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Interactions between Anticancer *trans*-Platinum Compounds and Proteins: Crystal Structures and ESI-MS Spectra of Two Protein Adducts of *trans*-(Dimethylamino)(methylamino)dichloridoplatinum(II)

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Supporting Information

ABSTRACT: The adducts formed between *trans*-(dimethylamino)(methylamino)dichloridoplatinum(II), [t-$PtCl_2$(dma)(ma)], and two model proteins, i.e., hen egg white lysozyme and bovine pancreatic ribonuclease, were independently characterized by X-ray crystallography and electrospray ionization mass spectrometry. In these adducts, the Pt^{II} center, upon chloride release, coordinates either to histidine or aspartic acid residues while both alkylamino ligands remain bound to the metal. Comparison with the cisplatin derivatives of the same proteins highlights for [t-$PtCl_2$(dma)(ma)] a kind of biomolecular metalation remarkably different from that of cisplatin.

Since the discovery of the antitumor activity of cisplatin and the establishment of the so-called "Hoeschele's rules" defining structure−activity relationships in cytotoxic platinum(II) complexes, the presence of a cis configuration with two adjacent leaving ligands has been considered to be an absolute requirement for anticancer action. For this reason, *trans*-platinum compounds have long been reputed as uninteresting and largely neglected. This trend was reversed in more recent times when it was realized that a number of *trans*-platinum compounds, including *trans*-[$PtCl_2${EHN=C(OCH_3)CH_3}$_2$], *trans*-[$PtCl_2$(NH_3) (thiazole)], *trans*-(dimethylamino)-(methylamino)dichloroplatinum(II) ([t-$PtCl_2$(dma)(ma)]), and *trans*-(isopropylamino)(dimethylamino)-dichloridoplatinum(II) ([t-$PtCl_2$(ipa)(dma)]), manifest remarkable cytotoxic properties toward several human tumor cell lines.[1−3] It was found that the presence of bulky amino ligands within a square-planar *trans*-platinum(II) configuration leads to a net decrease in the reactivity with biomolecules, commonly associated with a higher antitumor activity in vitro.[3]

Interactions of platinum-containing drugs with proteins are believed to play key roles in drug resistance processes and also in the mechanism of action of this class of compounds.[4−6] However, the formation of adducts between *trans*-platinum(II) compounds and proteins and their biological consequences have been poorly investigated so far.[4−6]

We describe here the X-ray structure of the hen egg white lysozyme (HEWL)/[t-$PtCl_2$(dma)(ma)] derivative and that of the adduct formed between the same platinum compound and bovine pancreatic ribonuclease (RNase A). We have chosen these two proteins because they have been used for more than half a century as model systems for studies on protein chemistry, including metalation caused by metal-based bioactive agents,[7,8] like cisplatin.[7a,8a] The structural characterization is supported and completed by independent electrospray ionization mass spectrometry (ESI-MS) measurements.

Crystals of the HEWL/[t-$PtCl_2$(dma)(ma)] adduct were obtained by cocrystallization experiments, using 0.6 M $NaNO_3$, 20% ethylene glycol, and 0.1 M sodium acetate, at pH 4.2, as the reservoir solution. Details of the crystallization procedure, data collection, structure determination, and refinement statistics are given in the Supporting Information (SI), Tables 1 and 2. The structure, which has been solved at 2.5 Å resolution, refines to *R* factor = 17.9 (R_{free} = 25.9). The overall HEWL structure in the adduct is very similar to that of the ligand-free enzyme (PDB code 193L):[9] the root-mean-square deviation in positions of the CA atoms is as low as 0.3 Å. Inspection of the electron density maps revealed platination of the only HEWL histidine residue, i.e., His15, at the imidazole NE2 atom (at the so-called left-handed site)[7a] (Figure 1). Pt^{II} has an occupancy equal to 0.8 and is also bound to the other three light atoms, which have been interpreted as two N atoms of two amine moieties and to a water molecule. The His(N)−Pt distance is 2.3 Å, whereas Pt−N distances are close to 2.0 Å. The geometry of this site is significantly distorted.

The X-ray structure also reveals the presence of an additional binding site close to Asp101 (Figure 1). In this site, Pt^{II} has an occupancy of 0.35. The Asp(OD1)−Pt distance is 2.1 Å, whereas Pt−N distances are close to 2.0 Å.

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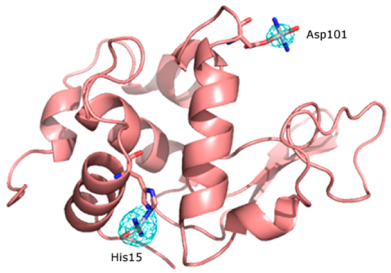

Figure 1. Cartoon representation of the adduct between HEWL and [t-$PtCl_2$(dma)(ma)]. The side chains of His15 and Asp101, which are exposed to the solvent, are shown along with platinum and its ligands. $2F_o - F_c$ electron density maps are contoured at the 3σ level. The structure has been deposited in the Protein Data Bank with code 4QGZ.

In both platinum binding sites, the quality of the electron density maps does not permit one to gain conclusive information on the nature of the platinum ligands. Therefore, electrospray ionization mass spectrometry (ESI-MS) spectra were registered to obtain further independent information on the same adduct. In particular, HEWL was dissolved in a suitable buffer (20 mM ammonium acetate buffer, pH 6.8) and treated with a 3:1 excess of a fresh solution of the platinum compound. Then, ESI-MS spectra were recorded after increasing time intervals (Figure 2a). The formation of metallodrug−protein adducts is well witnessed by the appearance of two main peaks of greater molecular mass than the native protein. Interestingly, these two peaks, which may be attributed to the same platinum adduct, show values of 14609.83 and 14633.87 Da (i.e., the previous peak plus a Na^+ ion), suggesting the formation of an adduct where the platinum compound loses one chlorine ligand, retaining the dma and ma ligands. Adduct formation is nearly complete after the first 24 h; no relevant further changes are detected at 72 h.

A similar approach was used to monitor the interactions of [t-$PtCl_2$(dma)(ma)] with RNase A. Crystals of the metallodrug−protein complex were obtained by soaking experiments. Ligand-free RNase A crystals grown by a hanging-drop vapor diffusion method using 20% PEG4000, 10 mM sodium citrate (pH 5.0), and a protein concentration of 20 mg mL^{-1} were incubated for 5 days with an excess of the platinum compound at a 1:10 protein/metal molar ratio. X-ray diffraction data were collected on these crystals at 2.00 Å resolution. Details of crystallization, data collection, and structure refinement are given in the SI.

The structure of this adduct, which contains two molecules in the asymmetric unit (Figure S1 in the SI), refines to R_{factor} = 21.4 (R_{free} = 27.5). The overall structure of the RNase A molecules in the complex is very similar to that of the ligand-free protein (PDB code 1JVT):[10] the root-mean-square deviations in the CA atom positions are in the range 0.4−0.6 Å. Platination just induces some conformational disorder of residues 16−22.

In both molecules, platination occurs at the NE2 atom of the imidazole ring of His105 (occupancy = 1.0) and at the ND1 atom of His48 (occupancy = 0.3 and 0.4 for molecules A and B, respectively). Moreover, platination also occurs at His119, i.e., at the enzyme active site (Figure 3a). In molecule A, platination occurs at the ND1 and NE2 atoms of the imidazole ring of His119 (occupancy = 0.3 and 0.4, respectively), which adopts two conformations. Also, in molecule B, platinated His119 adopts two distinct conformations, and, platinum binds the NE2 atom (Pt occupancy = 0.5).

Notably, the whole platinum compound has been modeled at the His105 binding site (Figure 3b). In this site, the His(N)−Pt distance is equal to 2.3 Å, whereas Pt−N distances are close to 1.9 Å. The fourth loosely coordinated ligand of the platinum, placed

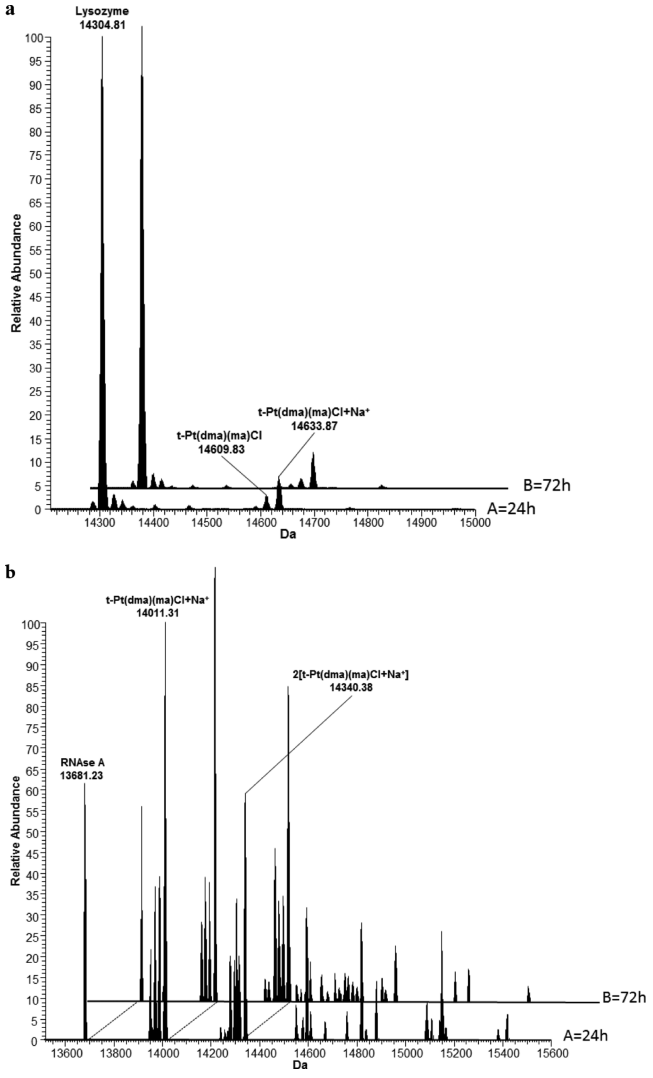

Figure 2. Deconvoluted ESI-MS spectra of 10^{-4} M HEWL (a) and RNase A (b) treated with [t-$PtCl_2$(dma)(ma)] (metal:protein ratio = 3:1) in a 20 mM ammonium acetate buffer (pH 6.8) recorded after 24 h (A) and 72 h (B) of incubation at 37 °C.

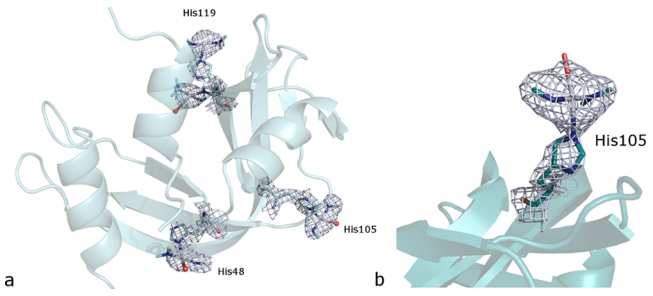

Figure 3. (a) Cartoon representation of the adduct between RNase A and [t-$PtCl_2$(dma)(ma)]. The side chains of His48, His105, and His119 are shown along with platinum and its ligands. The structure has been deposited in the Protein Data Bank under the accession 4QH3. (b) Zoom on His105 of molecule A. $2F_o - F_c$ electron density maps are shown at the 1.5σ level. A scheme showing coordination around the central Pt ion after coordination to His105 is reported in the SI (Figure S2).

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at 2.6 Å, has been modeled as a water molecule, suggesting that the reactive species, in the conditions used to obtain the RNase A/[t-$PtCl_2$(dma)(ma)] adduct, is the product of hydrolysis of [t-$PtCl_2$(dma)(ma)]. In the other binding sites, a water molecule is found 2.8−3.0 Å far apart from the metal.

A direct comparison between the structure of the RNase A/[t-$PtCl_2$(dma)(ma)] derivative and that of the same protein in a complex with cisplatin[8a] demonstrates that *trans*-platinum compounds are able to induce a type of protein metalation remarkably distinct from that inferred by cisplatin. In fact, we have shown that cisplatin binds RNase A through Met29 coordination.[8a] It seems that [t-$PtCl_2$(dma)(ma)] is not able to bind Met29 probably because of the greater steric hindrance of the alkylamino groups compared to that of amino groups. These results are in line with previous studies showing that transplatin and cisplatin have distinct binding sites upon reaction with ubiquitin.[11]

Even in the case of RNase A, ESI-MS analyses have been carried out. The ESI-MS spectra show two main peaks at 14011.31 and 14340.38 Da (Figure 2b), which are attributed to mono- and bis-coordination, respectively, of the same metallic fragment in which the Pt center loses a single chloride ligand (in both cases, these peaks are accompanied by the respective Na^+ species).

In spite of the fact that the same type of platinum coordination is afforded with the two proteins, a greater amount of adduct is formed in the case of RNase A (see Figure 2).

Although some differences are found upon comparison of the data obtained from ESI-MS spectra with those from X-ray diffraction experiments (which may be easily accounted for considering the different conditions of the two experiments), the results demonstrate that alkylamino ligands are invariantly retained upon reaction of the platinum drug with proteins.

In conclusion, we have offered here unambiguous structural evidence that the [t-$PtCl_2$(dma)(ma)] complex gives rise to stable adducts with two different model proteins. These adducts are formed through histidine or aspartic acid binding of a $[Pt(RNH_2)_2Cl]^+$ or $[Pt(RNH_2)_2OH_2]^{2+}$ fragment (Figure S2 in the SI), i.e., the products of hydrolysis of the original compound in water. These structures grow the crystallographic repertoire of platinum adducts with proteins and represent the first high-resolution characterization for adducts formed between *trans*-platinum compounds and proteins. Our data, in combination with previous ones,[3] reveal that *trans*-platinum compounds do bind proteins with full retention of the alkylamino ligands. The retention of nitrogen ligands in the reaction of *trans*-platinum complexes with proteins is in agreement with a recent study describing the interactions of other *trans*-platinum compounds with the Atox-1 protein.[12]

As expected, on the basis of the results from our previous studies of interactions between this platinum complex and oligonucleotides,[13] this kind of biomolecular metalation may result in different patterns of DNA platination compared to cisplatin and its analogues and, accordingly, in a different pharmacological profiles.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Materials and analytical techniques, crystallization, X-ray data collection, structure solution, and refinement, ESI-MS, Tables 1 and 2, and Figures S1 and S2. 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; they have approved the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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