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Communication

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Peculiar Features in the Crystal Structure of the Adduct Formed between *cis*-$PtI_2(NH_3)_2$ and Hen Egg White Lysozyme

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Supporting Information

ABSTRACT: The reactivity of *cis*-diammineiodidoplatinum(II), *cis*-$PtI_2(NH_3)_2$, the iodo analogue of cisplatin, with hen egg white lysozyme (HEWL) was investigated by electrospray ionization mass spectrometry and X-ray crystallography. Interestingly, the study compound forms a stable 1:1 protein adduct for which the crystal structure was solved at 1.99 Å resolution. In this adduct, the Pt^{II} center, upon release of one ammonia ligand, selectively coordinates to the imidazole of His15. Both iodide ligands remain bound to platinum, with this being a highly peculiar and unexpected feature. Notably, two equivalent modes of Pt^{II} binding are possible that differ only in the location of I atoms with respect to ND1 of His15. The structure of the adduct was compared with that of HEWL−cisplatin, previously described; differences are stressed and their important mechanistic implications discussed.

Cisplatin [*cis*-diamminedichloroplatinum(II)] is a leading anticancer drug in widespread clinical use for the treatment of several types of malignancies.[1] Since the discovery of cisplatin, research has been largely focused on the characterization of platinum−DNA adducts according to the concept that DNA is its primary biological target: these studies indicated the N7 atom of guanine as the preferential platination site.[2] Interestingly, bifunctional adducts involving two adjacent nucleobases are predominantly formed by cisplatin[3] that are believed to represent the major DNA lesions, ultimately leading to apoptotic cancer cell death. Yet, nucleobases are not the only biological targets for platinum drugs. Cys, Met, and His residues in proteins are alternative reactive sites to which platinum can efficiently coordinate.[4] The interaction between cisplatin and proteins is underscored by a number of crystallographic and electrospray ionization mass spectrometry (ESI MS) studies that include determination of the complexes between this 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 superoxide dismutase.[13]

In 2007, Casini et al. determined the X-ray structure of the HEWL−cisplatin derivative obtained by soaking experiments on pregrown HEWL crystals, at 1.9 Å resolution. The structure revealed selective platination of the only HEWL histidine residue, i.e., His15, at the imidazole ND1 atom (at the so-called right-handed site).[5a] Pt^{II} has an occupancy equal to 0.3 and is also bound to the N atoms of two ammonia molecules. The fourth ligand was not detected (Figure S1a in the Supporting Information, SI). This result was in substantial agreement with the ESI MS spectra of HEWL−cisplatin derivatives, which showed two peaks of similar intensity at 14569 and 14605 Da, formally corresponding to either a $[Pt(NH_3)_2Cl]^+$ fragment or intact cisplatin bound to HEWL. A similar situation was described by Dyson and co-workers in the case of the cisplatin−transferrin system and interpreted in terms of a two-step cisplatin-to-protein binding process.[6]

More recently, cocrystallization experiments conducted by Helliwell and co-workers revealed that cisplatin binds HEWL by coordinating either the ND1 or the NE2 atom (i.e., it can bind at the so-called "right-handed" and "left-handed" sites) of the imidazole ring of His15,[5b,c] after release of one chlorine ligand (Figure S1b in the SI).

Although cisplatin remains one of the most effective chemotherapeutic agents for cancer treatment (in particular for testicular and ovarian cancers), it shows some major drawbacks, such as cumulative toxicities of nephrotoxicity and ototoxicity, inherent or treatment-induced resistance, and relevant patient toxicity. This has provided the motivation for developing novel metal complexes (either platinum or nonplatinum) as prospective anticancer agents with a different mechanism of action. Recently, a number of *cis*- and *trans*-diammineiodidoplatinum compounds [such as *cis*-$PtI_2(ammine)_2$,[14] *trans*-$PtI_2(ammine)_2$[15] and *trans*-$PtI_2(ammine)(ammine')_2$[16]] showing unconventional reactivity have been synthesized and their cytotoxicities toward a variety of human tumor cell lines evaluated.

As a model for the interaction of iodido analogues of platinum(II) drugs with proteins[16] and for a direct comparison of the results with cisplatin, the cisplatin analogue *cis*-$PtI_2(NH_3)_2$, previously synthesized (see the SI for details)[17] and charac-

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terized, was chosen to perform a detailed investigation of its reaction with HEWL by X-ray crystallography and ESI MS.

Crystals of the adduct formed between HEWL and *cis*-$PtI_2(NH_3)_2$ were obtained by soaking experiments, in which pregrown HEWL crystals were incubated for 2 months with an excess of the platinum drug. Representative yellow crystals of the resulting adduct are reported in Figure 1.

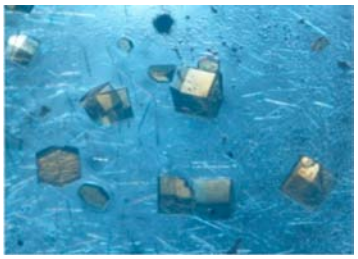

Figure 1. Crystals of the adduct between HEWL and *cis*-$PtI_2(NH_3)_2$.

These crystals were subjected to X-ray diffraction data collection, and the structure of the adduct was solved at 1.99 Å resolution. Details of crystallization, data collection, and structure refinement are given in the SI. The overall structure of the adduct between HEWL and *cis*-$PtI_2(NH_3)_2$ (Figure 2), which refines to R_{factor} = 18.4 (R_{free} = 23.4), is very similar to that of the native protein (PDB code 193L): the root-mean-square deviation in positions of CA atoms is 0.3 Å.

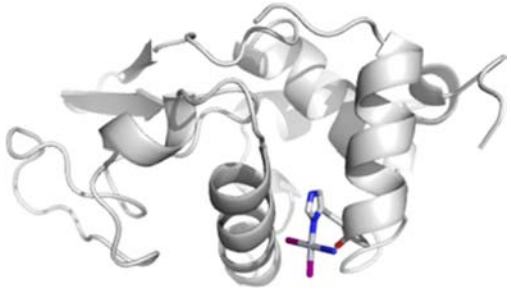

Figure 2. Ribbon representation of the structure of the adduct formed between HEWL and *cis*-$PtI_2(NH_3)_2$. The side chain of His15, which is solvent-accessible, being situated on the protein surface, is shown along with platinum, ammonium, and I ligands. For sake of clarity, just one of the two different modes of binding of *cis*-$PtI_2(NH_3)_2$ to HEWL is shown. The structure has been deposited in the Protein Data Bank under the accession code 4MR1.

In agreement with that found in the case of cisplatin by Casini et al.,[5a] platination occurs at the ND1 atom of the imidazole ring of His15, i.e., at the right-handed site. The local environment of the protein-bound Pt^{II} center was identified by observation of a very clear electron density map (Figure 3). Notably, the Pt^{II} ion is coordinated to the ND1 atom of His15, two I atoms, and a N atom in a classical square-planar geometry. Refinement of the temperature factors of platinum ligands and inspection of the residual F_o-F_c electron density maps indicate that two equivalent modes of platinum binding are present in the crystal structure, which differ only in the location of the I atoms with respect to the ND1 of His15 (Figure 4). The existence of two alternative modes of binding was further supported by inspection of the anomalous scattering map, which confirmed the presence of anomalous signals of I and Pt atoms in the binding moieties (Figure S2).

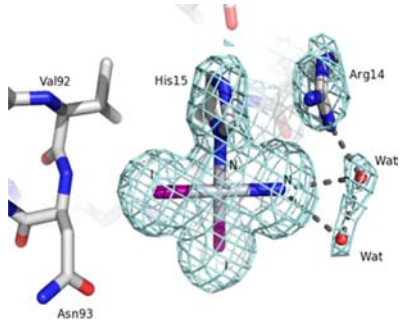

Figure 3. Details of the binding site of Pt^{II} in HEWL–*cis*-$PtI_2(NH_3)$ showing the Pt ion bound to His15. $2F_o-F_c$ electron density maps are contoured at the 1σ level. For the sake of clarity, just one of the two different modes of binding of *cis*-$PtI_2(NH_3)_2$ to HEWL is shown.

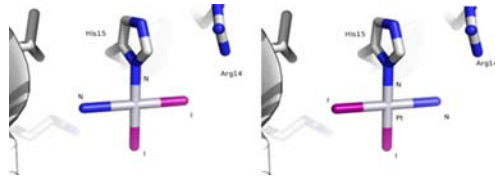

Figure 4. Two modes of binding of *cis*-$PtI_2(NH_3)_2$ to HEWL. I atoms are colored in purple, and N atoms are colored in blue.

In the former mode of binding, the NH_3 group is close to the N and OD1 atoms of Asn93 (distances 3.8 and 4.5 Å, respectively); in the latter, the NH_3 group is solvent-exposed and interacts with two water molecules, which, in turn, are in contact with Arg14, whereas the I atom occupies the position close to Asn93 (Figure 4a,b). Within these assumptions, the His(N)–Pt distance is 2.1 Å, whereas the Pt–I and Pt–N(NH_3) distances are, on average, both close to 2.4 Å.

Remarkably, the present structure shows that the Pt^{II} ion binds the protein with an occupancy value close to 1.0. To the best of our knowledge, this is the highest occupancy ever observed for platinum in adducts between proteins and cisplatin-like drugs. No other significant modifications of the electron density map were observed, ruling out the presence of additional (secondary) binding sites. This finding supports the view that interaction of this platinum drug with HEWL is highly selective for His15.

Independent valuable information on the same system was subsequently gained through application of ESI MS (see the SI for details). ESI MS is indeed a powerful and informative tool to characterize the interactions of metal-based drugs with small model proteins. Precise information may be derived on the stoichiometry and kinetics of binding as well as on the exact nature of the protein-bound metal fragments.[4b,15,18] HEWL dissolved in a suitable buffer (20 mM ammonium acetate buffer, pH 6.8) was treated with a 3:1 excess of a fresh solution of the study compounds, and ESI MS spectra were measured after increasing time intervals. Deconvoluted ESI MS spectra recorded at the indicated time intervals (6, 24, 48, and 72 h) are shown in Figure 5. The progressive formation of adducts is well witnessed by the appearance of a number of peaks of higher molecular mass than the native protein. Interestingly, the dominant peak within those attributed to platinum adducts shows a value of 14769.63 Da, which perfectly matches the mass of an adduct in which a single [PtI_2NH_3] fragment is bound to the protein. This observation offers further and independent support to the above-reported crystallographic results.

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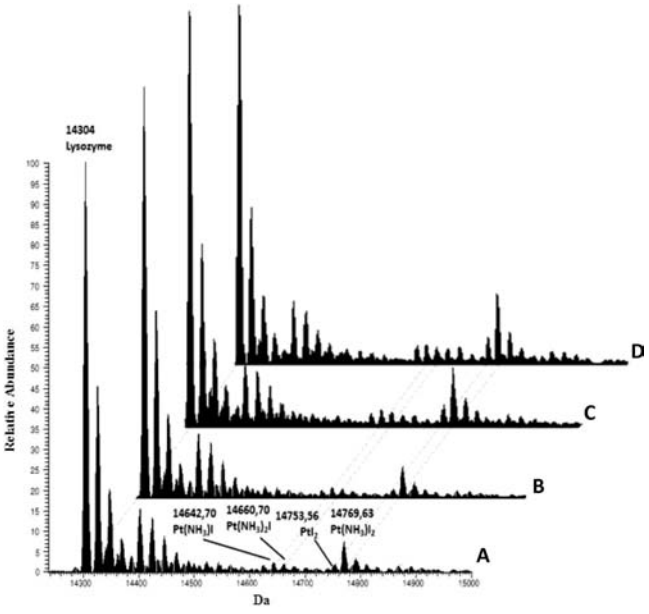

Figure 5. Deconvoluted ESI MS of HEWL treated with 10^{-4} M *cis*-$PtI_2(NH_3)_2$ (metal:protein ratio = 3:1) in a 20 mM ammonium acetate (pH 6.8) buffer recorded after 6 h (A), 24 h (B), 48 h (C), and 72 h (D) of incubation at room temperature.

In conclusion, we have provided here unambiguous structural evidence that the diiodido analogue of cisplatin forms stable protein adducts containing a different type of protein-bound metal fragment. While cisplatin typically affords protein platination through coordination of a $[Pt(NH_3)_2]$ fragment to selected protein side chains, *cis*-$PtI_2(NH_3)_2$ preferentially gives rise to a protein-bound $[PtI_2NH_3]$ fragment with full retention of the two halide ligands. This finding is of potential great interest, implying that the diiodido analogue produces a type of biomolecular metalation remarkably distinct from that inferred by cisplatin. It would be interesting to verify whether this kind of metalation is operative even toward double-helix DNA. This might result in different patterns of DNA platination and, accordingly, in a different pharmacological profile. Thus, the present results highlight that the simple chloride to iodide replacement in cisplatin may have spectacular consequences on the reactivity and biological actions of platinum(II) compounds and may lead to classes of innovative platinum agents not obeying classical Hoeschele's rules.[19]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Materials and analytical techniques, crystallization, X-ray data collection, structure solution, and refinement, scheme of cisplatin binding to HEWL at the right-handed site, and anomalous electron density map contoured at 4.0σ. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Wang, D.; Lippard, S. J. *Nat. Rev. Drug Discovery* **2005**, *4*, 307−320.
(2) (a) Brabec, V. *Prog. Nucleic Acid Res. Mol. Biol.* **2002**, *71*, 1−68. (b) Reedijk, J. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 3611−3616.
(3) Casini, A.; Gabbiani, C.; Mastrobuoni, G.; Messori, L.; Moneti, G.; Pieraccini, G. *ChemMedChem* **2006**, *1*, 413−417.
(4) (a) Hahn, M.; Kleine, M.; Sheldrick, W. S. *J. Biol. Inorg. Chem.* **2001**, *6*, 556−566. (b) Casini, A.; Guerri, A.; Gabbiani, C.; Messori, L. *J. Inorg. Biochem.* **2008**, *102*, 995−1006.
(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.; Coffey, J.; Johnson, N. *Rapid Commun. Mass Spectrom.* **2002**, *16*, 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.
(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. *ChemMedChem* **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**, No. 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*, 1113−1121.
(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*, 7009−7014.
(14) 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**, 381−385.
(15) Messori, L.; Cubo, L.; Gabbiani, C.; Alvarez-Valdés, A.; Michelucci, E.; Pieraccini, G.; Ríos-Luci, C.; León, L. G.; Padrón, J. M.; Navarro-Ranninger, C.; Casini, A.; Quiroga, A. G. *Inorg. Chem.* **2012**, *51*, 1717−1726.
(16) Parro, T.; Medrano, M. A.; Cubo, L.; Muñoz-Galván, S.; Carnero, A.; Navarro-Ranninger, C.; Quiroga, A. G. *J. Inorg. Biochem.* **2013**, *127*, 182−187.
(17) Dhara, S. C. *Indian J. Chem.* **1970**, *8*, 193−4.
(18) Timerbaev, A. R.; Pawlak, K.; Gabbiani, C.; Messori, L. *Trends Anal. Chem.* **2011**, *30*, 1120−1138.
(19) (a) Cleare, M. J.; Hoeschele, J. D. *Bioinorg. Chem.* **1973**, *2*, 187. (b) Cleare, M. J.; Hoeschele, J. D. *Platinum Met. Rev.* **1973**, *17*, 2.