

Structural insights into the interaction of platinum-based inhibitors with the Alzheimer's disease amyloid- β peptide[†]

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Extended X-ray absorption fine structure spectroscopy, mass spectrometry, dynamic light scattering and density functional theory are combined to derive structural models for the interaction of neurotoxicity-ablating platinum-based compounds with the amyloid- β peptide.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common form of dementia in humans. It is characterized pathologically by the presence of large extracellular amyloid plaques in the brain. The main component of these plaques is the 39–43 residue amyloid β peptide (A β) which is produced by the sequential cleavage of the amyloid precursor protein (APP) by β - and γ -secretases. However, soluble low molecular weight oligomers of A β are shown to exert the neurotoxic effects potentially impair synapse structure and function.^{1,2} Anti-AD therapeutic strategies have focused, on the inhibition of the production of A β , on the inhibition of aggregation of A β , or on antibody based clearance of A β .

The aggregation of A β is enhanced in the presence of Fe, Cu and Zn^{3–5} due to its relatively high affinity towards transition metal ions and cross-linking of peptides. A β -Cu and A β -Fe complexes are also involved in the production of reactive oxygen species (ROS) under reducing conditions.^{6,7} Unsurprisingly, altering the metal binding activity of A β can inhibit its neurotoxicity. Studies^{8–13} suggest that Cu²⁺, Zn²⁺ and Fe²⁺ coordinate with the three histidines in the A β (His6, His13 and His14). Methylation of the imidazole side chains on these histidines inhibits neurotoxicity of A β *in vitro*.¹⁴ In recent years, binding of Pt compounds^{15–18} to the metal binding site of A β have been studied. Barnham *et al.*¹⁵ showed that Pt(II)-1,10-phenanthroline complexes bind to A β , inhibit aggregation and the generation of

reactive oxygen species, and rescue A β -induced synaptotoxicity in mouse hippocampal slices.

Knowledge of the atomic structure of the complexes made by Pt compounds with A β would greatly facilitate A β -specific therapeutic and diagnostic development. Here we employed X-ray absorption spectroscopy (XAS), mass spectrometry (MS), dynamic light scattering (DLS) and density functional theory (DFT) to characterize and derive structural models of the interactions of cisplatin (cisPt) and Pt(4,7-diphenyl-[1,10]phenanthroline disulfonate)Cl₂ (Pt(BPS)Cl₂) with A β ₁₆ and A β ₄₂.

Barnham *et al.*¹⁵ suggests that Pt(II)-1,10-phenanthroline complexes coordinate to the histidine residues of A β while the methionine (Met35) also may be involved in the binding of cisplatin. Based on this and also more recent investigations^{16,17,19,20} using HPLC, ESI-MS, NMR, and EPR into the coordination of Pt(II) complexes to A β , His6, His13, and His14, and additionally Asp1 (N-terminus), Lys16 (A β ₁₆ C-terminus) and Met35 can be considered as the potential ligands in A β ₁₆ or A β ₄₂ binding cisPt and Pt(BPS)Cl₂.

Our DLS experiments demonstrated that Pt(BPS)Cl₂ inhibited aggregation of the A β ₄₂ (Fig. S1 and text in ESI[†]). We performed MALDI-TOF MS of complexes of A β ₁₆ and A β ₄₂ with cisPt and Pt(BPS)Cl₂ (Fig. 1). The MS data suggests that addition of cisPt and Pt(BPS)Cl₂ resulted in the formation of multiple binary complexes between the peptide and Pt-compounds. After 2 h of incubation with cisPt, the dominant metallated species observed was A β -Pt (species I in Fig. 1a) for both A β ₁₆ and A β ₄₂ peptides, followed by complexes where cisPt lost either one (species V) or both (species IV) chloride ions for A β ₁₆ or additionally either one or both NH₃ groups in case of A β ₄₂ (Fig. 1a). When Pt(BPS)Cl₂ binds to A β ₁₆ or A β ₄₂, it loses either one or both chloride ions as reported previously.¹⁵ Additionally, in case of A β ₄₂ it binds peptide with both chloride ions attached (species V in Fig. 1d) implying that this complex may bind through hydrophobic or/and π - π non-covalent interactions of the phenanthroline motif with the hydrophobic C-terminal region of A β ₄₂. Crosslinked oligomers and DMSO adducts were not observed by MS analysis.

Based on the MS results three-dimensional structural models for fitting the EXAFS spectra were constructed by performing

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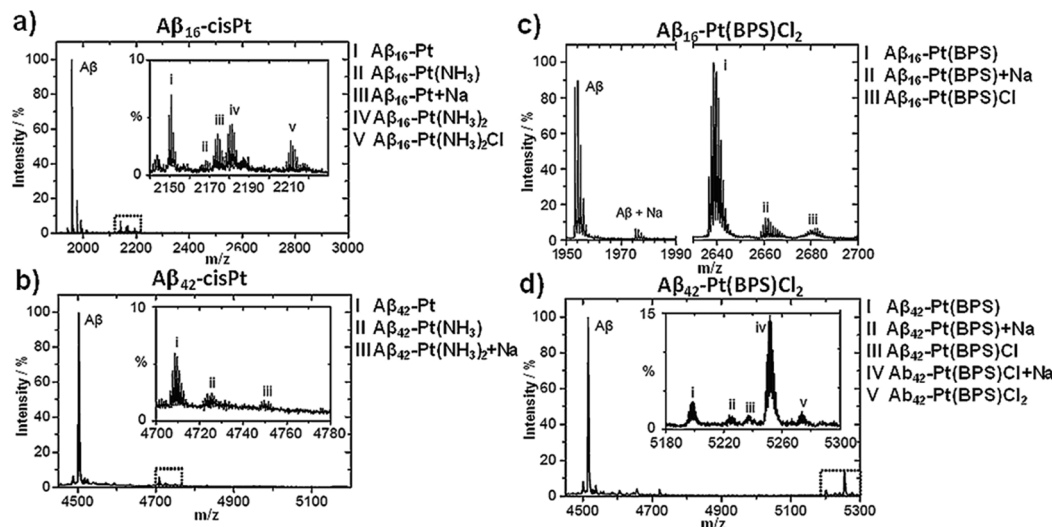


Fig. 1 MALDI-MS analysis of Aβ₁₆ (a, c) and Aβ₄₂ (b, d) complexes with cisPt (a, b) and Pt(BPS)Cl₂ (c, d).

quantum mechanical calculations, optimizing the geometry of each model system with DFT (details given in ESI†). The DFT optimized geometries of studied complexes (Table S2 in ESI†) were then refined against EXAFS spectra using multiple scattering theory²¹ considering an approximately planar, tetra-coordinated Pt atom. Fig. 2 presents refined Pt coordination geometries and the experimental and modelled EXAFS regions of Pt(II) spectra at Pt L_{III} edge and their Fourier Transforms (FT) for Aβ₁₆-cisplatin, Aβ₄₂-cisplatin, Aβ₁₆-Pt(BPS)Cl₂, and Aβ₄₂-Pt(BPS)Cl₂. The refined model included major species shown by MS (Fig. 1).

In the case of Aβ₁₆-cisPt, the best fit was obtained with the mixture of the models including gradually replaced Cl and NH₃ ligands with Aβ histidines and possibly with N-terminus (Asp1), C-terminus (Lys16), or Asp7:¹⁶ Pt-(NH₃)₂Cl₂, Aβ₁₆(His)-Pt-(NH₃)₂Cl, Aβ₁₆(His)₂-Pt-(NH₃)₂ and Aβ₁₆(His)₃(N/O)-Pt in accordance with MS data (Fig. 1a). The total populations of N(H₃), N(His) and Cl were refined with fixed to physically meaningful values of σ^2 (Debye-Waller terms). In the case of cisPt-Aβ₄₂ complex, the best fit was obtained with mixture of two species: Aβ₄₂(His)₃S(Met35)-Pt and Aβ₄₂(His)₂S(Met35)-Pt-(NH₃) in accordance

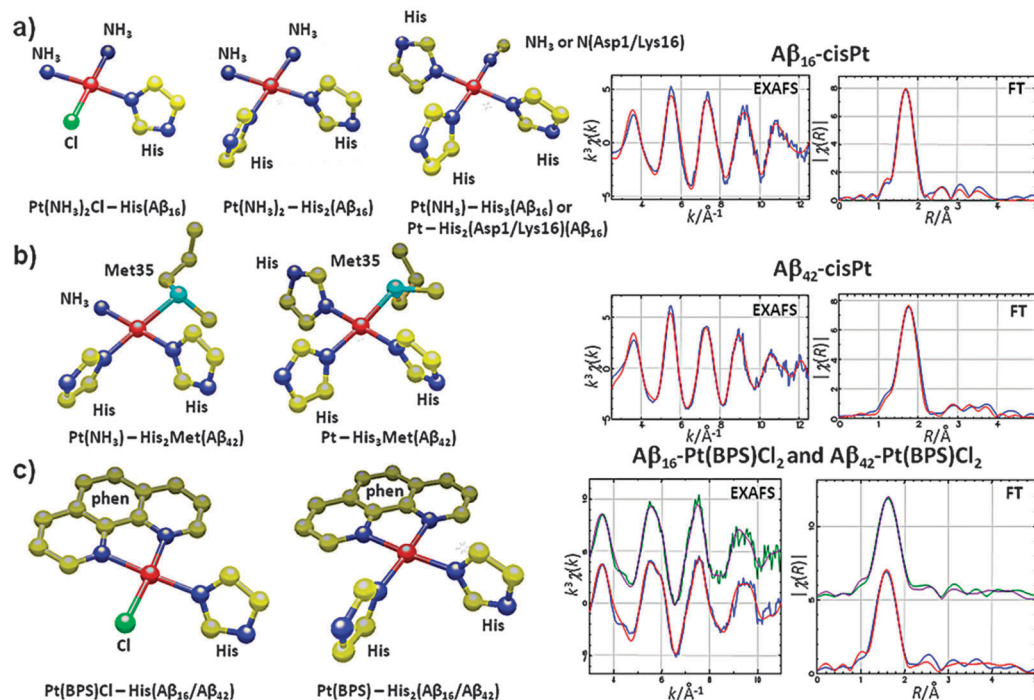


Fig. 2 Structural models for (a) Aβ₁₆ and (b) Aβ₄₂ complexed with cisPt and (c) Aβ₁₆ and Aβ₄₂ complexed with Pt(BPS)Cl₂ (phenanthroline (=phen) motif and sulfonate groups are not shown due to their negligible contribution to EXAFS). The corresponding Pt-L_{III}-edge k^3 -weighted (EXAFS) data and their Fourier Transforms (FT) are shown in blue lines for the experimental data and in red for the best model fits. Atom colors: Pt-red, N-blue, C-yellow, Cl-green, S-cyan.



with MS spectra (Fig. 1b). The result is consistent with previous ^1H NMR data for $\text{A}\beta_{40}$ which showed that cisPt formed adducts with $\text{A}\beta$ and coordinated predominantly at the S atom of Met35.¹⁵

Fig. 2c shows structures of the $\text{A}\beta_{16}$ -Pt(PBS) Cl_2 and $\text{A}\beta_{42}$ -Pt(PBS) Cl_2 complexes fitted simultaneously to both experimental data sets. The high similarity in the XANES and EXAFS regions for the $\text{A}\beta_{16}$ - and $\text{A}\beta_{42}$ -Pt(PBS) Cl_2 complexes suggested that geometry of the Pt^{2+} coordination did not change significantly across the samples. Therefore we have attempted a multiple EXAFS data refinement for these complexes. Each of the two DFT optimized global models were used to simultaneously fit the two experimental spectra and resulted in predominant (phen)-Pt-A β (His)Cl species in both cases.

The EXAFS refinement resulted (Table S3 in ESI[†]) in the average Pt-N(imidazole), Pt-N(phen), Pt-N(NH_3), Pt-Cl and Pt-S(Met35) distances of 2.03(1) Å, 1.993(5) Å, 2.05(1) Å, 2.235(7) Å, and 2.306(5) Å, respectively. The heavy ligand Cl and S distances tend to be shorter than those calculated by DFT. However, they are close to the distances for Pt-N (2.04(1)–2.05 Å), Pt-S (2.23–2.26(1) Å), and Pt-Cl (2.29–2.31(1) Å) from EXAFS fits for Pt-based anticancer drugs.^{22–24}

The Pt complex Pt(BPS) Cl_2 preferentially binds to the histidines of $\text{A}\beta$. In contrast, although cisPt did form adducts with $\text{A}\beta_{42}$, it coordinated predominantly at the S atom of Met35. cisPt has been reported to form DMSO adducts when prepared and kept in DMSO stocks.²⁵ The formation of the cisPt DMSO adduct dramatically changes the reactivity and thus binding, of this complex²⁵ and the reported lack of interaction of $\text{A}\beta_{16}$ with cisPt¹⁶ may be due to this phenomenon. In our hands incubating cisPt with DMSO for 30 min resulted in formation of complexes such as $\text{Pt}(\text{NH}_3)_2\text{S}(\text{DMSO})\text{Cl}$ identified by EXAFS analysis (not shown).

Even though the low affinity of ligand L (e.g. very low millimolar affinity of Pt-free 1,10-phenanthroline) for $\text{A}\beta$ should result in short residency times with the intended target, the kinetic inertness of Pt complexes means that, once bound to the target, the L-Pt-A β adducts are very stable and will not dissociate. The data presented here confirmed the hypothesis that the 1,10-phenanthroline complexes of Pt(II) coordinate to the histidine imidazole side chains of $\text{A}\beta$ and alter the biochemical and biophysical properties of the peptide. Because the Pt-complexes coordinate up to two histidine residues of $\text{A}\beta$, they occupy the Zn, Cu and Fe binding site on $\text{A}\beta$ and so inhibit metal-mediated phenomena, such as ROS generation and enhanced aggregation. The bio-inactivity of cisplatin¹⁵ may indicate that the aromatic 1,10-phenanthroline scaffold coordinated to Pt(II) confers the necessary specific targeting of the Pt preferably to histidines His6 and His14 of $\text{A}\beta^{16}$ compared with the predominantly Met35 binding of cisplatin in our studies. The results achieved with the L-Pt Cl_2 complexes support the future development of this class of compound¹⁸ as therapeutic agents for AD to ensure they efficiently cross the blood-brain barrier. The potent effects of these Pt complexes define the histidine residues of $\text{A}\beta$ as a viable therapeutic target to inhibit the neurotoxic and synaptotoxic actions of $\text{A}\beta$.

In this study XAS, MS and DLS experiments and DFT modelling were used to characterize and derive structural models of the interactions of Pt compounds with $\text{A}\beta$. Over the past few years, a number of small molecule compounds have been reported to bind to $\text{A}\beta$ and alleviate its toxic properties. Lack of experimentally determined structural models for many of their interactions with $\text{A}\beta$ is detrimental to developing effective therapeutics and diagnostics for AD. Our work reported here goes some way in addressing this deficiency.

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