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fac-$\{Ru(CO)_3\}^{2+}$ Selectively Targets the Histidine Residues of the β-Amyloid Peptide 1-28. Implications for New Alzheimer's Disease Treatments Based on Ruthenium Complexes

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The reaction of the ruthenium(II) complex *fac*-$[Ru(CO)_3Cl_2(N^1\text{-thz})]$ (**I** hereafter; thz = 1,3-thiazole) with human β-amyloid peptide 1-28 ($A\beta_{28}$) and the resulting $\{Ru(CO)_3\}^{2+}$ peptide adduct was investigated by a variety of biophysical methods. ^{1}H NMR titrations highlighted a selective interaction of $\{Ru(CO)_3\}^{2+}$ with $A\beta_{28}$ histidine residues; circular dichroism revealed the occurrence of a substantial conformational rearrangement of $A\beta_{28}$; electrospray ionization mass spectrometry (ESI-MS) suggested a prevalent 1:1 metal/peptide stoichiometry and disclosed the nature of peptide-bound metallic fragments. Notably, very similar ESI-MS results were obtained when **I** was reacted with $A\beta_{42}$. The implications of the above findings for a possible use of ruthenium compounds in Alzheimer's disease are discussed.

Alzheimer's disease (AD) is a high-incidence neurodegenerative disorder, leading to progressive and irreversible brain damage that is characterized by severe symptoms such as memory loss, confusion, personality changes, and impairment of language skills. The senile plaques, the major neuropathological hallmark of AD, are mainly composed of aggregated β-amyloid peptides (especially $A\beta_{40}$ and $A\beta_{42}$),[1−5] whose overproduction triggers a cascade of biochemical processes, eventually leading to apoptotic neuronal cell death.[6] Recent studies have pointed out that soluble oligomers rather than mature fibrils are the major neurotoxic $A\beta$ species in AD.[7−10] Moreover, a few transition-metal ions, such as Zn^{2+}, Cu^{2+}, and Fe^{2+}, were implicated in AD progression.[11−15]

No curative AD treatments have been developed so far. What has been attempted is trying to limit the side effects of AD, such as memory impairment, which, however, has no effect on the slowing down the disease.[16−18] The current medications are mainly aimed at sustaining the function of the acetylcholine and glutamate pathways, the two brain neurotransmitters primarily involved in learning and memory processes.[19−21]

It was recently shown that targeting His-13 and His-14 of $A\beta$s may result in a dramatic decrease of the cytotoxicity.[22] This observation offers a strong rationale for the development

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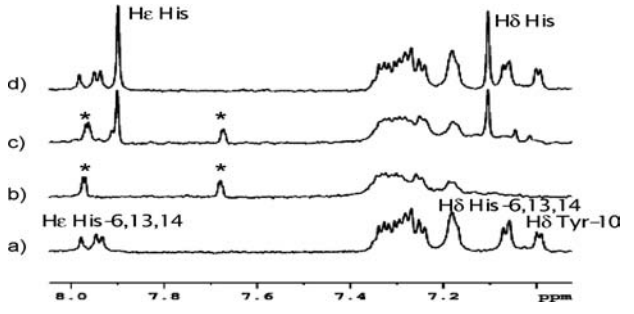
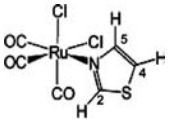


Figure 1. ^{1}H NMR spectra of (a) 0.20 mM Aβ_{28} at pH 7.5 and T = 298 K; (b) after the addition of 1.0 equiv of **I**, (c) after the addition of 1.0 equiv of **I** and 1.0 equiv of L-His, and (d) after the addition of 1.0 equiv of L-His. Proton signals of thz are labeled with asterisks.

Scheme 1. Chemical Structure of **I**



of new anti-AD therapeutic strategies directed to reduce β-amyloid neurotoxicity. Indeed, a few platinum compounds were successfully exploited in vitro for this purpose.[23] Experience previously gathered in our laboratory on a variety of metal-based drugs[24,25] led us to hypothesize that ruthenium compounds might offer a valid and even better alternative to platinum compounds for the selective modification of β-amyloid histidines. In fact, the well-known preference of ruthenium species for imidazole nitrogen atoms is typically accompanied by a more favorable toxicity profile compared to platinum drugs (i.e., *ruthenium compounds are on the average far less cytotoxic than platinum compounds*). With this in mind, we have explored the reaction taking place between the novel ruthenium(II) complex *fac*-[Ru(CO)$_3$Cl$_2$(N^1-thz)] (**I** hereafter; thz = 1,3-thiazole; Scheme 1), recently synthesized and characterized by some of us,[26] and the Aβ_{28} peptide (DAEFRHDSGYEVHHQKLVFFAEDVGSNK-NH$_2$), a good model for the longer Aβ_{40} and Aβ_{42} peptides. For a better description of such an interaction, other peptides and amino acids were investigated as well, such as Aβ_{28} protected at the N terminus (AcAβ_{28}), the rat Aβ_{28} (rAβ_{28}), L-His, GlyGlyHis (GGH), and the reduced glutathione (GSH). Our analysis mainly relies on NMR measurements but is independently supported by results arising from the application of other physicochemical methods.

One-dimensional (1D) ^{1}H NMR spectra of **I** at pH 7.5 clearly show two sets of signals belonging to thz, with those exactly matching the resonances of thz in the free state being the most abundant; this observation strongly suggests that a high percentage of thz is released from the complex (Figure S1 in the Supporting Information, SI).

Upon the addition of **I** to Aβ_{28}, large and selective broadenings of Aβ ^{1}H NMR resonances were detected. The direct comparison of 1D NMR spectra, recorded without (Figure 1a) or with 1.0 equiv of **I** (Figure 1b), highlights the nearly complete

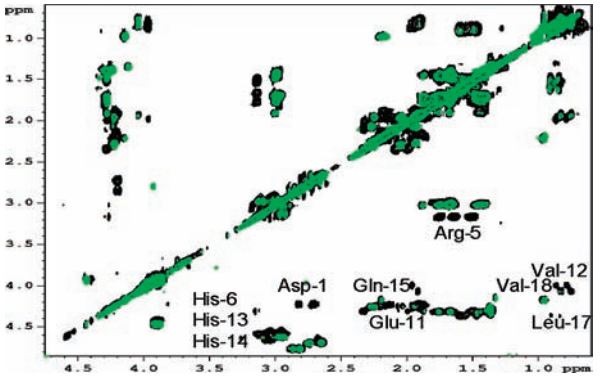


Figure 2. ^{1}H−^{1}H TOCSY of 0.20 mM Aβ_{28} at pH 7.5 and T = 298 K in the absence (black) and presence (green) of 1.0 equiv of **I**.

disappearance of the aromatic signals of the three histidine residues and of Tyr-10. The large broadening of these signals is diagnostic for relevant changes in the chemical environment of the corresponding nuclei upon metallodrug−peptide interaction and strongly suggests histidine binding to the ruthenium(II) center. As expected, no variations were detected for the thz protons, consistent with the fact that it does not interact with Aβ_{28}.

A deeper inspection of the Aβ_{28}−**I** interaction was obtained from ^{1}H−^{1}H TOCSY and NOESY maps. Figure 2 compares the spectra recorded without (black) or with (green) 1.0 equiv of **I**. Together with the histidine and tyrosine broadening, other spin systems were greatly affected. The correlations of Asp-1, Arg-5, Glu-11, Val-12, Gln-15, Leu-17, and Val-18 were no longer detectable in the presence of the ruthenium complex. A detailed analysis of the ^{1}H−^{1}H TOCSY fingerprint region indicated the additional broadenings of Asp-7, Gly-9, Phe-19, Phe-20, and Ala-21 (Figure S2 in the SI). Unfortunately, the extensive line broadening caused by **I** prevented the determination of any detailed structural feature for the metallodrug−Aβ_{28} species based on NOESY maps. Similar NMR experiments were also recorded on AcAβ_{28} and rAβ_{28}. Analysis of the ^{1}H−^{1}H TOCSY maps upon the addition of **I** (Figure S3 in the SI) shows that AcAβ_{28} behaves as Aβ_{28}, indicating that the N-terminal amino group is not essential for ruthenium(II) binding. On the contrary, rAβ_{28} was much less affected than Aβ_{28}. Because the rat β-amyloid bears three mutations relative to human β-amyloid (R5G, Y10F, and H13R), the detected differences strongly point out a pivotal role played by the His13−His14 pair in ruthenium(II) binding.

The selective broadenings observed on several NMR resonances might be explained with the occurrence of an intermediate exchange regime (on the NMR time scale) among different bound conformations. In fact, the addition of increasing amounts of **I** (up to 2.5 equiv) does not resolve the proton line broadening. The NMR spectra are strongly time-dependent: indeed, the same ^{1}H NMR spectrum, registered after 2 weeks, revealed greatly reduced signal intensities both for the amyloid peptide and for the ruthenium complex (Figure S4 in the SI). This generalized loss of the signal intensity might be accounted for by the formation of large insoluble aggregates because, a few days after sample preparation, the Aβ_{28}−**I** solutions showed the formation of an amorphous precipitate.

Finally, NMR spectra on samples containing Aβ_{28} and one of potential competitors for {Ru(CO)$_3$}$^{2+}$ (L-His, GGH, and

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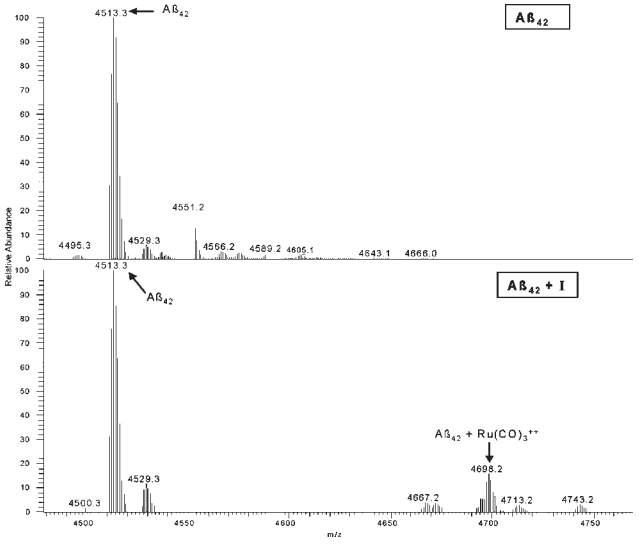

Figure 3. Deconvoluted ESI-MS spectra of Aβ_{42} and Aβ_{42} + **I**. The aqueous mixture of Aβ_{42} + **I** was prepared by mixing equivalent amounts of Aβ_{42} and **I**. The mixture was incubated at 37 °C for 1 h. For MS experiments, both the mixture and Aβ_{42} solution were diluted in 0.5% HCOOH to a final concentration of 10 μM.

GSH) with and without the additional presence of **I** were also performed to gain information on the Aβ_{28}−**I** affinity. Before analysis of the Aβ_{28}−**I** interaction in the presence of these possible competitors, the independent interaction between **I** and each of the above-mentioned systems was investigated (data not shown). The addition of either L-His and GGH to equimolar Aβ_{28}−**I** solutions does not largely affect the broadening induced by **I** on Aβ_{28} resonances, pointing out that Aβ_{28} is still bound to ruthenium in the presence of L-His (Figure 1c) and GGH (Figure S5 in the SI). Moreover, samples containing Aβ_{28} and L-His (Figure 1c) or GGH (Figure S5 in the SI) show histidine signals less affected by **I** than in samples without Aβ_{28} (data not shown), indicating a weaker interaction with ruthenium in the presence of Aβ_{28}. On the contrary, the addition of GSH resulted in a relevant narrowing of Aβ_{28}{Ru(CO)$_3$}$^{2+}$ resonances, revealing a strong competition between Aβ_{28} and GSH for {Ru(CO)$_3$}$^{2+}$ (Figure S6 in the SI). The same was observed by comparing ^{1}H−^{1}H TOCSY experiments: the presence of L-His and GGH does not substantially alter the **I**-induced line broadening of Aβ_{28} signals (Figure S7 in the SI). On the other hand, the presence of GSH restored nearly completely the intensity of Aβ_{28} TOCSY correlations (Figure S7 in the SI).

The interaction between **I** and Aβ_{28} was subsequently analyzed by circular dichroism (CD) spectroscopy (Figure S8 in the SI). The Aβ_{28} peptide exhibits a CD pattern typical of a random-coil conformation.[27] Immediately after the addition of **I**, a shift of the CD signal from 198 to 203 nm was clearly observed. Afterward, the intensity of the CD signal progressively decreased with time and nearly disappeared after 15 days. Such behavior, nicely matching the NMR findings, is explained by the formation of the insoluble aggregates, which dramatically reduce CD signal intensities.

To gain independent information on the investigated system, electrospray ionization mass spectrometry (ESI-MS) spectra were recorded, on both Aβ_{28} and Aβ_{42} peptides, before and after the addition of **I**. The resulting ESI-MS deconvoluted spectra are shown in Figures 3 and S9 in the SI. The free peptides are characterized by intense peaks, centered at 4513.3 and 3302.6 Da. Very interestingly, peptide treatment with **I** leads to the appearance of new intense peaks at 4698.2 and 3487.4 Da, which are straightforwardly assigned as metallodrug−peptide adducts. In both cases, the position of the new peaks is consistent with a mass increase of 185 Da, a value corresponding to the molecular fragment Ru(CO)$_3^{2+}$, suggesting that such a type of fragment is actually bound to the Aβ. In Figure S10 in the SI, the observed and theoretical MS spectra are shown for 5+ charged species Aβ_{28} + Ru(CO)$_3^{2+}$. The strict analogy of the ESI-MS behavior manifested by the two peptides in their reactions with **I** strongly supports the view that the same kind of interaction is taking place.

In conclusion, all of the collected results provide unambiguous evidence that **I**, after displacement of the labile chloride ligands and thz, binds tightly and quickly to Aβ_{28}. The NMR findings strongly support histidine coordination to the ruthenium(II) center, with the formation of a stable Ru-Aβ_{28} adduct. Such an adduct features a relevant structural rearrangement of the residues encompassing the 10−20 peptide portion. Detection of the broadening on residues 17−21, in fact, is ascribed to relevant changes in the local conformation upon metal binding. The ruthenium-induced NMR line broadening resembles the zinc-induced one,[28,29] suggesting the occurrence of a similar type of interaction. In turn, ESI-MS data suggest the predominant formation of a monoruthenated derivative. Finally, CD analysis showed that the peptide experiences an important and rapid local conformational transition upon ruthenium binding. In view of the selective binding of ruthenium(II) to Aβ histidine residues, of the favorable effects caused by histidine modification on β-amyloid neurotoxicity and of the usually safer biological profile of ruthenium compared to platinum agents, we strongly suggest to consider ruthenium compounds further as possible candidates for new experimental AD treatments.

Supporting Information Available: Material and methods; ^{1}H−^{1}H TOCSY regions of Aβ_{28}, acAβ_{28}, and rAβ_{28} in the absence and presence of **I**; ^{1}H NMR spectra of Aβ_{28} in the presence of **I**, L-His, GGH, and GSH; ^{1}H−^{1}H TOCSY regions of Aβ_{28} in the absence and presence of **I**, L-His, GGH, and GSH; CD spectra of Aβ_{28} and Aβ_{28} + **I**; deconvoluted ESI-MS spectra of Aβ_{28} and Aβ_{28} + **I**; observed and theoretical MS spectra of a five-charged Aβ_{28} + Ru(CO)$_3^{2+}$ fragment. This material is available free of charge via the Internet at http://pubs.acs.org.

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