

Minimal Zn²⁺ Binding Site of Amyloid- β

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ABSTRACT Zinc-induced aggregation of amyloid- β peptide (A β) is a hallmark molecular feature of Alzheimer's disease. Here we provide direct thermodynamic evidence that elucidates the role of the A β region 6–14 as the minimal Zn²⁺ binding site wherein the ion is coordinated by His⁶, Glu¹¹, His¹³, and His¹⁴. With the help of isothermal titration calorimetry and quantum mechanics/molecular mechanics simulations, the region 11–14 was determined as the primary zinc recognition site and considered an important drug-target candidate to prevent Zn²⁺-induced aggregation of A β .

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Alzheimer's disease, a fatal neurodegenerative disorder of the elderly, is characterized by extracellular depositions of amyloid- β peptide (A β) saturated with metal ions (1). It is believed that Zn²⁺ ions play a key role in pathological aggregation of A β and therefore affect the pathogenesis of Alzheimer's disease (2–5). The amino acid region 1–16 (A β (1–16)) is generally considered as the metal binding domain of A β (6). N-acetylated and C-amidated peptide A β (1–16) is soluble and stable in the presence of zinc ions under physiological conditions (7), and the three-dimensional structures of this domain in Zn²⁺-loaded and Zn²⁺-free states have been solved by NMR (8). In the Zn²⁺-A β (1–16) complex, zinc is tetrahedrally coordinated to His⁶, His¹³, and His¹⁴ through their N- δ 1, N- ϵ 2, and N- δ 1 atoms, respectively, and to Glu¹¹ through its O- δ atom.

At the same time, several alternative coordination modes of zinc binding to A β with nonacetylated N-terminus are discussed (6,9). While there is general agreement regarding the participation of the three histidine residues, the role of Asp¹ as fourth zinc chelator instead of Glu¹¹ has been proposed (10). In addition, a pentacoordination of zinc ion by Asp¹, His⁶, Glu¹¹, His¹³, and His¹⁴ has been also suggested (11). However, in contrast to the data published by Zirah et al. (8), none of the alternative complexes has been defined structurally. Thus, the exact mode of zinc coordination by A β deserves further investigation.

In this work, we characterize the thermodynamics of the binding of Zn²⁺ to A β fragments and A β (1–16) mutants by isothermal titration calorimetry (ITC) in order to determine the minimal Zn²⁺-binding site of A β under physiological conditions. We also use the quantum mechanics/molecular mechanics (QM/MM) method to model Zn²⁺ recognition by A β .

Thermodynamic parameters of Zn²⁺ binding to A β fragments and A β (1–16) mutants obtained by ITC (Fig. 1 and Fig. S1 in the Supporting Material) are presented in Table 1.

It can be seen that acetylation of N-terminus does not dramatically change Zn²⁺ binding to A β (1–16).

The truncation of five amino acids from the N-terminal part and two from the C-terminus of A β (1–16) leads to the fivefold increase in its affinity to Zn²⁺ (Fig. 1, Table 1). This could be explained by the formation of a hydrogen bond between His⁶ and either Asp¹ or Glu³ in A β (1–16) in the absence of zinc (Fig. S2). At the same time, titration of the A β (1–5) fragment with Zn²⁺ ions did not produce any heat. It is important to note that angiotensin-converting enzyme proteolytically cleaves this fragment from A β (12). Thus, angiotensin-converting enzyme can increase zinc affinity to A β and act as a modulator of zinc binding. Visible aggregation of A β (6–14) peptide was observed in the calorimetric cell upon interaction with zinc. However, this aggregation does not affect stoichiometry of Zn²⁺ binding (Table 1), suggesting that formation of oligomers occurs not via Zn²⁺ ions, but through interpeptide interactions of Zn²⁺-loaded A β (6–14) in the same manner as for A β which first binds to zinc and then is conformationally driven to aggregates (13). Further removing of one of the terminal histidines from A β (6–14) results in the remarkable drop in association constant K_a from $8.6 \times 10^4 \text{ M}^{-1}$ to $1.7 \times 10^3 \text{ M}^{-1}$ for peptide A β (7–14) and to $3.4 \times 10^3 \text{ M}^{-1}$ for peptide A β (6–13).

These data indicate that all Zn²⁺-chelating amino acids are located within the A β (6–14) peptide and that the A β (1–5) segment of A β is not necessary for Zn²⁺ binding. Amino acids located between Asp⁷ and Tyr¹⁰ of A β do not participate in Zn²⁺ binding, because their removal (A β (7–14) \rightarrow A β (11–14)) only slightly changes the affinity of the peptide to zinc. Substitution of Glu¹¹ to Ala as well as

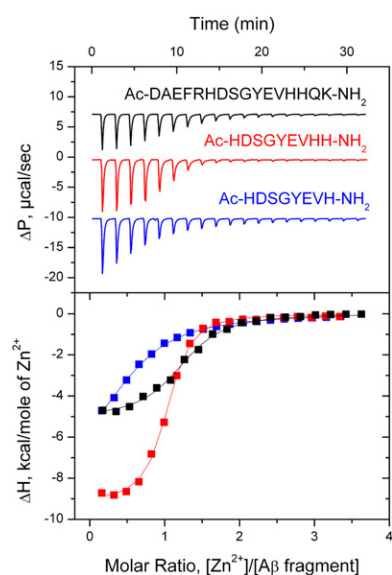


FIGURE 1 ITC titration curves (upper panel) and binding isotherms (lower panel) for zinc interactions with A β (1–16), A β (6–14), and A β (6–13) (black, red, and blue curves, respectively) at 25°C in 50 mM Tris buffer, pH 7.3.

His⁶ to Arg in A β (1–16) causes strong decrease of Zn²⁺ affinity (Table 1). These data clearly demonstrate that His⁶, Glu¹¹, and His¹⁴ are necessary for Zn²⁺ coordination, which is in good agreement with NMR structural data (8).

Zinc binding to all studied A β fragments and A β (1–16) mutants was enthalpy-driven (Table 1). Contrary to the A β (1–16) domain, Zn²⁺ binding to A β (6–14), A β (7–14), and A β (6–13) peptides was entropically unfavorable. This is explained by burying hydrophobic surfaces upon Zn²⁺ binding to A β (1–16), which is in line with the structural

TABLE 1 Thermodynamic parameters of Zn²⁺ ions binding to A β fragments and A β (1–16) mutants obtained by ITC at 25°C in 50 mM Tris buffer, pH 7.3

Peptide*	N [†]	K_a^{\ddagger} (M ⁻¹ × 10 ⁴)	ΔH^{\dagger} (kcal M ⁻¹)	$T\Delta S$ (kcal M ⁻¹)
A β (1–16)	1.1	1.80	–4.0	1.8
NH ₂ -A β (1–16)	1.1	3.90	–5.4	0.9
A β (6–14)	1.0	8.60	–9.2	–2.5
A β (6–13)	0.6	0.34 [¶]	–8.1	–3.3
A β (7–14)	0.9	0.17	–7.9	–3.5
A β (11–14)	0.5	0.27 [¶]	–2.6	2.1
A β (1–5)	No binding			
A β (1–16) H6R	0.8	0.25	–6.4	–1.8
A β (1–16) E11A	1.0	0.54	–6.9	–1.8

All experiments were carried out on an iTC₂₀₀ instrument (MicroCal, Northampton, MA).

*All peptides were purchased from Biopeptide (San Diego, CA). The N- and C-termini of each peptide were protected with acetyl and amide, respectively; peptide NH₂-A β (1–16) was protected at the C-terminus with amide.

[†]Standard deviation did not exceed $\pm 10\%$.

[‡]Standard deviation did not exceed $\pm 20\%$.

[¶]Due to peptide dimerization, K_a dimension is equal to M⁻².

data (8). Stoichiometry of Zn²⁺ binding to A β (6–13) and A β (11–14) fragments is close to 0.5 (Table 1), indicating dimer formation via Zn²⁺ ion which is in a good agreement with a recent simulation study (14). High positive entropy of Zn²⁺ binding to A β (11–14) dimer is explained by formation of hydrophobic contacts between the two subunits.

This is also confirmed by an NMR study of the Zn²⁺-A β (11–14) dimer. ¹H NMR spectra (Fig. S3) demonstrate that resonance peaks of both valine methyl groups are strongly upshifted upon Zn²⁺ binding to A β (11–14) (from 0.83 and 0.75 to 0.45 and 0.69 ppm), due to the proximity of the zinc ion. The peak that displays the stronger shift undergoes significant broadening, also indicating that the corresponding methyl group is very close to Zn²⁺. The coordination of Zn²⁺ by two peptide molecules resulting in the formation of a symmetrical complex is in good agreement with NMR data as well. In such a complex, valine methyl groups within the immediate neighborhood of Zn²⁺ ion are oriented inward, which means that they are not exposed considerably to the solution.

Thus, we propose that the tetrapeptide A β (11–14) containing three Zn²⁺ chelating amino acids functions as a Zn²⁺ recognition site. In line with this hypothesis, NMR data demonstrate that this site has a definite structure in the absence of Zn²⁺ (8). We suppose that Zn²⁺ capture by the structured EVHH region is the initial step in the formation of the Zn²⁺-A β (1–16) complex.

The dimerization of the A β (11–14) peptide upon Zn²⁺ binding makes it impossible to determine the thermodynamic parameters of binding for the monomeric peptide, i.e., to reveal the role of this fragment in the mechanism of Zn²⁺ recognition by A β in more detail. Therefore, we applied an approach similar to that described by Furlan and La Penna (15). Theoretical calculations of the stability of a transitional EVHH complex with zinc were performed. QM/MM simulations were applied to analyze Zn²⁺ binding by A β (11–14), A β (6–14), and A β (1–16) peptides. Starting conformation for each peptide complexed with Zn²⁺ was derived from the A β (1–16) structure (PDB ID: 1ZE9).

Simulations showed that both A β (6–14) and A β (1–16) peptides where Zn²⁺ was chelated by His⁶, Glu¹¹, His¹³, and His¹⁴ kept stable tetrahedral coordination of zinc during 8 ps of the QM/MM simulation trajectory. In the case of A β (6–14) energy, the QM part (see the Supporting Material for details) was slightly smaller because His¹³ is better oriented in the complex (Table 2). For A β (11–14), fast (1-ps) formation of the tetrahedral Zn²⁺ coordination environment (Table 2) with a water molecule as the fourth chelator was observed (see Movie S1 in the Supporting Material). In the longer (8-ps) simulation, this water molecule was stable in terms of orientation and position. Summarizing our simulation results, we conclude that initially Zn²⁺ is recognized and captured by the EVHH region of A β and temporarily coordinated by water as the fourth chelator (Fig. 2 A). This state exists until His⁶ comes close to Zn²⁺ due to

TABLE 2 Energy and geometry values of zinc coordination by A β (1–16), A β (6–14), and A β (11–14) peptides obtained by QM/MM simulations

	A β (1–16)	A β (6–14)	A β (11–14)
Energy of QM subsystem, kJ/mol	-618571 \pm 27	-618621 \pm 64	NA
Distance, nm			
Zn ²⁺ \rightarrow His ⁶ (N δ)	0.202 \pm 0.006	0.203 \pm 0.005	0.206 \pm 0.010
Zn ²⁺ \rightarrow H ₂ O (O)	NA	NA	0.206 \pm 0.010
Zn ²⁺ \rightarrow Glu ¹¹ (O δ)	0.220 \pm 0.014	0.226 \pm 0.015	0.200 \pm 0.007
Zn ²⁺ \rightarrow His ¹³ (N ϵ)	0.207 \pm 0.006	0.207 \pm 0.006	0.202 \pm 0.007
Zn ²⁺ \rightarrow His ¹⁴ (N δ)	0.209 \pm 0.006	0.209 \pm 0.006	0.199 \pm 0.006
Angle, degrees			
His ⁶ -Zn ²⁺ -Glu ¹¹	96.5 \pm 4.6	95.6 \pm 5.2	NA
His ⁶ -Zn ²⁺ -His ¹³	131.0 \pm 3.7	134.2 \pm 4.2	NA
His ⁶ -Zn ²⁺ -His ¹⁴	110.8 \pm 4.2	110.4 \pm 4.6	NA
Glu ¹¹ -Zn ²⁺ -His ¹³	85.2 \pm 4.5	83.0 \pm 3.2	98.1 \pm 6.1
Glu ¹¹ -Zn ²⁺ -His ¹⁴	96.3 \pm 4.6	96.9 \pm 5.2	112.6 \pm 7.0
His ¹³ -Zn ²⁺ -His ¹⁴	117.0 \pm 5.2	114.0 \pm 4.2	116.5 \pm 5.7
H ₂ O-Zn ²⁺ -Glu ¹¹	NA	NA	97.5 \pm 6.9
H ₂ O-Zn ²⁺ -His ¹³	NA	NA	111.9 \pm 8.0
H ₂ O-Zn ²⁺ -His ¹⁴	NA	NA	115.5 \pm 7.9

NA: not applicable.

thermal fluctuations and replaces the water molecule in the zinc coordination environment, resulting in the formation of the final complex (Fig. 2 B).

In summary, we used ITC to characterize the interactions of Zn²⁺ with fragments of native A β and A β (1–16) mutants. The amino acid region 6–14 of A β was determined as the minimal Zn²⁺-binding site wherein the ion is coordinated by His⁶, Glu¹¹, His¹³, and His¹⁴. Both ITC and QM/MM showed that three of four residues from the A β region 11–14 (EVHH) contribute to zinc binding, and that this tetrapeptide readily forms dimers linked through a zinc ion, similarly to a model proposed for A β aggregation in a recent molecular dynamics study (14).

These data allow us to consider A β (11–14) tetrapeptide as a primary Zn²⁺-recognition site of A β and an important drug target candidate to prevent Zn²⁺-induced aggregation of A β .

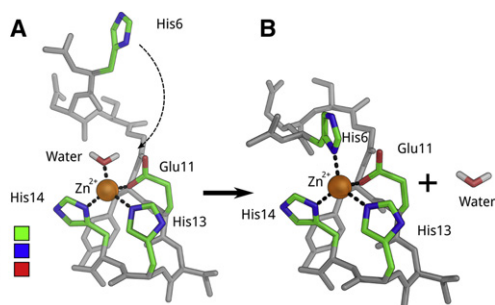


FIGURE 2 Schema of Zn²⁺ ion recognition by the A β binding site 6–14 according to QM/MM calculations performed with the GROMACS/CPMD package (<http://www.tougaloo.edu/research/qmmm/index.htm>) based on PDB ID: 1ZE9. (A) structure of the intermediate Zn²⁺-A β complex with a water molecule as the fourth chelator; (B) structure of the final Zn²⁺-A β complex. See also Movie S2 in the Supporting Material.

SUPPORTING MATERIAL

Three figures, materials and methods, and two movies are available at [http://www.biophysj.org/biophysj/supplemental/S0006-3495\(10\)S0006-3495\(10\)01161-6](http://www.biophysj.org/biophysj/supplemental/S0006-3495(10)S0006-3495(10)01161-6).

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