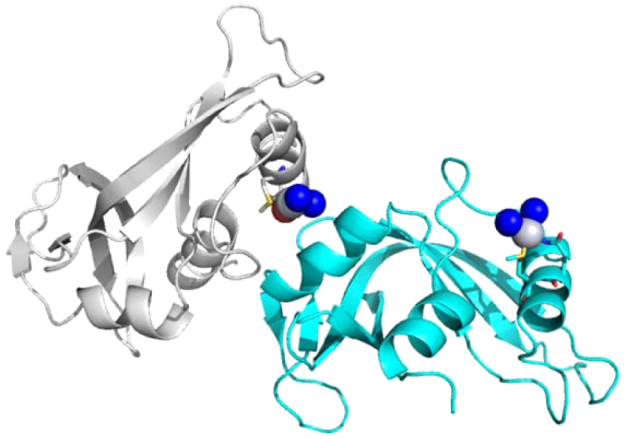

Figure 1. Ribbon representation of the asymmetric unit of the RNase A−cisplatin structure. Molecules A and B are colored in gray and cyan, respectively. The side chain of Met29 of the two molecules in the asymmetric unit and the side chain of Gln28 of molecule B are shown along with platinum centers. The structure has been deposited in the Protein Data Bank under the accession code 4OT4.

(molecules A and B hereafter) occurs nearly exclusively at the SD atom of side chain of Met29. The local environment of the protein-bound platinum(II) center was identified by the observation of a very clear electron density map (Figure 2).

The first platinum(II) binding site is in a cavity formed between molecules A and B (Figure 1). A platinum(II) ion is coordinated to the SD atom of Met29 and to other three atoms, which are hydrogen-bonded to the NE atom of Arg33, to the OG atom of Ser32, and to a water molecule, which, in turn, is hydrogen-bonded to the OD1 atom of Asp53 of molecule B (Figures 2A and S1 in the SI). The lack of an anomalous signal attributable to chlorine ions suggests that these ligands are released from the platinum ion. This is in line with hydrolysis studies performed at acidic pH and at low chlorine concentration.[21] Thus, it is likely that a water molecule and two ammine groups complete the coordination sphere of platinum, although because of the strong trans-labilizing effect of sulfur, we cannot exclude the loss of one ammine group in favor of a water molecule. Inspection of the residual electron density map of this region shows the presence of an additional peak close to the SD atom, which is attributed to an alternative, low-occupancy position of the platinum ion bound to methionine. Because of the low occupancy and the possible conformational disorder of this site, the coordination sphere of the alternative platinum is not clearly defined, and just one ligand (NH_3) beyond the SD atom of Met29 has been modeled (Figures 2A and S1B in the SI). This ligand is taken in its place by the hydrogen bond that it forms with the OG atom of Ser59 of molecule B.

In molecule B, the platinum(II) ion coordinates to the SD atom of Met29, to the NE (or OD) atom of the side chain of Gln28, and to two light atoms, interpreted as ammine ligands, in a classical square-planar geometry (Figure 2B). It is not possible to distinguish between the NE or OD atom of the Gln side chain. However, following previous indications,[22] we positioned the NE atom close to the platinum ion. In this site, the ammine groups are hydrogen-bonded to two water molecules and to the OD2 atom of Asp14. The refinement of *B* factors of platinum ligands and the inspection of residual $F_o - F_c$ electron density maps suggest that the platinum ions have high occupancy, 0.75 and

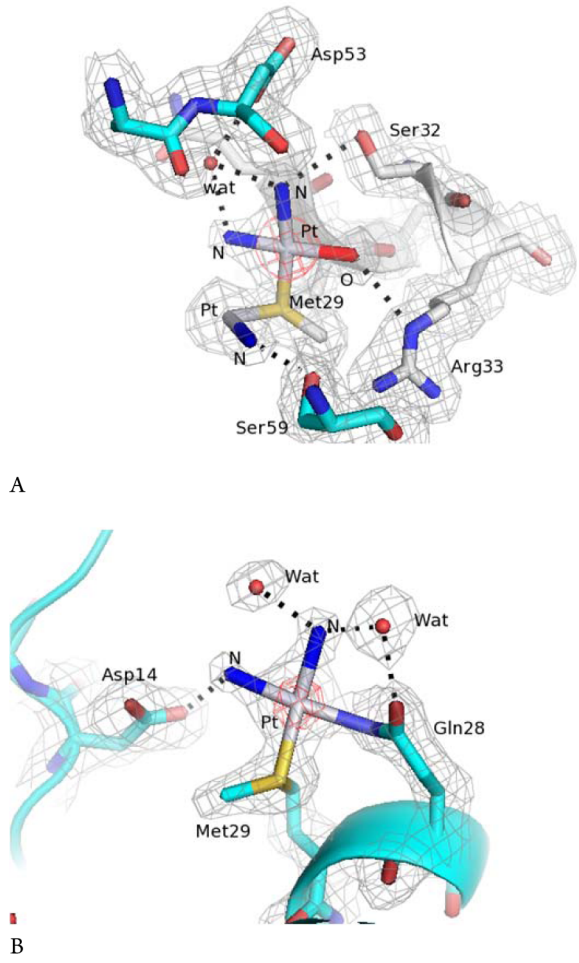

Figure 2. Details of the binding sites of platinum(II) in the RNase A−cisplatin structure. A platinum ion is bound to Met29 in molecule A (panel A) and to Met29 and Gln28 in molecule B (panel B). In molecule A, two alternative binding modes of the platinum ion have been modeled (see also Figure S1 in the SI). $2F_o - F_c$ electron density maps are contoured at the 0.8σ (gray) and 8.0σ (red) levels.

0.25 for the two alternative conformations close to Met29 of molecule A and 0.55 in molecule B.

Altogether these data support a clear tendency of platinum for the binding of accessible Met29; this is in line with the established thermodynamic preference of the "soft" platinum(II) ion for sulfur donors compared to nitrogen donors.[22,23]

In conclusion, we have reported here the X-ray structure of the RNase A−cisplatin adduct. The present structure shows the following:

(a) Cisplatin binds RNase A mainly through the Met29 residue. To the best of our knowledge, this is the first X-ray structural characterization of a cisplatin adduct with a methionine residue in a protein. This is a valuable result because platinum binding to sulfur-donor ligands has often been experimentally observed and is expected to play a relevant role in the cisplatin metabolism.[21,22] In this respect, it should also be recalled that, up to now, the possibility that sulfur-donor binding of platinum drugs could be an intermediate in platinum transport to nuclear DNA has not been ruled out.[23−25]

(b) Different binding modes are observed for cisplatin toward RNase A. Indeed, the platinum center is anchored to the protein either in a monodentate (i.e., like in molecule A) or in a bidentate (i.e., like in molecule B) fashion. This might result in different

dx.doi.org/10.1021/ic500360f | *Inorg. Chem.* 2014, 53, 3929−3931

patterns of protein platination. Interestingly, in molecule A, two alternative positions of the platinum ion can be modeled. This indicates that alternative binding modes are possible within the same metal binding site. The existence of monodentate and bidentate binding modes of cisplatin to proteins has been suggested by other authors on the basis of mass spectrometry studies carried out on the adduct formed between cisplatin and insulin.[26]

(c) NH_3 groups have an important role in protein−cisplatin recognition because they are responsible for important hydrogen bonds. These groups can drive the drug approach toward the biological target,[27] stabilizing the final structure. This is in line with the expected donor properties of NH.

(d) Because His residues in RNase A are well accessible and have already been shown to be involved in the formation of an adduct with metallodrugs,[18] the selective binding of cisplatin to Met residues suggests a strong preference of this molecule for available sulfur donors. This result is in agreement with the established thermodynamic preference of platinum(II) ions for sulfur donors over nitrogen donors.[23]

■ ASSOCIATED CONTENT

Supporting Information

Crystallization, X-ray data collection, and structure solution and refinement. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: antonello.merlino@unina.it.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We gratefully acknowledge Beneficentia Stiftung and COST Action CM1105 for financial support. The authors thank G. Sorrentino and M. Amendola for technical assistance.

■ REFERENCES

(1) (a) Wang, D.; Lippard, S. J. *Nat. Rev. Drug Discovery* **2005**, *4*, 307−320. (b) Rabik, C. A.; Dolan, M. E. *Cancer Treat. Rev.* **2007**, *33*, 9−23. (c) Ozols, R. F. *Cancer Treat. Rev.* **1991**, *18*, 77−83.

(2) (a) Brabec, V. *Prog. Nucleic Acid Res. Mol. Biol.* **2002**, *71*, 1−68. (b) Reedijk, J. *Pure Appl. Chem.* **1987**, *2*, 181−192.

(3) (a) Pil, P.; Lippard, S. J. In *Encyclopedia of Cancer*; Bertino, J. R., Ed.; Academic Press: San Diego, CA, 1997; Vol. *1*, pp 392−410. (b) Casini, A.; Gabbiani, C.; Mastrobuoni, G.; Messori, L.; Moneti, G.; Pieraccini, G. *Chem. Med. Chem.* **2006**, *1*, 413−417.

(4) Pinato, O.; Musetti, C.; Sissi, C. *Metallomics* **2014**, *6*, 380−395.

(5) (a) Casini, A.; Mastrobuoni, G.; Temperini, C.; Gabbiani, C.; Francese, S.; Moneti, G.; Supuran, C. T.; Scozzafava, A.; Messori, L. *Chem. Commun.* **2007**, *2*, 156−158. (b) Tanley, S. W. M.; Schreurs, A. M. M.; Kroon-Batenburg, L. M. J.; Meredith, J.; Prendergast, R.; Walsh, D.; Bryant, P.; Levy, C.; Helliwell, J. R. *Acta Crystallogr.* **2012**, *D68*, 601−612. (c) Helliwell, J. R.; Tanley, S. W. M. *Acta Crystallogr.* **2013**, *D69*, 121−125. (d) Tanley, S. W. M.; Schreurs, A. M. M.; Kroon-Batenburg, L. M. J.; Helliwell, J. R. *Acta Crystallogr.* **2012**, *F68*, 1300−1306.

(6) Allardyce, C. S.; Dyson, P. J.; Coffrey, J.; Johnson, N. *Rapid Commun. Mass Spectrom.* **2002**, *26*, 933−935.

(7) (a) Boal, A. K.; Rosenzweig, A. C. *J. Am. Chem. Soc.* **2009**, *131*, 14196−14197. (b) Arnesano, F.; Banci, L.; Bertini, I.; Felli, I. C.; Losacco, M.; Natile, G. *J. Am. Chem. Soc.* **2011**, *133*, 18361. (c) Palm, M. E.; Weise, C. F.; Lundin, C.; Wingsle, G.; Nygren, Y.; Björn, E.; Naredi, P.; Wolf-Watz, M.; Wittung-Stafshede, P. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 6951−6.

(8) Mandal, R.; Kalke, R.; Xing-Fang, L. *Rapid Commun. Mass Spectrom.* **2002**, *17*, 2748−935.

(9) (a) Zhao, T.; King, F. L. *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 1141−1147. (b) Casini, A.; Gabbiani, C.; Mastrobuoni, G.; Messori, L.; Moneti, G.; Pieraccini, G. *Chem. Med. Chem.* **2006**, *1*, 413−417.

(10) (a) Gibson, D.; Costello, C. E. *Eur. J. Mass Spectrom.* **1999**, *5*, 501−510. (b) Hartinger, C. G.; Ang, W. H.; Casini, A.; Messori, L.; Keppler, B. K.; Dyson, P. J. *J. Anal. At. Spectrom.* **2007**, *22*, 960−967.

(11) Damsma, D. E.; Alt, A.; Brueckner, F.; Carrel, T.; Cramer, P. *Nat. Struct. Mol. Biol.* **2007**, *14*, 1127−1133.

(12) (a) Casini, A.; Karotki, A.; Gabbiani, C.; Rugi, F.; Vasak, M.; Messori, L.; Dyson, P. J. *Metallomics* **2009**, *1*, 434−441. (b) Zhang, G.; Hu, W.; Du, Z.; Lv, S.; Zheng, W.; Luo, Q.; Li, X.; Wu, K.; Han, Y.; Wang, F. *Int. J. Mass Spectrom.* **2011**, *307*, 79−84. (c) Esteban-Fernandez, D.; Canas, B.; Pizarro, I.; Palacios, M. A.; Gomez-Gomez, M. M. *J. Anal. At. Spectrom.* **2007**, *22*, 1113−21.

(13) (a) Calderone, V.; Casini, A.; Mangani, S.; Messori, L.; Orioli, P. L. *Angew. Chem.* **2006**, *45*, 1267−9. (b) Banci, L.; Bertini, I.; Blazevits, O.; Calderone, V.; Cantini, F.; Mao, J.; Trapananti, A.; Vieru, M.; Amori, I.; Cozzolino, M.; Carrì, M. T. *J. Am. Chem. Soc.* **2012**, *134*, 7009−14.

(14) Messori, L.; Marzo, T.; Gabbiani, C.; Alvarez-Valdes, A.; Quiroga, A.; Merlino, A. *Inorg. Chem.* **2013**, *52*, 13827−9.

(15) Chivers, P. T.; Laboissiere, M. C. A.; Raines, R. T. *EMBO J.* **1996**, *15*, 2659−67.

(16) (a) Messori, L.; Scaletti, F.; Massai, L.; Cinellu, M. A.; Gabbiani, C.; Vergara, A.; Merlino, A. *Chem. Commun.* **2013**, *49*, 10100−2. (b) Vergara, A.; D'Errico, G.; Montesarchio, D.; Mangiapia, G.; Paduano, L.; Merlino, A. *Inorg. Chem.* **2013**, *52*, 4157−9. (c) Santos-Silva, T.; Mukhopadhyay, A.; Seixas, J. D.; Bernardes, G. J.; Romao, C. C.; Romao, M. J. *J. Am. Chem. Soc.* **2011**, *133*, 1192.

(17) (a) Sze, C. M.; Khairallah, G. N.; Xiao, Z.; Donnelly, P. S.; O'Hair, R. A. J.; Wedd, A. G. *J. Biol. Inorg. Chem.* **2009**, *14*, 163−165. (b) Zhao, T.; King, F. L. *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 1141−1147. (c) Hahn, M.; Kleine, M.; Sheldrick, W. S. *J. Biol. Inorg. Chem.* **2001**, *6*, 556−566. (d) Casini, A.; Guerri, A.; Gabbiani, C.; Messori, L. *J. Inorg. Biochem.* **2008**, *102*, 995−1006.

(18) (a) Messori, L.; Scaletti, F.; Massai, L.; Cinellu, M. A.; Russo Krauss, I.; di Martino, G.; Vergara, A.; Paduano, L.; Merlino, A. *Metallomics* **2014**, *6*, 233−6. (b) Vergara, A.; Montesarchio, D.; Russo Krauss, I.; Paduano, L.; Merlino, A. *Inorg. Chem.* **2013**, *52*, 10714.

(19) Neault, J. F.; Novetta-Delen, A.; Tajmir-Riahi, H. A. *J. Biomol. Struct. Dyn.* **1999**, *17*, 101−109.

(20) (a) Vitagliano, L.; Merlino, A.; Zagari, A.; Mazzarella, L. *Proteins* **2002**, *46*, 97. (b) Vitagliano, L.; Merlino, A.; Zagari, A.; Mazzarella, L. *Protein Sci.* **2000**, *9*, 1217.

(21) Miller, S. E.; House, D. A. *Inorg. Chim. Acta* **1989**, *161* (1), 131−137.

(22) Rothenburger, C.; Galanski, M.; Arion, V. B.; Gorls, H.; Weigand, W.; Keppler, B. *Eur. J. Inorg. Chem.* **2006**, 3746−3752.

(23) (a) Reedijk, J. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 3611−3616. (b) Quintal, S. M. O.; Qu, Y.; Quiroga, A. G.; Moniodis, J.; Nogueira, H. I. S.; Farrell, N. *Inorg. Chem.* **2005**, *44*, 5247−5253.

(24) (a) Lempers, E. L. M.; Inagaki, K.; Reedijk, J. *Inorg. Chim. Acta* **1988**, *152*, 201. (b) Lempers, E. L. M.; Reedijk, J. *Inorg. Chem.* **1990**, *29*, 217. (c) van Boom, S. S. G. E.; Reedijk, J. *J. Chem. Soc., Chem. Commun.* **1993**, 1397.

(25) Reedijk, J. *Platinum Met. Rev.* **2008**, *52*, 2−11.

(26) Moreno-Gordaliza, E.; Cañas, B.; Palacios, M. A.; Gómez-Gomez, M. *Anal. Chem.* **2009**, *81*, 3507−3516.

(27) Messori, L.; Casini, A.; Gabbiani, C.; Michelucci, E.; Cubo, L.; Rios-Luci, C.; Padron, J. M.; Navarro-Ranninger, C.; Quiroga, A. G. *ACS Med. Chem. Lett.* **2010**, *1* (8), 381−385.