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# Metallomics

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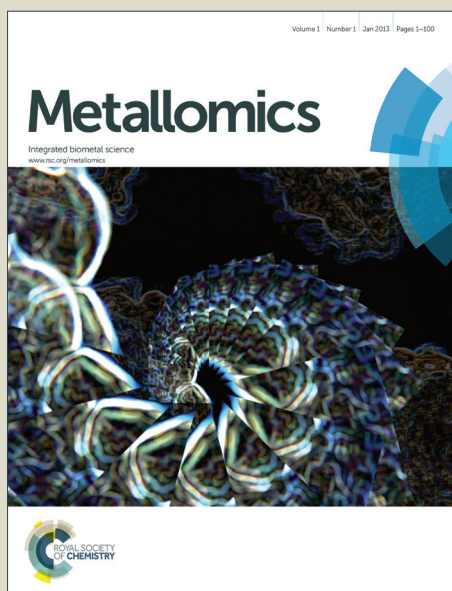
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# Metallomics

## COMMUNICATION

## The English (H6R) familial Alzheimer's disease mutation facilitates zinc-induced dimerization of the amyloid-β metal-binding domain†

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**Interaction of $Zn^{2+}$ with the metal-binding domain of the English (H6R) amyloid-β mutant results in formation of peptide dimers. The mutation causes exclusion of His6 from the zinc chelation pattern observed in the intact domain and triggers assembly of the dimers via zinc ion coordinated by [11]EVHH[14] fragments.**

Alzheimer's disease (AD), the most common type of dementia, is associated with oligomerization and aggregation of amyloid-β peptide (Aβ).[1] There are several familial mutations located within Aβ that cause early onset AD: English (H6R), Tottori (D7N), Taiwan (D7H), Flemish (A21G), Dutch (E22Q), Italian (E22K), Arctic (E22G), and Iowa (D23N).[2,3] Pathological Aβ aggregation is mediated by zinc ions,[4-7] therefore the English familial AD mutation (H6R),[3] that affects His6 residue from zinc coordination of the metal-binding domain (residues 1-16, Aβ$_{1-16}$) of Aβ,[8] is of particular interest. It has been shown that English mutation increases neurotoxicity of respective Aβ (H6R-Aβ) oligomers[9] and accelerates H6R-Aβ fibril formation.[10] However, the impact of zinc ions on the aggregation state of H6R-Aβ has not been yet investigated. Synthetic peptides corresponding to Aβ$_{1-16}$ are good soluble models to examine zinc binding to Aβ.[8,11-15] In the present work, we have characterized interaction of zinc ions with the H6R mutant of Aβ$_{1-16}$ amidated at the C-terminus, with either acetylated ($^{Ac}$H6R-Aβ$_{1-16}$) or free (H6R-Aβ$_{1-16}$) N-terminus, using surface plasmon resonance (SPR), isothermal titration calorimetry (ITC) and NMR spectroscopy.

The ability of $^{Ac}$H6R-Aβ$_{1-16}$ to form oligomers in the presence of zinc ions has been tested using SPR biosensing. This has been done by registering interactions between the dissolved $^{Ac}$H6R-Aβ$_{1-16}$ peptides and the same peptides immobilized on an optical chip. A set of sensograms has been obtained corresponding to the injections of solution with different concentrations of $^{Ac}$H6R-Aβ$_{1-16}$ in the presence and absence of zinc ions (Fig. 1, details provided in the ESI). The results given in Figure 1 indicate that oligomers are formed only in the presence of zinc ions.
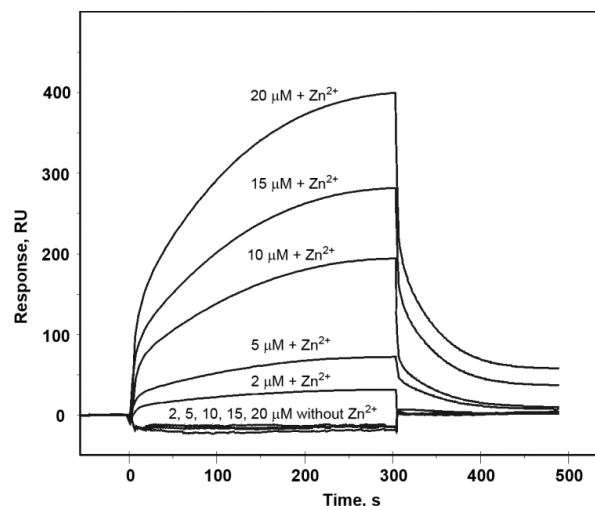


**Fig. 1.** SPR sensograms of $^{Ac}$H6R-Aβ$_{1-16}$ (analyte) interactions with the immobilized ligand ($^{Ac}$H6R-Aβ$_{1-16}$) at 25 ºC in 10 mM HEPES, in the presence and absence of 0.1 mM ZnCl$_2$, pH 6.8. Total analyte concentrations in the running buffer were 2, 5, 10, 15 and 20 µM.

The stoichiometry of $Zn^{2+}$ binding to the $^{Ac}$H6R-Aβ$_{1-16}$ determined by ITC (Fig. 2B, N=0.5) suggests the formation of a complex where two peptide molecules interact with one zinc ion. In contrast, non-modified $^{Ac}$Aβ$_{1-16}$ forms monomeric complex in presence of zinc ions with stoichiometry of the peptide-metal interaction 1:1 (Fig.

2A).[11,12] This process can be described by the following equilibrium scheme,[16,17] where P denotes the peptide $^{Ac}$H6R-Aβ$_{1-16}$:

$$Zn + P \overset{K1}{\leftrightarrow} Zn \cdot P \qquad (1)$$

$$P + Zn \cdot P \overset{K2}{\leftrightarrow} P \cdot Zn \cdot P \qquad (2)$$
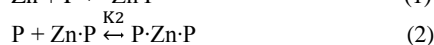
By fitting the binding isotherm, the value of apparent association constant $K_a = K_1 \times K_2 = 0.24 \times 10^4$ M$^{-2}$ was determined. In order to rule out participation of the N-terminal amino group in chelation of zinc ion, we have compared the thermodynamic parameters of Zn$^{2+}$-binding to the peptides with acetylated ($^{Ac}$H6R-Aβ$_{1-16}$) and free N-terminus (H6R-Aβ$_{1-16}$) (Table S1). Both peptides bind Zn$^{2+}$ similarly, indicating that the N-terminus of H6R-Aβ$_{1-16}$ does not participate in Zn$^{2+}$ chelation.
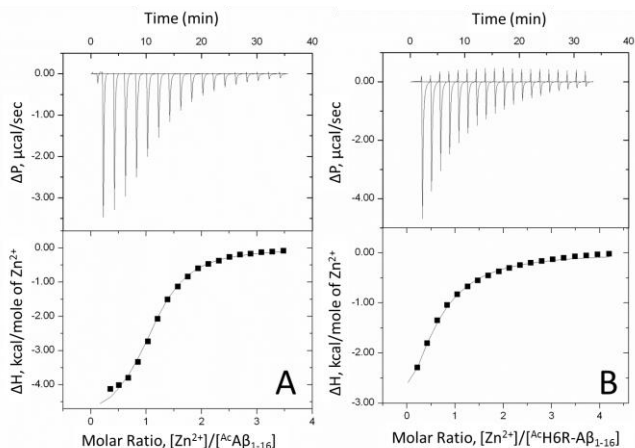


**Fig. 2**. ITC titration curves (upper panel) and the binding isotherms (lower panel) for the zinc interactions with $^{Ac}$Aβ$_{1-16}$[12] (A) and $^{Ac}$H6R-Aβ$_{1-16}$ (B) at 25°C in 50 mM Tris buffer, pH 7.3.

Formation of the dimer Zn·($^{Ac}$H6R-Aβ$_{1-16}$)$_2$ in solution was unambiguously confirmed by NMR. Sharp signals observed in NMR spectra of the peptide $^{Ac}$H6R-Aβ$_{1-16}$ (Fig. 3A, Table S1) are broadened upon addition of zinc ions (Fig. 3B, Table S2), which is typical for the formation of zinc complexes with Aβ fragments containing the metal binding domain.[18-20] However, in contrast to other previous studies of Aβ peptides,[11,13,19] interaction of $^{Ac}$H6R-Aβ$_{1-16}$ with Zn$^{2+}$ leads to the appearance of the additional set of the peptide resonances (see assignments in Table S3). For example, in addition to signals at 0.76 and 0.87 ppm which correspond to the methyl groups of Val12 of Zn·$^{Ac}$H6R-Aβ$_{1-16}$ (Table S2), new signal appears at 0.23 ppm (Fig. 3B, Table S3). This signal along with the resonances from several other residues (Table S3) belongs to the dimeric form of the complex Zn·($^{Ac}$H6R-Aβ$_{1-16}$)$_2$. Indeed, increase of the total peptide concentration results in higher intensities of the signals assigned to the dimeric form (Fig. S2). Moreover, Job's plot for intensity changes of the signal at 0.23 ppm (Fig. 3B) observed in experiments carried out using the method of continuous variations[21] (details provided in the ESI) indicate that the stoichiometry of peptide-zinc interaction is 2:1 (Fig. 4, curve 1). Meanwhile Job's plot of the changes of His13 and His14 Hε1 chemical shifts of the monomeric Zn·($^{Ac}$H6R-Aβ$_{1-16}$) form (Fig. 4, curve 2) shows 1:1 stoichiometry of peptide-zinc interaction.
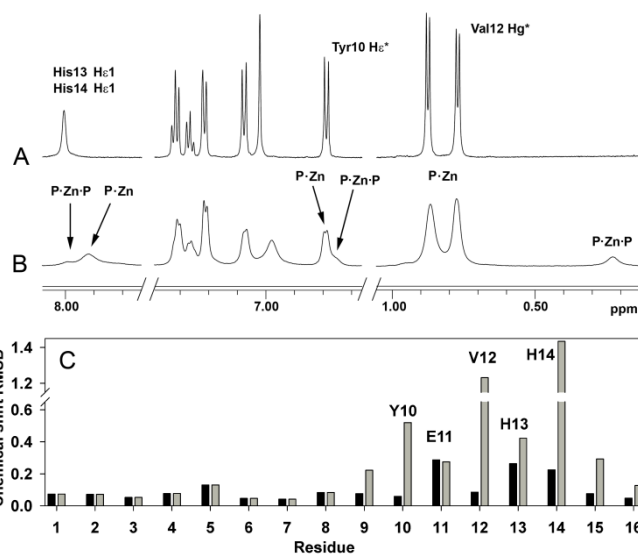


**Fig. 3.** Representative parts of the $^1$H NMR spectra of 1.1 mM $^{Ac}$H6R-Aβ$_{1-16}$ peptide (denoted P) in the free state (A) and in the presence of a two-fold molar excess of ZnCl$_2$ (B). Root-mean-square deviations (RSMD) of the $^1$H and $^{13}$C chemical shifts between the free and zinc-bound states of $^{Ac}$H6R-Aβ$_{1-16}$ (C). Data for the peptide monomer Zn·P are shown in black, and for the dimer P·Zn·P in grey. Experiments were carried out in 10 mM Bis-Tris-d$_{19}$ buffer in D$_2$O, pH 6.8, 283K and 600 MHz.

Chemical shift changes measured in Zn$^{2+}$ titration NMR experiments (Fig. S3-S6) were used to identify metal binding sites both in monomeric Zn·($^{Ac}$H6R-Aβ$_{1-16}$) and dimeric Zn·($^{Ac}$H6R-Aβ$_{1-16}$)$_2$ complexes (Fig. 3C). Observed changes highlight the group of residues involved in the binding of zinc ion in monomeric complex (Fig. 3C, black bars). Such binding site is formed by two histidine residues (His13 and His14) and Glu11. It should be noted that these three residues compose the primary zinc-recognition fragment $^{11}$EVHH$^{14}$.[12] Changes of the chemical shifts that accompany formation of the dimeric complex are much higher than those observed for monomer (Fig. 3C, grey bars). They indicate that zinc interaction interface in the Zn·($^{Ac}$H6R-Aβ$_{1-16}$)$_2$ complex is also formed by the fragment $^{11}$EVHH$^{14}$, where the most probable zinc ion chelators are Glu11 and His14 of two interacting subunits. At the same time considerable chemical shift changes of the residues that are unable to bind zinc ions (i.e. Tyr10 and Val12) are observed. Such changes can only be explained by the conformational reorganization of the peptide molecule upon formation of stable a dimeric complex.

Exchange cross-peaks in the 2D ROESY spectrum (Fig. S7) between the resonances that belong to monomeric and dimeric peptide complexes indicate that there is an equilibrium between the two forms. For example, such exchange peaks with positive intensities are seen between the Hγ* signals of Val12 at 0.21 and 0.84 ppm. However, existence of two separate sets of signals corresponding to the Zn·($^{Ac}$H6R-Aβ$_{1-16}$) and Zn·($^{Ac}$H6R-Aβ$_{1-16}$)$_2$ complexes evidences that the dimeric complex is stable in the NMR time scale in contrast to all other studied zinc-bound Aβ$_{1-16}$ dimers.[11,13,19] Comparison of the NMR spectra of $^{Ac}$H6R-Aβ$_{1-16}$ and H6R-Aβ$_{1-16}$ recorded under identical conditions in the presence of ZnCl$_2$ (Fig. S8, S9) confirms our ITC data (Table S1) showing that the N-terminal amino group does not participate in interaction with zinc ions at neutral pH.

Previously it was shown that complexes of Aβ$_{1-16}$ with zinc ions exist as either dimers,[22] where residues from the $^{11}$EVHH$^{14}$ regions of two interacting peptide chains jointly coordinate the zinc ion, or

monomers.[11,12] In monomers zinc ion is coordinated by three residues from the region [11]EVHH[14] together with the distant His6 residue of the $A\beta_{1-16}$ molecule. The first possibility can be realized in the pathogenic dimers of full-length $A\beta$ molecules,[23,24] while the existence of soluble monomeric $A\beta$ complexes with one zinc ion can be associated to a normal physiological state of $A\beta$ in biological fluids. Recently we have shown that withdrawal of His6 from zinc ion chelation in conjunction with the [11]EVHH[14] site upon Ser8 phosphorylation leads to zinc-dependent dimerization of the respective $A\beta_{1-16}$ peptide ($pS8\text{-}A\beta_{1-16}$).[13] In the present study we
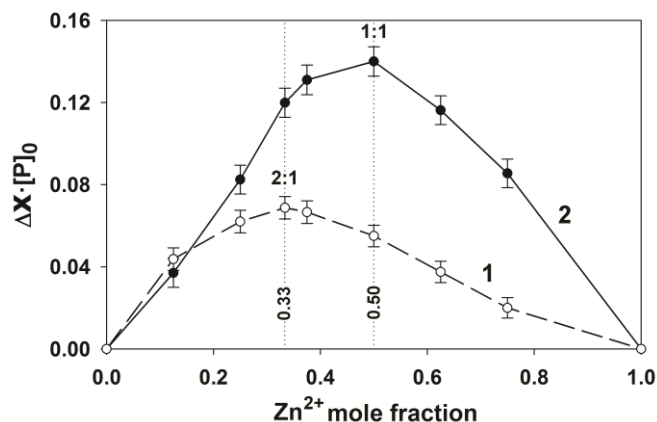


**Fig. 4.** Results of the $Zn^{2+}$ isomolar NMR titration of $^{Ac}H6R\text{-}A\beta_{1-16}$. Abscissa represents the mole fraction of $ZnCl_2$, and the ordinate axis shows the product of $\Delta X \cdot [P]_0$, where $\Delta X$ is either $\Delta I$ (change of the relative intensity of the Val12 $H\gamma^*$ signal at 0.2 ppm) for the curve 1 (open circles), or $\Delta\delta$ (change of the chemical shift of histidine $H\delta 2$ signals) for the curve 2 (black circles), and $[P]_0$ – represents the total $^{Ac}H6R\text{-}A\beta_{1-16}$ peptide concentration in the sample. Curve 2 corresponds to the formation of zinc-peptide complex with the stoichiometry 1:1. Curve 1 corresponds to the formation of the peptide dimer in complex with single zinc atom (stoichiometry 2:1). Experimental details are given in the ESI.

have for the first time documented the formation of water-soluble zinc-bonded dimers of $^{Ac}H6R\text{-}A\beta_{1-16}$. NMR spectra of human $^{Ac}A\beta_{1-16}$ in the presence of $Zn^{2+}$ ions highlight formation of dimers structurally similar to the $Zn\cdot(^{Ac}H6R\text{-}A\beta_{1-16})_2$, as evidenced by characteristic resonances of the Val12 methyl group and His14 HN (Fig. S10). However, the quantity of dimer formed by the wild-type peptide is substantially smaller compared to the H6R analog, a result which can be explained by the involvement of His6 residue in coordination of zinc ion in the monomeric peptide-zinc complex formed by $^{Ac}A\beta_{1-16}$. Dimerization interface in $^{Ac}H6R\text{-}A\beta_{1-16}$ is virtually identical to that observed for $Zn\cdot(pS8\text{-}A\beta_{1-16})_2$.[13] Each of the peptides $^{Ac}H6R\text{-}A\beta_{1-16}$ and $pS8\text{-}A\beta_{1-16}$, has only one amino acid change (due to mutation or chemical modification) distinguishing their sequences from the native $A\beta_{1-16}$ form. Although the changes have different origins (genetic versus kinase dependent) they result in the formation of zinc-bound dimers stabilized by the same dimerization interface as in the intact $A\beta_{1-16}$,[22] suggesting a general mechanism of zinc-bound $A\beta$ dimer formation.

## Conclusions

We have demonstrated that interaction of the metal-binding domain of the English (H6R) $A\beta$ mutant with zinc ions results in the formation of stable soluble dimers under physiologically relevant conditions. These findings suggest that possible molecular mechanism of the AD pathogenicity of English mutation could be related to the increased propensity of respective $A\beta$ species to undergo zinc-dependent oligomerization. The interface of zinc-mediated complexes is formed by the residues [11]EVHH[14] from the

two $^{Ac}H6R\text{-}A\beta_{1-16}$ chains and appears to be identical to other zinc-induced $A\beta$ dimers,[13,16,22,23] thus making the $A\beta$ site 11-14 a putative drug target to prevent zinc-induced aggregation of $A\beta$ in AD progression.

## Notes and references

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