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Exploring the Reactions of β-Amyloid (Aβ) Peptide 1−28 with Al^{III} and Fe^{III} Ions†

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Supporting Information

ABSTRACT: The reactions of human β-amyloid peptide 1−28 (Aβ28) with Al^{III} and Fe^{III} ions were investigated by ^{1}H NMR and electrospray ionization mass spectrometry (ESI-MS) under pH conditions close to physiological ones. ^{1}H NMR titrations, performed in the 5.3−8.0 pH range, revealed that no measurable amounts of Aβ28-Al^{III} or Aβ28-Fe^{III} adducts are formed; such metal adducts could not be obtained even by changing a number of experimental conditions, e.g., temperature, buffer, nature of the salt, etc. These observations were later confirmed by ESI-MS. It is thus demonstrated that Aβ28, at physiological pH, is not able to form binary complexes with Al^{III} and Fe^{III} ions of sufficient stability to compete with metal hydroxide precipitation. The biological implications of these findings are discussed in the frame of current literature.

Alzheimer's disease (AD) is a progressive and irreversible brain disorder with a very high health and social impact.[1,2] Although the etiopathogenesis of AD is far from being understood, it is widely accepted that β-amyloid (Aβ) plaques are a major pathological hallmark of neurodegeneration in AD.[1−4] Notably, Aβ, a 38−43 amino acid long peptide derived from the transmembrane amyloid precursor protein, is intrinsically unstructured in its monomeric form but can easily aggregate into oligomers, protofibrils, and fibrils, adopting a well-organized β-sheet structure.[1−7] Out of these, Aβ oligomers are known to be highly neurotoxic species.

Transition-metal ions like Fe^{III}, Cu^{II}, and Zn^{II} as well as Al^{III} ions are very likely involved, as factors or cofactors, in the etiopathogenesis of many neurodegenerative disorders.[8−14] Notably, these metals are abundant in Aβ plaques of AD patients. The exact mechanisms through which metals may participate in AD pathogenesis are a matter of intense debate.[2−4,10,15−22] Besides metal-induced Aβ aggregation,[23−26] redox-active metals such as Cu^{II} and Fe^{III} might further modulate Aβ neurotoxicity through redox cycling; indeed, iron or copper ions, bound to Aβ peptides, promote the generation of reactive oxygen species capable of producing severe oxidative damage and of triggering neurodegeneration.[2−4,10,15−18,27,28]

While zinc, copper, and iron are essential biometals, aluminum is a nonphysiological, environmentally abundant metal, showing a marked neurotoxicity, even in low concentration.[11] Though not having specific transport or accumulation machineries at its disposal, Al^{III} may exploit the molecular mechanisms of similar metal ions, e.g., Fe^{III}, and eventually accumulates in brain tissues.[11] Remarkably, Al^{III} was reported to induce peculiar changes in the Aβ conformation that stabilize oligomeric states, increase the surface hydrophobicity and membrane permeability, and enhance toxicity.[11,29]

While the reactions of amyloid peptides with Zn^{II} and Cu^{II} ions have been investigated in depth through several recent studies[17−22] and the formation of the respective adducts has been carefully documented, fewer investigations are available on their reactions with Al^{III} and Fe^{III} ions.[11,30−32] Moreover, these latter studies were often carried out under highly heterogeneous experimental conditions, so that an exhaustive and convincing description of adduct formation has not been achieved yet. This prompted us to explore such reactions further. The present study is grounded on the β-amyloid peptide 1−28 (Aβ28), which is far more amenable to ^{1}H NMR and electrospray ionization mass spectrometry (ESI-MS) analysis than full-length Aβ40 and Aβ42 peptides.[33] Yet, Aβ28 remains an excellent model for longer Aβ peptides because it retains the ability to form regular amyloid fibrils and also contains, in full, the major metal binding site, corresponding to the 1−16 peptide portion.[17−22]

The reactions of Aβ28 with the above metal ions were investigated, at first, through ^{1}H NMR. Indeed, Aβ28 shows remarkable solubility in water, and samples suitable for ^{1}H NMR analysis may be prepared and are stable for several hours. The reactions of Aβ28 with metals were monitored under conditions progressively closer to the physiological ones starting from acidic pH.

Initially, Aβ28 was challenged with aluminum(III) chloride, under moderately acidic conditions (pH 5.3), to limit aluminum hydroxide precipitation. Up to 1.8 equiv if Al^{III} was added to the peptide solution without observation of any relevant change in the ^{1}H NMR signals (Figure 1 in the Supporting Information). ^{1}H NMR spectra, recorded at temperatures ranging from 278 to 318 K, showed no appreciable variations after Al^{III} addition. In

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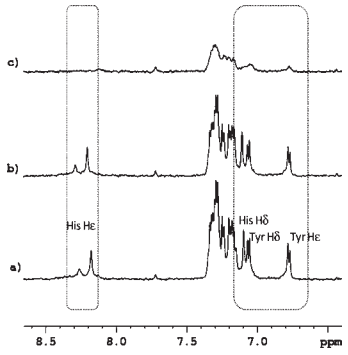

Figure 1. Selected regions of ^{1}H NMR spectra of 0.15 mM Aβ28 (pH 6.4, T = 298 K): (a) free; (b) +0.9 equiv of aluminum lactate; (c) 0.9 equiv of Zn^{II}.

particular, no effects were detected on the side-chain protons of potential metal binding residues, such as His, Asp, and Glu. The virtual absence of perturbations in Aβ28 proton resonances strongly suggests that no Aβ28-Al^{III} adduct is formed at this pH.

Later on, NMR analysis was extended to higher pH values (pH 6.4, 7.4, and 8.0) and to either water or buffer solutions (phosphate or MES) to rule out any critical role of pH and/or buffer in modulating Aβ28-Al^{III} interactions. In all cases, evident aluminum(III) hydroxide precipitation was observed. ^{1}H NMR analysis performed with both equimolar and saturated $AlCl_3$ solutions revealed the virtual absence of any Aβ28 signal perturbation (Figure 2 in the Supporting Information). Thus, all 1D and 2D ^{1}H NMR spectra, obtained through changes of the pH, buffer, or temperature, were completely unaffected by metal addition, ruling out the occurrence of Aβ28-Al^{III} interactions.

The impossibility to form adducts with aluminum(III) chloride prompted us to repeat the experiments using a different aluminum(III) source, namely, aluminum lactate $[Al(lact)_3]$. The lactate ligand solubilizes aluminum in the form of its 1:3 complex, effectively contrasting hydroxide precipitation. Moreover, aluminum lactate might form, at least in principle, ternary complexes with the Aβ28 peptide, possibly of higher stability than the binary complex, as was previously reported for the Aβ16-Iron-NTA system.[31] The direct comparison of ^{1}H NMR spectra recorded in the absence or in the presence of 0.9 equiv of aluminum lactate (Figure 1, traces a and b) again ruled out the formation of any stable metal−peptide complex.

For comparison purposes, the spectra of the free peptide and of the peptide reacted with Zn^{II} ions are also shown. Notably, the addition of Zn^{II} results in significant spectral changes (Figure 1, trace c) consistent with Zn^{II} binding to the major metal binding site of Aβ28;[19,20,34−38] in particular, the large broadening of the imidazole resonances is in agreement with all three histidines being directly involved in metal coordination.

In contrast, the addition of aluminum lactate does not cause any specific alteration of NMR resonances (Figure 1, trace b). A quite generalized line broadening is instead noticed at mixing; with time, the line broadening progressively decreases and an evident precipitate is eventually formed. These observations point out that aluminum lactate, too, fails to form stable adducts with Aβ28; invariantly, an insoluble aluminum-containing species slowly forms and separates as a solid.

The reaction of Al^{III} with Aβ28 was independently assayed by ESI-MS, using a previously established experimental protocol.[32] Again, no evidence of metal−peptide complexation was obtained,

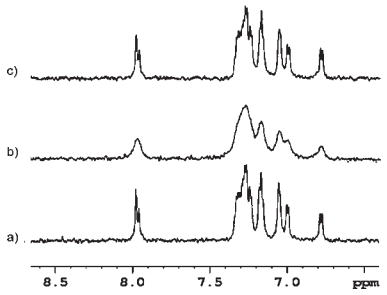

Figure 2. ^{1}H NMR spectra of 0.15 mM Aβ28 and 0.1 mM phosphate buffer (pH 7.2, T = 298 K): (a) free; (b) +0.9 equiv of Fe^{III}; (c) +0.9 equiv of Fe^{III} after 24 h.

at variance with the cases of *fac*-$\{Ru(CO)_3\}^{2+}$ and of Cu^{II} and Zn^{II} ions, previously described.[21,33]

Next, the reactions of Aβ28 with Fe^{III} ions were explored according to similar experimental strategies. ^{1}H NMR spectra, recorded after the addition of stoichiometric amounts of Fe^{III} ions, are shown in Figure 2. A quite evident line broadening with no appreciable resonance shifts is noticed at mixing (Figure 2, traces b); afterward, the formation of a precipitate slowly takes place with nearly complete restoration of the ^{1}H NMR spectrum of the free peptide (Figure 1, trace c). We interpret this spectral behavior as follows. Fe^{III} causes, at first, some generalized line broadening because of its paramagnetic nature and the formation of oligomeric iron hydroxo species; then, iron(III) hydroxide precipitates, and the broadening effects are reverted. Possible Fe^{III} binding to Aβ28 was evaluated by independent ESI-MS measurements at pH 7.0. Similar to the case of Al^{III}, it was not possible to observe any peak corresponding to the formation of stable metal−peptide adducts.

These results, altogether, offer final and unambiguous evidence that both Al^{III} and Fe^{III} ions are not able to form binary complexes with Aβ28 under physiologically relevant pH conditions. In other words, the interaction of these metal ions with Aβ28 is not sufficiently strong to contrast precipitation of the respective hydroxides. The fact that the intensity of the NMR signals is not altered by Al^{III} or Fe^{III} further implies that no appreciable aggregation is induced by these metal ions.

Our findings are in apparent contrast with a number of previous papers where the formation of aluminum(III) and iron(III) complexes was claimed for a variety of β-amyloid peptides.[25,29−32,39,40] However, those studies were carried out under very different solution conditions and often at far more acidic pH values. Tight control of the pH conditions, carefully pursued in the present study, allows us to state that for both tripositive ions formation and precipitation of the respective hydroxides are definitely preferred compared to Aβ28 complexation. It might be just speculated that Aβ28 differs appreciably from Aβ42 and Aβ40 in its metal-complexing properties and that the 29−40/42 peptide portion has some role in stabilizing metal−peptide interactions. Another possibility in justifying Aβ complexation to Al^{III} or Fe^{III} would be that stable ternary complexes are formed as in the case of the Aβ16-iron-NTA system,[31] but the nature of the physiological ligand capable of stabilizing such ternary adducts is still elusive.

In conclusion, with the present study, we have explored the chances of forming aluminum(III) and iron(III) complexes for the Aβ28 peptide, under pH conditions close to physiological ones. It clearly emerges that Aβ28 cannot form binary complexes with Fe^{III} and Al^{III} sufficiently stable to prevent or limit precipitation

of the respective metal hydroxides. These experimental results are in our opinion of great interest in relation to the metallobiology of AD because they imply that the formation of binary complexes between Aβ28 and Al^{III} or Fe^{III} is strictly disfavored at physiological pH and unlikely. It follows that the presence of relevant amounts of iron and aluminum in the AD senile plaques, well documented in previous studies,[12,13] should be better interpreted in terms of metal binding to other components of the senile plaques. Conversely, the studies on metal interactions of Aβ peptides should mainly focus on Cu^{II} and Zn^{II} ions.

■ ASSOCIATED CONTENT

Supporting Information. Experimental methods and ^1H NMR and ESI-MS spectra. 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. All authors have given approval to the final version of the manuscript.

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