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Interaction of the Human Prion Protein PrP106–126 with Metal Complexes: Potential Therapeutic Agents Against Prion Disease

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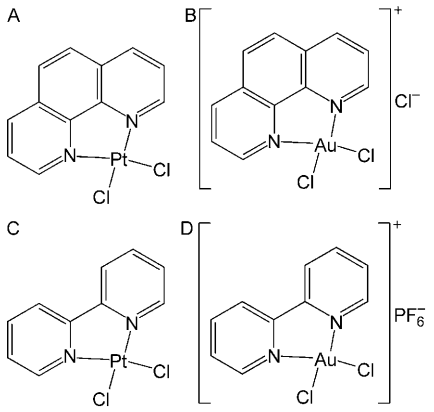
Prion diseases are fatal neurodegenerative disorders that include transmissible spongiform encephalopathies (TSEs) in animals and humans.[1] The biological functions of prion proteins are not well understood; however, current data suggests that these proteins are involved in enzymatic activity and cellular signal transduction processes.[2] An aberrant conformational isomer of the prion protein PrP^{c}, named PrP^{Sc}, is thought to be the infectious agent. The PrP^{c}–PrP^{Sc} conversion may occur without any chemical modification, but the two isomers, identical in primary structure, show different secondary structures and thus distinct physicochemical properties.[3] With an increase in prion-related diseases worldwide, there is an urgent need to design effective therapeutics against such disorders. One potential approach is the design of drugs that destabilize the structure of PrP^{Sc}.

The synthetic peptide PrP106–126 (106-KTNMKHMAGAAA-AGAVVGGLG-126), an N-terminal fragment of the human prion protein, has been thoroughly investigated as it shares many physicochemical and biological properties with PrP^{Sc}, including cellular toxicity, fibrillogenesis, and membrane-binding affinity.[4] Divalent metal cations that bind PrP106–126 modulate its aggregation and neurotoxic properties. The imidazole side chain of His111 has been identified to be the primary contributor to the high metal affinity.[4b] Recent studies indicate that alkylating the imidazole side chain of this histidine could change the metal–peptide interaction and affect the physicochemical properties of the related peptide.[5] This implies that alkylating the peptide may have therapeutic potential.

Platinum anticancer drugs have been used in the clinic for more than 30 years. Anticancer drugs based on ruthenium and gold are also of particular interest.[6] To target the imidazole side chain and change the physicochemical properties of PrP106–126, 1,10-phenanthroline (phen) was initially selected as a ligand and the ability of three complexes, [Pt(phen)Cl_2], [Au(phen)Cl_2]Cl (Scheme 1), and [Ru(phen)$_2Cl_2$] (see the Supporting Information), to interact with PrP106–126 was evaluated.[4b,7] This study demonstrated that PrP106–126 can selectively bind the metal complexes with distinguishable binding affinities and significantly alter the PrP106–126 physicochemical properties.



Scheme 1. Structures of metal complexes: A) [Pt(phen)]Cl_2; B) [Au(phen)Cl_2]Cl; C) [Pt(bipy)]Cl_2; and D) [Au(bipy)Cl_2]PF_6.

To determine whether these complexes bind directly to PrP106–126, the metal complexes were incubated with PrP106–126, at pH 5.8, and the final solutions were monitored by ^{1}H NMR spectroscopy. Figure 1 shows the NMR spectra of PrP106–126 in the presence of [Pt(phen)Cl_2] and [Au(phen)Cl_2]Cl. The ^{1}H NMR spectrum of PrP106–126 was consistent with the published spectrum and the $C_\delta H_s$ resonance of His111 was easily resolved at $\delta = 7.08$ ppm.[4b] Incubation of PrP106–126 with [Pt(phen)Cl_2] induced a change in the chemical shift of His111 $C_\delta H_s$. The resonance shifted from $\delta = 7.08$ to 7.23 ppm, which indicated that Pt^{II} was bound to PrP106–126. Surprisingly, incubation of PrP106–126 with [Au(phen)Cl_2]Cl showed a very different spectrum

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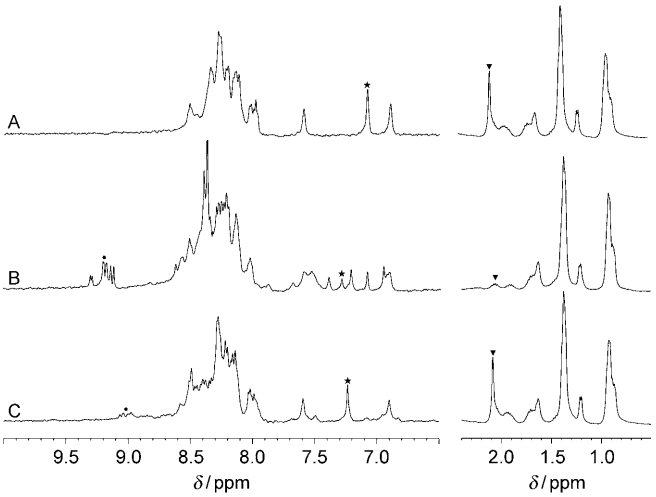

Figure 1. ^{1}H NMR spectra of 0.5 mM PrP106–126 in H_2O/DMSO at pH 5.8, 298 K. A) PrP106–126; B) PrP106–126 in the presence of [Au(phen)Cl_2]Cl (1.0 equiv); C) PrP106–126 in the presence of [Pt(phen)]Cl_2 (1.0 equiv). The signal at 2.08 ppm (▼) representing an $C_\varepsilon H_s$ group of methionine was significantly decreased when incubated with [Au(phen)Cl_2]Cl. The signal at 7.08 ppm (✱) represents the $C_\delta H_s$ of His111, which was clearly perturbed by the metal complex. The signals at around $\delta = 9.1$ ppm (●) represents mainly the metal complexes.

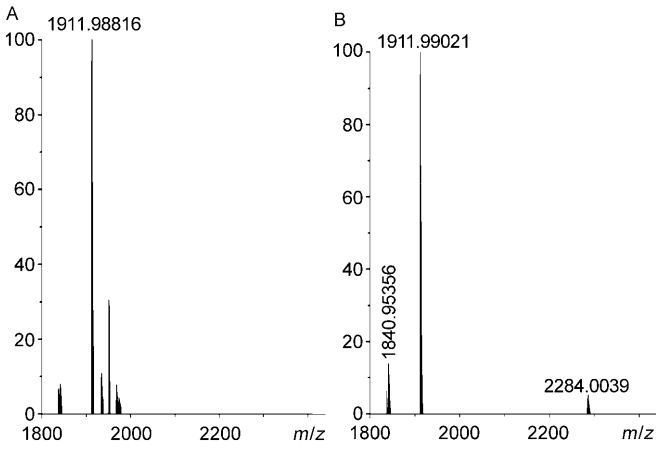

Figure 2. Deconvoluted ESI-MS spectra of PrP106–126 in the absence (A) and presence (B) of [Pt(phen)Cl_2]. The aqueous mixture was prepared by adding equivalent amounts of [Pt(phen)Cl_2] to PrP106–126. The solution was diluted in water to a final concentration of 50 μM.

in the downfield region compared with that of PrP106–126. Besides the chemical shift change for the $C_\delta H_s$ resonance of His111, new resonances, including those from the free complex and those from the bound state of PrP106–126, were observed. Interestingly, for the PrP106–126–Au complex, a distinct change in the upfield region was also observed. The signal intensity at $\delta = 2.08$ ppm assigned to an $C_\varepsilon H_s$ group of methionine had clearly decreased, indicating the possible binding site of [Au(phen)Cl_2]Cl at Met112 or Met109. In contrast, the $C_\varepsilon H_s$ resonances of the methionine residues showed negligible perturbation in the presence of [Pt(phen)Cl_2], implying different modes of binding.

The NMR spectrum of PrP106–126 was unperturbed when [Ru(phen)$_2$$Cl_2$] was mixed with the protein. This observation indicates that [Ru(phen)$_2$$Cl_2$] does not interact with PrP106–126. The absence of an interaction between PrP106–126 and [Ru(phen)$_2$$Cl_2$] is possibly due to the steric obstruction of the two phenanthroline ligands, which inhibits the binding of [Ru(phen)$_2$$Cl_2$] to PrP106–126.

To further identify the binding of PrP106–126 with the metal complexes, the samples were analyzed by ESI mass spectroscopy. PrP106–126 gave a single peak at (1911 ± 1) Da, corresponding to the expected mass. Incubation with [Pt(phen)Cl_2] produced an additional peak at (2284 ± 1) Da (Figure 2). The increase of 373 Da matches the formation of a [Pt(phen)]–PrP106–126 adduct in which the two chloro ligands have been displaced. The mass spectrum of the incubation of [Au(phen)Cl_2]Cl with PrP106–126 showed the formation of a similar adduct (see the Supporting Information).

Circular dichroism (CD) spectroscopy was used to examine the effect of the Pt and Au complexes on the conformation of PrP106–126. The CD spectrum of PrP106–126 showed a negative absorption at 197 nm. The addition of [Pt(phen)Cl_2] or [Au(phen)Cl_2]Cl led to a clear decrease in the negative absorption (see the Supporting Information). These data indicate that the conformation of PrP106–126 was changed remarkably.

PrP^{Sc} and PrP106–126 both bind the cellular prion protein at residues 112–119.[8] In addition, PrP106–126 is crucial for the aggregation of PrP^{Sc}. The aggregation of PrP106–126 was investigated by fluorescence spectroscopy using thioflavin T (ThT), which produces a featured emission when bound to PrP106–126.[9] Both [Pt(phen)Cl_2] and [Au(phen)Cl_2]Cl inhibited ThT fluorescence (see the Supporting Information), indicating that aggregation of the peptide was inhibited in the presence of the metal complexes. The aggregation was also investigated by transmission electron microscopy (TEM). The results revealed that [Au(phen)Cl_2]Cl noticeably reversed the aggregation of PrP106–126 (Figure 3). In contrast, the incubation of [Pt(phen)Cl_2] with PrP106–126 generated aggregates that appeared as thin long hairs (see the Supporting Information).

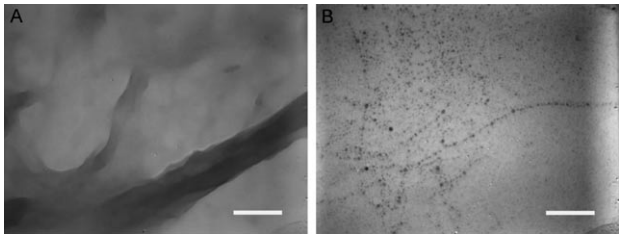

Figure 3. TEM images of PrP106–126 fibrils in A) the absence and B) the presence of [Au(phen)Cl_2]Cl.

The ThT and TEM experiments revealed that the [Au(phen)Cl_2]Cl complex was inhibiting the aggregation of PrP106–126 more effectively. For metal-based therapeutics

to be effective, a high affinity metal binding site is required. The analysis of the $[Ru(phen)_2Cl_2]$ results and mass spectral data indicate that an adequate ligand coordination is essential. In the case of the Ru complex, no interaction was observed between the metal and PrP106–126. The evaluation of a bipyridine (bipy) ligand was performed by using $[Pt(bipy)Cl_2]$ and $[Au(bipy)Cl_2]PF_6$. The resultant MS and TEM analysis provided valuable data (see the Supporting Information). The two complexes both bound PrP106–126 as metal–phenanthroline complexes. The use of single metal cations represents a poor selection because the metal cation binds nonspecifically. This nonspecific binding was observed for the interaction of $HAuCl_4$ with PrP106–126 (see the Supporting Information).

The Au complex showed better inhibition of peptide aggregation, and because $[Au(bipy)Cl_2]PF_6$ is more soluble and structurally similar to $[Au(phen)Cl_2]Cl$, it was used as a representative compound to study the binding mechanism. 1D NMR titration experiments were carried out for the $[Au(bipy)Cl_2]PF_6$–PrP106–126 system. The titration results showed a 1:1 binding mode for the system (see the Supporting Information). Therefore, a 2D TOCSY spectrum was acquired of the $[Au(bipy)Cl_2]PF_6$–PrP106–126 complex.

As mentioned above, the chemical shifts of the $C_\delta H_s$ resonance of His111 and the $C_\varepsilon H_s$ resonances of the methionines were significantly affected when PrP106–126 was mixed with $[Au(bipy)Cl_2]PF_6$. A region of the TOCSY spectrum (see the Supporting Information) showed that after incubation of $[Au(bipy)Cl_2]PF_6$ with PrP106–126 the chemical shift of His111 $C_\varepsilon H_s$ was shifted 0.13 ppm downfield. Moreover, the chemical shift of the amide protons of the methionines was shifted by 0.15 ppm and the signal intensities decreased dramatically. Furthermore, the spin systems assigned to Met109 and Met112 clearly varied with respect to the same spin systems in the absence of the metal complex, and resonances from one of the Met spin systems, likely to be Met112, showed a significantly greater change when the metal complex was present. The PrP aggregation is produced by nonfibril oligomers.[10] The oligomers are stacked by the interface of hydrophobic C-terminal residues 113–126, which might increase the rate of fibril growth. Considering the change in the aggregation properties induced by the Au complex and the position of Met112 near the hydrophobic C terminus of PrP106–126, the sulfur atom of Met112 may represent an additional binding site besides the imidazole nitrogen atom of His111.[4c] This binding mode could significantly affect the array of C-terminal residues and hence the oligomerization and further aggregation of PrP106–126.

As for the Pt complex, besides the imidazole nitrogen atom of His111, another binding site may arise from a backbone amide nitrogen. However, the inhibition of the PrP106–126 aggregation by the Pt complex is not satisfactory. In fact, we have compared the PrP106–126 binding affinity of Cu^{II} to that of $[Pt(phen)Cl_2]$ and $Au[(phen)Cl_2]Cl$. The NMR spectroscopy data indicate that $Au[(phen)Cl_2]Cl$ has a stronger binding affinity than Cu^{II} (see the Supporting Information). In contrast, Cu^{II} may interfere with the binding of the Pt complex to PrP106–126. Hence, the modulation of the Au complex to PrP106–126 aggregation is preferred.

PrP106–126 is a good example for probing the structural plasticity of PrP^{Sc}, and chemical reagents have the power to probe such structural plasticity. The interaction of a metal complex with PrP106–126 represents a new approach to better understand the design and development of metallodrugs against neurodegenerative diseases. Herein, an important discovery is demonstrated, in which, tetracoordinated Pt- and Au-based complexes inhibit the aggregation of PrP106–126, and dramatically affect the conformation of this peptide. The results show that His111 and Met112 of PrP106–126 coordinate the Au complex and that this complex has a higher affinity for PrP106–126 than the Pt complex. Importantly, the ligand must be carefully selected to ensure optimum solubility, binding affinity, the lowest cellular toxicity, and strong transmembrane permeability properties. These parameters are currently under investigation.

Experimental Section

Materials: Human prion protein fragment PrP106–126 was chemically synthesized by SBS(Beijing, China) and further purified and identified by HPLC and MS. The sample purity was more than 95%. The metal complexes were prepared as described previously,[11] dissolved in DMSO or $[D_6]$DMSO, and stored at −20 °C for later use.

NMR spectroscopy: The NMR samples were prepared in H_2O with 10% $[D_6]$DMSO, at pH 5.8. NMR spectroscopy experiments were carried out on Bruker 400 and 600 MHz spectrometers at 298 K. Suppression of the residual water signal was achieved by using a watergate pulse program with gradients. 2D TOCSY experiments were acquired with a total spin–lock time of 120 ms using a regular MLEV-17 mixing sequence.

ESI-MS: The spectra were recorded in the positive mode by direct introduction of the samples at a flow rate of 3 $\mu L\,min^{-1}$ in an APEX IV FT-ICR high-resolution mass spectrometer (Bruker, USA). For acquisition, DataAnalysis 4.0 software (Bruker) was used and the masses were obtained by using the integrated deconvolution tool.

CD analysis: The spectra were measured on a Jasco J-810 spectropolarimeter (Japan Spectroscopy, Japan). The samples were prepared in 10 mM phosphate buffer at pH 7.2. A scan rate of 100 $nm\,min^{-1}$ with 1 s response time was employed. The final spectrum for each sample was the average of three repeated experiments.

ThT assay: The metal compounds were added in equimolar amounts to PrP106–126 (0.1 mM) in a 10 mM phosphate buffer, pH 7.2. The samples were incubated with ThT (10 μM) and the fluorescence was monitored by using a LS55 spectrofluorometer (Perkin–Elmer, USA). The ThT signal was quantified by averaging the fluorescence emission at 500 nm over 10 s when the sample was excited at 432 nm.

TEM experiments: After aging PrP106–126, equivalent amounts of metal complex was added and then incubated at 37 °C for 24 h. The final peptide concentration was 0.1 mM with 1% DMSO. Each sample was spotted onto a carbon-coated 600-mesh copper grid and negatively stained by 2% phosphotungstic acid. Air-dried specimens were examined and photographed by a Hitachi H-800 electron microscope (Hitachi, Japan) operating at 200 kV.

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Keywords: aggregation · interactions · medicinal chemistry · metal complexes · prion proteins

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