

A case of Extensive Protein Platination: the Reaction of Lysozyme with a Pt(II) terpyridine complex

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Abstract

5 *An antiproliferative platinum(II)–terpyridine complex bearing two piperidine substituents at positions 2 and 2' (compound 1, hereafter), interacts non-covalently with DNA and induces cell death through necrosis, i.e. a mode of action that is distinct from that exhibited by cisplatin (Suntharalingam et al., Metallomics 2013, 5, 514). Here, the interaction between this Pt compound and the model protein hen egg white lysozyme (HEWL) was studied by both electrospray ionization mass spectrometry (ESI MS) and X-ray crystallography. ESI MS data collected after 24 h protein incubation with compound 1 at two different pH values offer evidence that the metal complex degrades upon reaction with HEWL, forming adducts with 1:1, 2:1 and 3:1 Pt/protein ratios. Two different X-ray structures of Pt-protein adducts, obtained by reaction of HEWL with the Pt compound under different experimental conditions and incubation times, are then reported. An unexpected extensive platination of the protein is clearly*
15 *observed: Pt containing fragments bind close to NZ atom of Lys1 and OE1 atom of Glu7, NE2 atom of His15 and NH1 atom of Arg14, ND1 atom of His15, NZ atom of Lys96, NZ atom of Lys97 and ND1 atom of Asn93, NZ atom of Lys13 and the C-terminal carboxylate, and the N-terminal amine. An additional binding site was observed close to NZ atom of Lys33. These results suggest that both N- and C-terminal tails, as well as Lys side chains, have to be considered as potential binding sites of Pt*
20 *containing drugs.*

The peculiar reactivity of compound 1 with biological macromolecules could play a role in its mode of action.

INTRODUCTION

Pt(II)-terpyridine complexes are well known cytotoxic agents owing to their ability to form tight adducts with double helix DNA through intercalation [1-3]. Many structurally diverse Pt(II)-terpyridine compounds have been synthesized and characterized during the last 20 years [1-3]. Some of these compounds show potent antiproliferative properties in vitro against several cancer cell lines comparable to or even better than cisplatin and show little or no cross resistance with cisplatin itself; interestingly, a few Pt(II)-terpyridine compounds also manifest relevant anti-parasitic activities [1-4]. Remarkably, Pt(II)-terpyridine complexes were reported to be effective inhibitors of mammalian topoisomerases and human thioredoxin reductase 1 (hTrxR1)[5-6].

Recently, a new Pt(II)-terpyridine compound bearing two piperidine substituents was synthesized and its interactions with different topologies of DNA were studied [7]. The piperidine substituents in compound **1** (Figure 1) were placed in positions 2 and 2' positions with the aim of hindering direct coordination of Pt to DNA, thus driving the binding towards intercalation, or towards the grooves and/or the phosphate groups. It was also reported that compound **1** is cytotoxic toward cancerous (U2OS and SH-SY5Y) and proliferating NIH 3T3 cell lines and it was found to induce cell death through necrosis [7].

Spectrophotometric studies carried out on this system demonstrated that it is stable under a variety of experimental conditions [7]. Furthermore, spectrophotometric and computational data point out that compound **1** selectively binds DNA in the minor groove, non-covalently. Since cellular uptake studies indicated that this compound penetrates the cell membrane and could be localized in the nucleus in relatively large amounts, genomic DNA was supposed to be its possible target [7]. However, proteins might be used to transport this molecule towards the genomic DNA target.

Furthermore, it cannot be ruled out that DNA is not the final and/or exclusive target for this cytotoxic agent, at variance with the case of clinically established Pt-based drugs.

With the aim of exploring the ability of compound **1** to interact with proteins, we have studied its reactivity with hen egg white lysozyme (HEWL), a relatively small protein, widely used as model system in metalation studies [8-12], through a well established combined mass spectrometry/X ray crystallography approach [13-14].

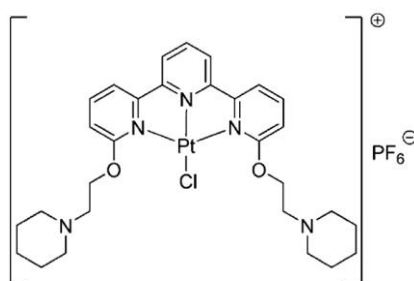


Figure 1. Structure of compound **1**.

RESULTS

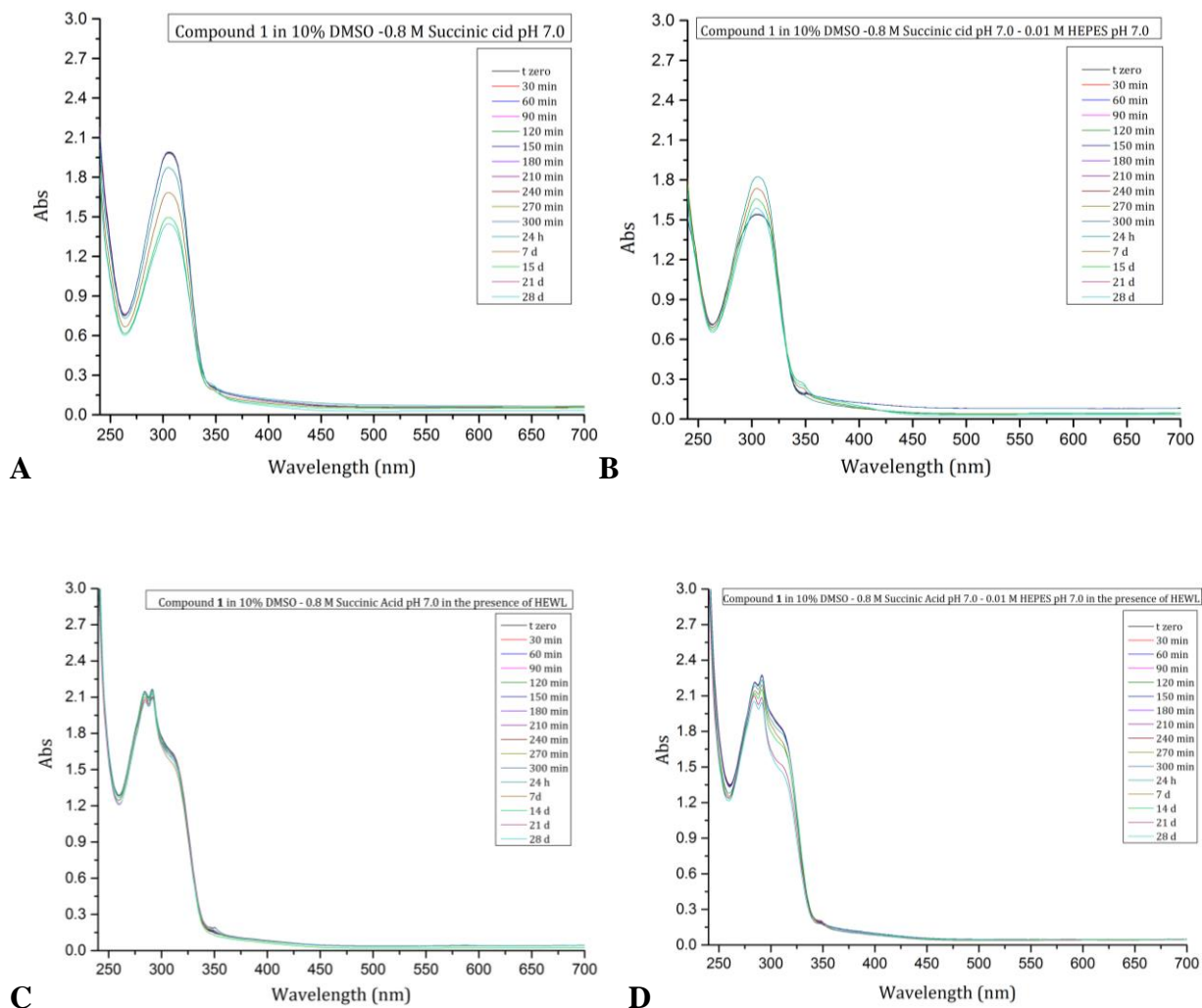
1. Solution behaviour of compound **1**

5 The stability of compound **1** in mixed DMSO/aqueous solutions and in 10 mM Tris-HCl /100 mM KCl with and without a cell lysate was previously established [7]. Here new experiments have been carried out to check the stability of the compound under the experimental conditions that are needed to allow a study on the reactivity of the compound with proteins by electrospray ionization mass spectrometry and X-ray crystallography.

10 UV-Vis spectra of compound **1** were thus collected in 100% DMSO; 50% DMSO – 50% PBS at pH 7.4; 10% DMSO – 90% PBS pH 7.4; 10% DMSO, 0.8 M succinic acid/NaOH at pH 7.0; 10% DMSO, 0.8 M succinic acid/NaOH and 0.010 M HEPES pH 7.0; 15% DMSO and 0.020 M ammonium acetate at pH 4.5 and pH 6.8. Spectra are reported in Figure S1.

15 All these spectra indicate that the main MLCT band shows no signs of platinum ligand dissociation/chemical transformations over 24 h. Analysis of the spectra of compound **1** collected after several days (from 7 to 28 days) reveals that it remains stable, although in part it precipitates after long incubation times, as demonstrated by data collected in 10% DMSO and 0.8 M succinic acid/NaOH at pH 7.0 and 10% DMSO, 0.8 M succinic acid/NaOH and 0.010 M HEPES at pH 7.0
20 that are reported Figures 2A and B. Notably, UV-Vis absorption spectra collected in the presence of the model protein hen egg white lysozyme (HEWL) under the same experimental conditions also reveal only very subtle differences (Figures S2A-G), even in 10% DMSO and 0.8 M succinic acid/NaOH at pH 7.0 and 10% DMSO, 0.8 M succinic acid/NaOH and 0.010 M HEPES at pH 7.0 and after several days (Figures 2C and D). These findings indicate that the precipitation of

compound **1** occurring after 7 days is attenuated by the presence of the protein and suggest that compound **1** slowly reacts with HEWL under these experimental conditions.



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Figure 2. Time dependent UV-Vis absorption spectra of compound **1** (10^{-4} M) dissolved in A) 10% DMSO – 0.8 M succinic acid/NaOH at pH 7.0, B) 10% DMSO – 0.8 M succinic acid/NaOH, 0.010 M HEPES at pH 7.0, C) 10% DMSO – 0.8 M succinic acid/NaOH at pH 7.0, D) 10% DMSO – 0.8 M succinic acid/NaOH, 0.010 M HEPES at pH 7.0 and monitored over 28 days. In panels C and D the spectra of the compound have been registered in the presence of HEWL (3:1 metal to protein ratio).

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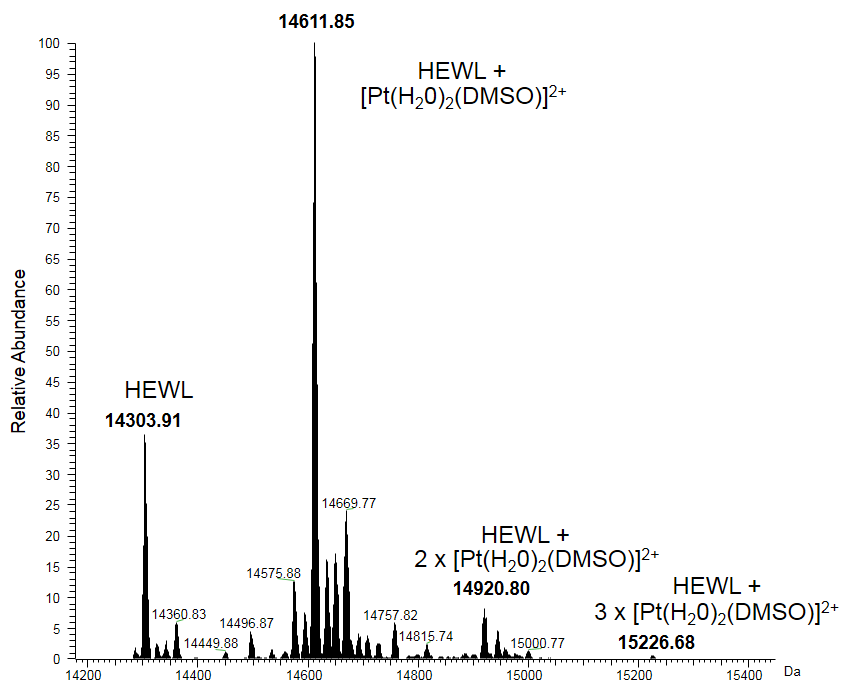
2. ESI MS studies of the reaction of compound **1** with HEWL

ESI MS data were then collected for a solution of compound **1** dissolved in DMSO and mixed with HEWL in 3:1 metal to protein ratio. Mass spectra, collected after 24 h of incubation in ammonium acetate at pH 4.5 and 6.8 (Figure 3), clearly reveal that compound **1** reacts with HEWL.

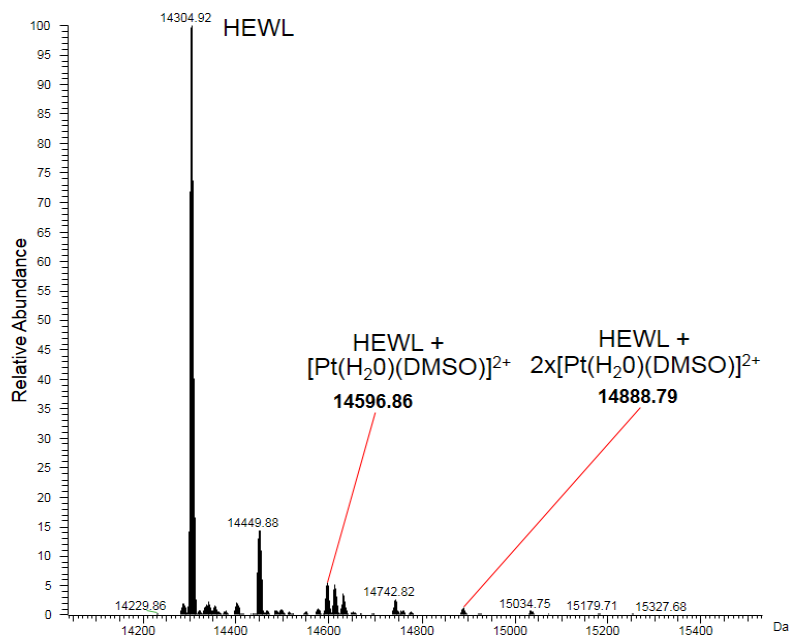
5 In particular, Pt fragments $[\text{Pt}(\text{H}_2\text{O})_2(\text{DMSO})]^{2+}$ and $[\text{Pt}(\text{H}_2\text{O})(\text{DMSO})]^{2+}$ bind HEWL with different metal to protein stoichiometries. This result indicates that the Pt complex degrades in the presence of the protein, contrarily to what was expected on the basis of the stability studies of the compound **1** in aqueous solutions and to what was previously found in the case of the adduct formed between another Pt(II)-terpyridine complex and hTrxR1 [6]. In the latter case the Pt centre retains its terpyridine ligand
10 when binding the protein close to two Cys side chains, with the terpyridine ligand that stacks with tryptophan residue side chains [6].

It is possible that compound **1** has to dissociate to interact with the protein, since Pt coordination of the intact compound to protein residue side chains is hindered by the piperidine substituents on the terpyridine ligand.

15 Comparison between the results of the above reported ESI MS experiments and those previously collected under similar experimental conditions for the same protein in the presence of cisplatin [15-17 and references therein], carboplatin [15], oxaliplatin [11,15] and other Pt-based drugs [8, 18] suggests that compound **1** shows a generally greater reactivity with HEWL than other known Pt compounds.



A



B

Figure 3. Deconvoluted ESI mass spectra of HEWL (10^{-4} M) treated with compound **1** in 1 : 3 protein to metal ratio. Spectra were registered after 24 h of incubation at 37 °C, in the presence of 15% DMSO and 0.020 M ammonium acetate pH 4.5 (A) and 6.8 (B).

A mass spectrometry characterization of the HEWL-compound 1 adduct has been also carried out using a lower amount of DMSO (3%), in order to study whether the high concentration of this solvent used in previous experiments could affect the interaction between the Pt complex and the protein. Results of this analysis, which are reported in Figure S3, indicate that even in presence of lower DMSO concentrations the metalation process does not change significantly, involving complex disassembling with coordination of $[\text{Pt}(\text{H}_2\text{O})_x(\text{DMSO})]^{2+}$ fragments to protein. The stoichiometry and the number of the adducts found in these experiments fully agree with those found using 25% of DMSO.

3. Crystallographic results

Two high resolution (1.49-1.96 Å) X-ray structures of the adduct formed in the reaction between HEWL and compound 1 were finally refined. These structures were obtained under different experimental conditions (see below). In both cases, the overall protein conformation was not significantly affected by binding of Pt atoms, implying that HEWL structure in the adduct is almost identical to that of native protein. Root mean square deviations in positions of the carbon alpha atoms between the present structures and that of the native enzyme used as a starting model are 0.19 and 0.23 Å.

The first molecular model of the Pt-protein adduct was obtained analysing data collected using crystals grown in ethylene glycol, sodium acetate and nitrate and exposed to the Pt compound for 6 months (see Methods for further details and reference [19] for the soaking procedure). In this structure, which was solved at 1.49 Å resolution, eight metal binding sites were found (Figure 4). In particular, Pt ions were observed close to NZ atom of Lys1 and OE1 atom of Glu7, ND1 atom of His15, NE2 atom of His15 and NH1 atom of the side chain of Arg14, NZ atom of Lys13 and the C-terminal carboxylate, NZ atoms of Lys96, NZ atom of Lys97 and ND2 atom of Asn93, and the N-terminal

amine (Figure 5). An additional Pt atom was also modeled as $\text{Pt}(\text{H}_2\text{O})_3\text{X}^{n+}$ (with $n=1$ or 2 depending on the charge of X, which is an undefined monoatomic ligand that is not visible in the electron density map). This is not directly coordinated to protein residues.

The side chains of Lys1 and Glu7 bind a $[\text{Pt}(\text{H}_2\text{O})(\text{DMSO})]^{2+}$ fragment, with the water molecule
5 coordinated to the Pt centre that is also in contact with two solvent water molecules and the side chain of the Arg14 from a symmetry related molecule (distance 3.6 Å). Since this residue is involved, together with the side chain of His15, in the recognition of another Pt centre, two Pt containing fragments are close each other in this structure. His15 was identified as the primary Pt binding site in other adducts between the protein and Pt-based drugs [8,15,16], including cisplatin
10 [8,15-16] and carboplatin [20-21].

$[\text{Pt}(\text{H}_2\text{O})(\text{DMSO})]^{2+}$ is the fragment observed close to the NE2 atom of His15 and to the side chain of Arg14, whereas a $[\text{Pt}(\text{H}_2\text{O})(\text{DMSO})_2]^{2+}$ fragment is found close to ND1 atom of the imidazole of the same His. The water molecule of the $[\text{Pt}(\text{H}_2\text{O})(\text{DMSO})]^{2+}$ fragment forms a hydrogen bond with the N atom of Ile88, whereas the water molecule of the $[\text{Pt}(\text{H}_2\text{O})(\text{DMSO})_2]^{2+}$ fragment
15 interacts with the O atom of Thr89 and with the O atom of the side chain of Asn93, which adopts two different conformations in this structure. One of the two DMSO molecules of the latter fragment is very close (3.2 Å) to the $[\text{Pt}(\text{H}_2\text{O})_3]^{2+}$ fragment bound to the side chain of Lys96. $[\text{Pt}(\text{H}_2\text{O})_2]^{2+}$ is the fragment bound to the side chain of Lys97 and to the second conformation of the side chain of Asn93. This fragment is in proximity of the binary axis. Two Pt centres are very
20 close to each other also at the C-terminal tail, again close to the binary axis. Here, two Pt centres, alternative to each other, bind the C-terminal carboxylate, the NZ atom of the side chain of Lys13 a DMSO molecule. The definition of the ligands of these Pt centres is complicated by the presence of overlapping electron density maps. It is possible that a water molecule is the ligand that completes the square planar Pt coordination sphere.

The N-terminal tail binds a $[\text{Pt}(\text{OH}_2)_2\text{X}]^{n+}$ ($n=1$ or 2 , depending on the X charge) fragment.

A Pt^{2+} ion was found close to the N-terminal tail also in the adduct formed in the reaction between HEWL and cisplatin by Helliwell and coworkers [22].

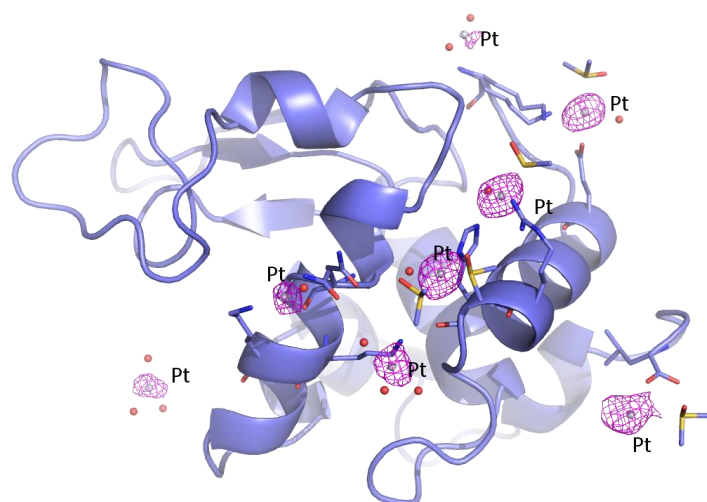


Figure 4. Overall structure of the first adduct formed in the reaction between HEWL and compound **1**. This adduct is obtained when HEWL crystals are soaked in a solution of compound **1** in the presence of ethylene glycol, sodium acetate and sodium nitrate. Anomalous difference electron density map at 3.0σ is reported in violet.

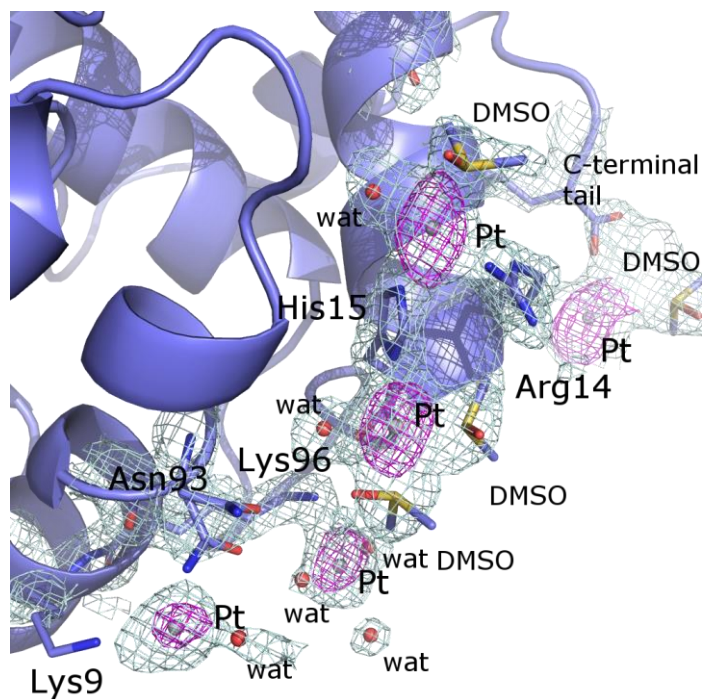


Figure 5. Details of five out of the eight Pt binding sites in the structure of the Pt-HEWL adduct formed within crystals of HEWL which were exposed for 6 months to compound **1**. 2Fo-Fc

electron density map is contoured at 1.0 σ level and reported in cyan. Anomalous difference map is depicted in violet at 3.0 σ .

5

The second crystal structure of the HEWL-compound **1** adduct was determined at 1.96 Å resolution using X-ray diffraction data collected on a protein crystal grown in succinic acid/NaOH, HEPES and MPEG exposed for twelve days at nearly neutral pH to a 0.005 M solution of compound **1** in 25% DMSO. In this structure, five out of the eight Pt binding sites described above were found (Figure 9).
10 In particular, Pt binds the ND1 and NE2 atoms of the side chain of His15 (Figure 6A), NZ atom of Lys1 and OE1 atom of Glu7, NZ atom of Lys13 and the C-terminal carboxylate, NZ atom of Lys97. Close to ND1 atom of His15 a $[\text{Pt}(\text{OH}_2)_3]^{2+}$ fragment was modelled (Figure 10A), whereas close to NH1 atom of Arg14 and to NE2 atom of His15 a $[\text{Pt}(\text{OH}_2)(\text{DMSO})]^{2+}$ fragment was found. Furthermore, an additional binding site was observed close to NZ atom of Lys33 (Figure 10B). Here,
15 the Pt centre coordinates three water molecules. Metal ligands were not modelled in the other Pt binding sites.

Altogether these results provide for the first time structural evidences that HEWL possesses multiple Pt binding sites, as had been previously proposed by Zhang and coworkers [23]. Notably, previous structural studies on crystals exposed for a long time to cisplatin (even 5 years) did not reveal
20 additional Pt binding sites beyond the side chains of His15 and Arg14 [24].

Overall the crystallographic data agree with recent structural analyses indicating that N-terminal amines and lysine side chains can unexpectedly bind Pt ions, particularly in the case of proteins with low abundance or low accessibility of His, Met and Cys residues [25] and with literature data indicating that Lys and Asn side chains are not known to react with Pt(II) terpyridine complexes [26-
25 27]. On the contrary, our results indicate that even carboxylates (C-terminal tail and side chains of

Glu) should be considered as possible targets of platination, in contrast to what previously hypothesised [27].

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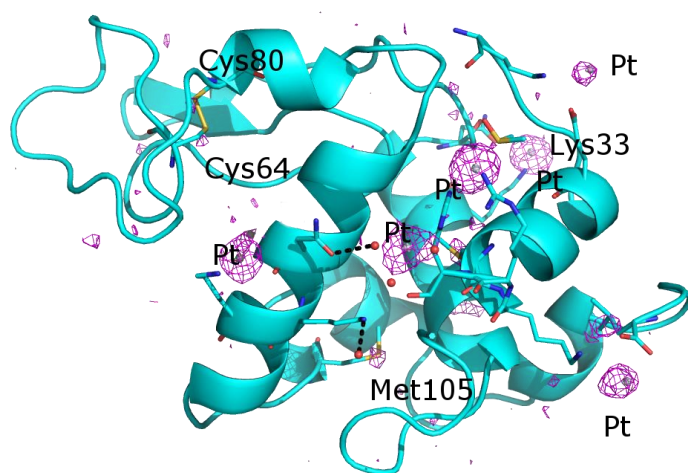
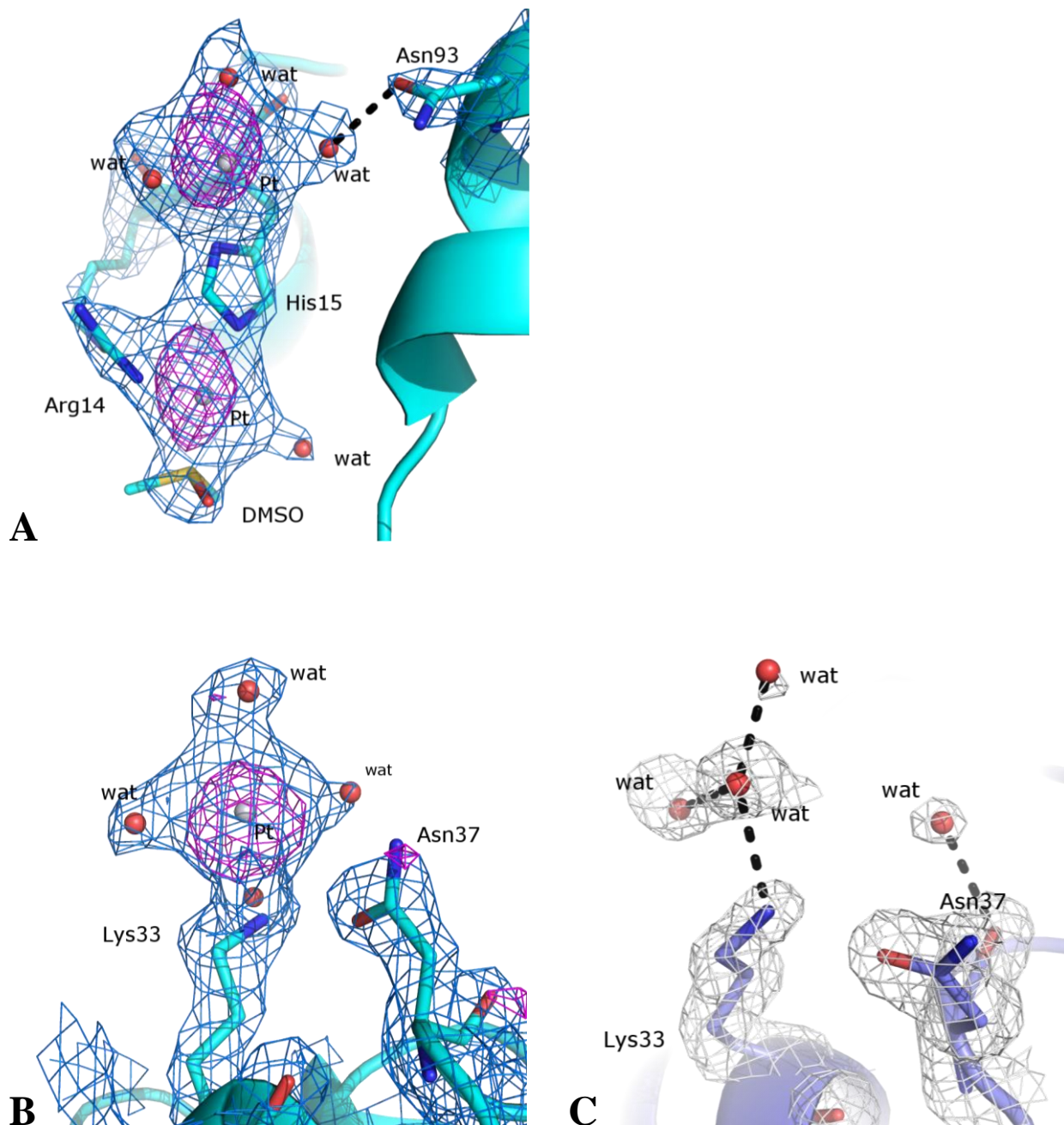


Figure 6. Overall structure of the adduct formed within HEWL crystals treated with compound **1** in succinic acid/NaOH, HEPES and MPEG at pH 7.0. Anomalous difference electron density map at 3.0σ is reported in violet. The high quality of the anomalous map is evidenced by the appearance of peaks
10 at $>3.0 \sigma$ in correspondence of the positions of sulphur atoms of Met and Cys residues.

15



5 **Figure 7.** Details of Pt binding sites close to His15 (panel A) and Lys33 (panel B) in the structure of the Pt-HEWL adduct formed within crystals of HEWL which were exposed for 12 days to compound **1** at neutral pH. 2Fo-Fc electron density map is contoured at 1.0 σ level and reported in cyan. Anomalous difference map is depicted in violet at 3.0 σ . For comparison, the electron density map of the side chain of Lys33 of the Pt-HEWL adduct formed within crystals of HEWL which were exposed
 10 for 6 months to compound **1** at acid pH is reported (panel C).

Conclusions

5 In conclusion, the main results that emerge from our combined crystallographic-mass spectrometric investigation are the following:

i) Compound **1** dissociates in the presence of HEWL, forming several adducts bearing various Pt²⁺ centers anchored to distinct protein residues with solvent molecules (DMSO and water). It is likely that the steric hinderance imposed by the piperidine substituents on the terpyridine ligand prevents
10 compound **1** from coordinating directly to the protein.

The observed Pt anchoring sites are the following: side chains of Lys1 and Glu7, His15, Arg14 and His15, Lys13 and C-terminal carboxylate, Lys96, Lys97 and Asn93, and N-terminal amine. The majority of these binding sites are observed under two different experimental conditions, i.e. at neutral pH in the presence of succinic acid/NaOH and at acid pH in acetate. At neutral pH and in the presence
15 of succinic acid/NaOH an additional binding site was found at the side chain of Lys33.

ii) The binding of this large number of Pt centres on the surface of HEWL does not significantly affect the overall conformation of the protein: only minor local changes in the structure of HEWL were observed.

iii) The Pt-HEWL adducts obtained by the reaction of compound **1** with the protein are
20 characterized by different metal to protein ratios, depending on the experimental conditions used to study the adduct.

The investigated Pt-terpyridine compound is thus a molecule that interacts non-covalently with DNA [7], whereas it can degrade upon reaction with proteins. This peculiar reactivity with biological macromolecules might be at the origin of its unusual mechanism of action and might trigger necrosis,

but further studies are still needed to verify this hypothesis.

EXPERIMENTAL

Materials and methods

5 Compound **1** was synthesised and characterized as previously reported [7]. HEWL and the chemical reagents used in this work were purchased from Sigma Chemical Co and used without further purifications.

Crystallization and X-ray diffraction data collection

10 HEWL crystals were grown using the hanging drop vapour diffusion method and the following reservoir:

a) 20% ethylene glycol and 0.60 M sodium nitrate and 0.10 M sodium acetate pH 4.4 as buffer;

b) 0.8 M succinic acid/NaOH, 0.10 M HEPES pH 7.0, 1% MPEG 2K.

Crystals of the protein adduct with compound **1** were obtained by soaking procedure. HEWL crystals
15 grown in ethylene glycol were soaked in a solution consisting of 83% reservoir, 17% DMSO and 0.003 M compound **1**. X-ray diffraction data on these crystals have been collected at ESRF synchrotron, Grenoble, France, after 6 months of soaking. These data have been processed with Mosflm [28].

Crystals of HEWL grown at pH 7 were soaked in a solution consisting of 75% reservoir, 25% DMSO and 0.005 M compound **1**. Data collections on these crystals have been carried out at CNR Institute of
20 Biostructures and Biomages using a Saturn944 CCD detector equipped with CuK α X-ray radiation from a Rigaku Micromax 007 HF generator. Processing and scaling were performed using HKL2000 [29].

The structures of the Pt-HEWL adducts were solved by molecular replacement method, using the reported coordinates of HEWL deposited in the PDB under the accession code 193L [30] as a

molecular search model. Refinements were carried out with CCP4 Refmac5 [31], model building or adjustments and inspection of the electron density maps have been manually carried out using Wincoot [32].

Pt binding sites were unambiguously identified by comparing 2Fo-Fc, residual Fo-Fc and anomalous difference electron density maps. The two structures refine to R-factor/R-free values of 0.193/0.188 and 0.240/0.250, respectively. Details on crystallographic and refinement parameters are reported in Table 1. The refined models and structure factors were deposited in the Protein Data Bank under accession codes 6G5Y and 6G5V. Coordinates and structure factors, including anomalous data, were provided to reviewers and Editor for review process. Comments on uninterpreted peaks of electron density are reported in Table S1. It is possible that Wat13 and Wat27 in the 6G5Y and 6G5V structures could be Na⁺ ions. The structure of the adduct obtained from crystals grown in 20% ethylene glycol and 0.60 M sodium nitrate and 0.10 M sodium acetate pH 4.4 and solved at 1.49 Å resolution has been also refined anisotropically to R-factor=0.145 and Rfree=0.218. Coordinates of the anisotropically-refined model are attached as supporting information.

15

Solution behavior of compound 1 in mixed solvents

Stability of compound **1** in different solvents was assessed through spectrophotometric studies performed with a Varian Cary 50 Bio UV-Vis spectrophotometer. Solutions of compound **1** (10⁻⁴ M) were prepared in 100% DMSO, 50% DMSO – 50% PBS at pH 7.4 and 10% DMSO – 90% PBS pH 7.4, 10% DMSO e 0.8 M succinic acid/NaOH at pH 7.0, 10% DMSO, 0.8 M succinic acid/NaOH and 0.010 M HEPES pH 7.0, 15% DMSO and 0.020 M ammonium acetate at pH 4.5 and 6.8; spectra were registered monitoring the absorbance in the wavelength between 240 and 700 nm for 24 h at 25 °C. Spectra were also collected under the same experimental conditions in the presence of HEWL and followed over 24 h or up to 28 days.

ESI MS experiments

ESI MS spectra of a solution of compound **1** (10^{-4} M) in 15% DMSO and ammonium acetate buffer (0.020 M, pH 4.5 or pH 6.8) in the presence of HEWL (3:1 metal to protein ratio) were recorded after 24 h incubation at 37 °C. Spectra were obtained by direct injection at 5 μ L/min flow rate in an Orbitrap high-resolution mass spectrometer (Thermo, San Jose, CA, USA), equipped with a conventional ESI source, according to a previously reported protocol [33].

The ESI-Q-TOF spectrum reported in Figure S3 was recorded by direct injection at 10 μ l min⁻¹ flow rate in an TripleTOF® 5600+ System mass spectrometer (Sciex, Framingham, MA, U.S.A.), equipped with DuoSpray® interface operating with an ESI probe. The MS source parameters were optimized and were as follows: positive polarity; Ionspray Voltage Floating 5500 V, Temperature OFF, Ion source Gas 1 (GS1) 40; Ion source Gas 2 (GS2) 0; Curtain Gas (CUR) 30, Declustering Potential (DP) 100 V, Collision Energy (CE) 10 V. For acquisition, Analyst TF software 1.7.1 (Sciex) was used and deconvoluted spectra were obtained by using the Bio Tool Kit version 2.2 tool for Peakview 2.2 software (Sciex). The MS was operated with a resolving power greater than 30 000fwhm for TOF MS scans.

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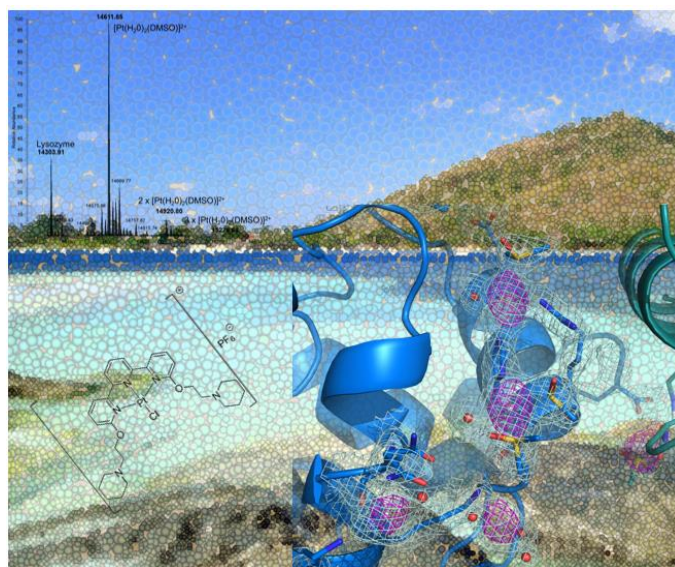
Highlights

The reaction of a Pt(II)-terpyridine compound with the model protein lysozyme is investigated.

Protein-Pt adducts have been studied by X-ray crystallography and mass spectrometry.

5 Several Pt binding sites have been characterized in the structure of lysozyme.

Synopsis and graphical abstract



10

The interaction between a Pt(II)-terpyridine cytotoxic compound and the model protein lysozyme has been investigated by X-ray crystallography and electrospray mass spectrometry under different experimental conditions. The compound shows a high reactivity with the model protein. An extensive
15 platination of lysozyme has been observed.

Abbreviations

HEWL: hen egg white lysozyme

ESI MS: Electrospray Ionization Mass Spectrometry

UV-Vis: UltraViolet-Visible

^s DMSO: dimethylsulfoxide

hTrxR1: human thioredoxin reductase 1

MLCT: metal-to-ligand charge-transfer

Table 1. Data collection and refinement statistics

	Structure 1	Structure 2
Crystallization conditions	20% ethylene glycol, 0.10 M sodium acetate pH 4.4, 0.60 M sodium nitrate	0.8 M succinic acid/NaOH at pH 7.0, 0.10 M HEPES pH 7.0, 1% MPEG 2K
Soaking solution	3 mM compound 1 , 17 % DMSO	5 mM compound 1 , 25 % DMSO
Soaking times	6 months	Twelve days
Data Collection statistics		
Wavelength (Å)	0.96771	1.5418
Space Group	P4 ₃ 2 ₁ 2	P4 ₃ 2 ₁ 2
Cell parameters a=b, c (Å); α=β=γ(°)	78.49, 36.73; 90	79.46, 36.49; 90
Resolution (Å)	55.51-1.49 (1.57-1.49)	56.18-1.96 (1.99-1.96)
Number of observed reflections	131438	44501
Unique reflections	18842	8823
Completeness (%)	98.3 (100)	99.3 (100)
Anomalous molteplicity	3.7 (3.7)	2.8 (2.4)
Rmerge†	0.039 (0.514)	0.084 (0.478)
Rpim	0.025 (0.219)	0.040 (0.256)
CC _{1/2}	1.00 (0.672)	0.812
I/σ(I)	18.1 (3.1)	8.1 (3.1)
Multiplicity	7.0 (7.2)	5.0 (4.4)
Anomalous completeness	97.1 (100)	99.5 (99.9)
Refinement statistics		
Resolution (Å)	55.51-1.49	56.18-1.96
n. of reflections in working set	17866	8356
n. of reflections in test set	952	435
R factor/Rfree /Rall(%)	19.3/24.0/19.6	18.8/25.0/19.1
Number of non-H atoms	1253	1124
Occupancy of Pt centre	0.75, 0.60, 0.75, 0.40, 0.45, 0.30, 0.30, 0.30	0.50, 0.60, 0.30, 0.30, 0.40, 0.30
B-factor of Pt centre (Å ²)	33.8, 28.5, 31.5, 41.3, 47.2, 45.1, 43.2, 49.1	47.7, 47.9, 66.3, 66.4, 36.1, 41.0
Overall B-factor	26.4	34.6
Deviations from ideality values		
R.m.s.d. bonds (Å)	0.018	0.016
R.m.s.d. angles (Å)	2.08	1.82