Biophysical Journal Volume 99 November 2010 L84–L86

Minimal Zn^{2+} Binding Site of Amyloid-β

Philipp O. Tsvetkov,[†‡] Alexandra A. Kulikova,[†] Andrey V. Golovin,[§†] Yaroslav V. Tkachev,[†] Alexander I. Archakov,[‡] Sergey A. Kozin,[†‡*] and Alexander A. Makarov[†*]

[†]Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia; [‡]Orekhovich Institute of Biomedical Chemistry, Russian Academy of Medical Sciences, Moscow, Russia; and [§]Bioengineering and Bioinformatics Department, M. V. Lomonosov Moscow State University, Moscow, Russia

ABSTRACT Zinc-induced aggregation of amyloid-β peptide (Aβ) is a hallmark molecular feature of Alzheimer's disease. Here we provide direct thermodynamic evidence that elucidates the role of the Aβ region 6–14 as the minimal Zn^{2+} binding site wherein the ion is coordinated by His^6, Glu^{11}, His^{13}, and His^{14}. 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 Zn^{2+}-induced aggregation of Aβ.

Received for publication 28 July 2010 and in final form 14 September 2010.

*Correspondence: kozinsa@gmail.com or aamakarov@eimb.ru

Alzheimer's disease, a fatal neurodegenerative disorder of the elderly, is characterized by extracellular depositions of amyloid-β peptide (Aβ) saturated with metal ions (1). It is believed that Zn^{2+} ions play a key role in pathological aggregation of Aβ and therefore affect the pathogenesis of Alzheimer's disease (2–5). The amino acid region 1–16 (Aβ(1–16)) is generally considered as the metal binding domain of Aβ (6). N-acetylated and C-amidated peptide Aβ(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 Zn^{2+}-loaded and Zn^{2+}-free states have been solved by NMR (8). In the Zn^{2+}-Aβ(1–16) complex, zinc is tetrahedrally coordinated to His^6, His^{13}, and His^{14} through their N-δ1, N-ε2, and N-δ1 atoms, respectively, and to Glu^{11} through its O-δ atom.

At the same time, several alternative coordination modes of zinc binding to Aβ with nonacetylated N-terminus are discussed (6,9). While there is general agreement regarding the participation of the three histidine residues, the role of Asp^1 as fourth zinc chelator instead of Glu^{11} has been proposed (10). In addition, a pentacoordination of zinc ion by Asp^1, His^6, Glu^{11}, His^{13}, and His^{14} 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 Aβ deserves further investigation.

In this work, we characterize the thermodynamics of the binding of Zn^{2+} to Aβ fragments and Aβ(1–16) mutants by isothermal titration calorimetry (ITC) in order to determine the minimal Zn^{2+}-binding site of Aβ under physiological conditions. We also use the quantum mechanics/molecular mechanics (QM/MM) method to model Zn^{2+} recognition by Aβ.

Thermodynamic parameters of Zn^{2+} binding to Aβ fragments and Aβ(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 Zn^{2+} binding to Aβ(1–16).

The truncation of five amino acids from the N-terminal part and two from the C-terminus of Aβ(1–16) leads to the fivefold increase in its affinity to Zn^{2+} (Fig. 1, Table 1). This could be explained by the formation of a hydrogen bond between His^6 and either Asp^1 or Glu^3 in Aβ(1–16) in the absence of zinc (Fig. S2). At the same time, titration of the Aβ(1–5) fragment with Zn^{2+} ions did not produce any heat. It is important to note that angiotensin-converting enzyme proteolytically cleaves this fragment from Aβ (12). Thus, angiotensin-converting enzyme can increase zinc affinity to Aβ and act as a modulator of zinc binding. Visible aggregation of Aβ(6–14) peptide was observed in the calorimetrical cell upon interaction with zinc. However, this aggregation does not affect stoichiometry of Zn^{2+} binding (Table 1), suggesting that formation of oligomers occurs not via Zn^{2+} ions, but through interpeptide interactions of Zn^{2+}-loaded Aβ(6–14) in the same manner as for Aβ which first binds to zinc and then is conformationally driven to aggregates (13). Further removing of one of the terminal histidines from Aβ(6–14) results in the remarkable drop in association constant K_a from $8.6 \times 10^4\ M^{-1}$ to $1.7 \times 10^3\ M^{-1}$ for peptide Aβ(7–14) and to $3.4 \times 10^3\ M^{-1}$ for peptide Aβ(6–13).

These data indicate that all Zn^{2+}-chelating amino acids are located within the Aβ(6–14) peptide and that the Aβ(1–5) segment of Aβ is not necessary for Zn^{2+} binding. Amino acids located between Asp^7 and Tyr^{10} of Aβ do not participate in Zn^{2+} binding, because their removal (Aβ(7–14) → Aβ(11–14)) only slightly changes the affinity of the peptide to zinc. Substitution of Glu^{11} to Ala as well as

Editor: Ruth Nussinov.

doi: 10.1016/j.bpj.2010.09.015

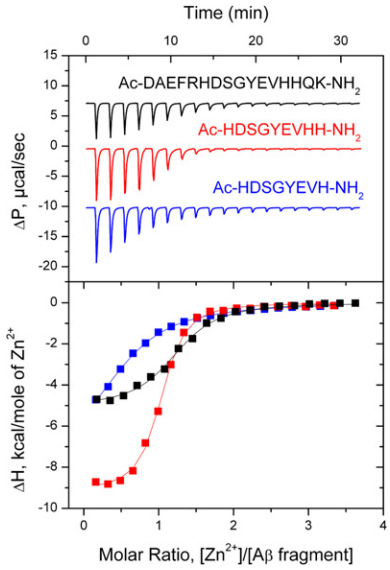

FIGURE 1 ITC titration curves (*upper panel*) and binding isotherms (*lower panel*) for zinc interactions with Aβ(1–16), Aβ(6–14), and Aβ(6–13) (*black*, *red*, and *blue curves*, respectively) at 25°C in 50 mM Tris buffer, pH 7.3.

His^6 to Arg in Aβ(1–16) causes strong decrease of Zn^{2+} affinity (Table 1). These data clearly demonstrate that His^6, Glu^{11}, and His^{14} are necessary for Zn^{2+} coordination, which is in good agreement with NMR structural data (8).

Zinc binding to all studied Aβ fragments and Aβ(1–16) mutants was enthalpy-driven (Table 1). Contrary to the Aβ(1–16) domain, Zn^{2+} binding to Aβ(6–14), Aβ(7–14), and Aβ(6–13) peptides was entropically unfavorable. This is explained by burying hydrophobic surfaces upon Zn^{2+} binding to Aβ(1–16), which is in line with the structural data (8). Stoichiometry of Zn^{2+} binding to Aβ(6–13) and Aβ(11–14) fragments is close to 0.5 (Table 1), indicating dimer formation via Zn^{2+} ion which is in a good agreement with a recent simulation study (14). High positive entropy of Zn^{2+} binding to Aβ(11–14) dimer is explained by formation of hydrophobic contacts between the two subunits.

TABLE 1 Thermodynamic parameters of Zn^{2+} ions binding to Aβ fragments and Aβ(1–16) mutants obtained by ITC at 25°C in 50 mM Tris buffer, pH 7.3

Peptide*	$N^{\dagger}$	$K_a^{\S}$ ($M^{-1} \times 10^4$)	$\Delta H^{\dagger}$ (kcal M^{-1})	$T\Delta S$ (kcal M^{-1})
Aβ(1–16)	1.1	1.80	−4.0	1.8
NH_2-Aβ(1–16)	1.1	3.90	−5.4	0.9
Aβ(6–14)	1.0	8.60	−9.2	−2.5
Aβ(6–13)	0.6	0.34¶	−8.1	−3.3
Aβ(7–14)	0.9	0.17	−7.9	−3.5
Aβ(11–14)	0.5	0.27¶	−2.6	2.1
Aβ(1–5)	No binding			
Aβ(1–16) H6R	0.8	0.25	−6.4	−1.8
Aβ(1–16) E11A	1.0	0.54	−6.9	−1.8

All experiments were carried out on an iTC_{200} 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 NH_2-Aβ(1–16) was protected at the C-terminus with amide.

†Standard deviation did not exceed ±10%.

§Standard deviation did not exceed ±20%.

¶Due to peptide dimerization, K_a dimension is equal to M^{-2}.

This is also confirmed by an NMR study of the Zn^{2+}-Aβ(11–14) dimer. ^{1}H NMR spectra (Fig. S3) demonstrate that resonance peaks of both valine methyl groups are strongly upshifted upon Zn^{2+} binding to Aβ(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 Zn^{2+}. The coordination of Zn^{2+} 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 Zn^{2+} ion are oriented inward, which means that they are not exposed considerably to the solution.

Thus, we propose that the tetrapeptide Aβ(11–14) containing three Zn^{2+} chelating amino acids functions as a Zn^{2+} recognition site. In line with this hypothesis, NMR data demonstrate that this site has a definite structure in the absence of Zn^{2+} (8). We suppose that Zn^{2+} capture by the structured EVHH region is the initial step in the formation of the Zn^{2+}-Aβ(1–16) complex.

The dimerization of the Aβ(11–14) peptide upon Zn^{2+} 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 Zn^{2+} recognition by Aβ 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 Zn^{2+} binding by Aβ(11–14), Aβ(6–14), and Aβ(1–16) peptides. Starting conformation for each peptide complexed with Zn^{2+} was derived from the Aβ(1–16) structure (PDB ID: 1ZE9).

Simulations showed that both Aβ(6–14) and Aβ(1–16) peptides where Zn^{2+} was chelated by His^6, Glu^{11}, His^{13}, and His^{14} kept stable tetrahedral coordination of zinc during 8 ps of the QM/MM simulation trajectory. In the case of Aβ(6–14) energy, the QM part (see the Supporting Material for details) was slightly smaller because His^{13} is better oriented in the complex (Table 2). For Aβ(11–14), fast (1-ps) formation of the tetrahedral Zn^{2+} 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 Zn^{2+} is recognized and captured by the EVHH region of Aβ and temporarily coordinated by water as the fourth chelator (Fig. 2 *A*). This state exists until His^6 comes close to Zn^{2+} due to

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

	Aβ(1–16)	Aβ(6–14)	Aβ(11–14)
Energy of QM subsystem, kJ/mol			
	−618571 ± 27	−618621 ± 64	NA
Distance, nm			
Zn^{2+} → His^{6} (Nδ)	0.202 ± 0.006	0.203 ± 0.005	0.206 ± 0.010
Zn^{2+} → H_2O (O)	NA	NA	0.206 ± 0.010
Zn^{2+} → Glu^{11} (Oδ)	0.220 ± 0.014	0.226 ± 0.015	0.200 ± 0.007
Zn^{2+} → His^{13} (Nε)	0.207 ± 0.006	0.207 ± 0.006	0.202 ± 0.007
Zn^{2+} → His^{14} (Nδ)	0.209 ± 0.006	0.209 ± 0.006	0.199 ± 0.006
Angle, degrees			
His^{6}-Zn^{2+}-Glu^{11}	96.5 ± 4.6	95.6 ± 5.2	NA
His^{6}-Zn^{2+}-His^{13}	131.0 ± 3.7	134.2 ± 4.2	NA
His^{6}-Zn^{2+}-His^{14}	110.8 ± 4.2	110.4 ± 4.6	NA
Glu^{11}-Zn^{2+}-His^{13}	85.2 ± 4.5	83.0 ± 3.2	98.1 ± 6.1
Glu^{11}-Zn^{2+}-His^{14}	96.3 ± 4.6	96.9 ± 5.2	112.6 ± 7.0
His^{13}-Zn^{2+}-His^{14}	117.0 ± 5.2	114.0 ± 4.2	116.5 ± 5.7
H_2O-Zn^{2+}-Glu^{11}	NA	NA	97.5 ± 6.9
H_2O-Zn^{2+}-His^{13}	NA	NA	111.9 ± 8.0
H_2O-Zn^{2+}-His^{14}	NA	NA	115.5 ± 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 $Zn^{2+}$with fragments of native Aβ and Aβ(1–16) mutants. The amino acid region 6–14 of Aβ was determined as the minimal Zn^{2+}-binding site wherein the ion is coordinated by His^{6}, Glu^{11}, His^{13}, and His^{14}. Both ITC and QM/MM showed that three of four residues from the Aβ 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 Aβ aggregation in a recent molecular dynamics study (14).

These data allow us to consider Aβ(11–14) tetrapeptide as a primary Zn^{2+}-recognition site of Aβ and an important drug target candidate to prevent Zn^{2+}-induced aggregation of Aβ.

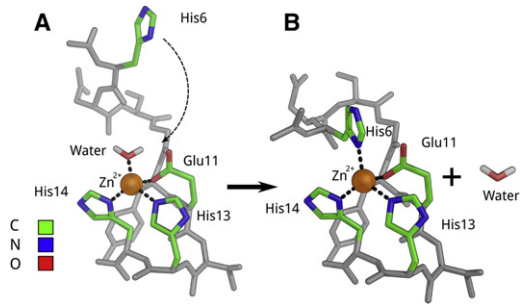

FIGURE 2 Schema of Zn^{2+} ion recognition by the Aβ binding site 6–14 according to QM/MM calculations performed with the GROMACS/CPMD package (http://www.tougaloo.edu/research/qmmm/index.htm) based on PDB ID: 1ZE9. (*A*) structure of the intermediate Zn^{2+}-Aβ complex with a water molecule as the fourth chelator; (*B*) structure of the final Zn^{2+}-Aβ 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.

ACKNOWLEDGEMENTS

Computer resources were provided by the Research Computing Center of Moscow State University. Supercomputer "Chebyshev" was used for all modeling studies.

This work was supported by the Molecular and Cellular Biology Program of the Russian Academy of Sciences, by the Russian Foundation for Basic Research (grant No. 08-04-01465), by the International Centre for Genetic Engineering and Biotechnology (grant No. CRP/RUS08-02), by the Russian Federal Program (contract No. 02.740.11.0776), by the Dmitry Zimin Dynasty Foundation, and by the "Proteomics in Medicine and Biotechnology" Program of the Russian Academy of Medical Sciences.

REFERENCES and FOOTNOTES

1. Goedert, M., and M. G. Spillantini. 2006. A century of Alzheimer's disease. *Science.* 314:777–781.
2. Bush, A. I. 2003. The metallobiology of Alzheimer's disease. *Trends Neurosci.* 26:207–214.
3. Cuajungco, M. P., and K. Y. Faget. 2003. Zinc takes the center stage: its paradoxical role in Alzheimer's disease. *Brain Res. Brain Res. Rev.* 41:44–56.
4. Maynard, C. J., A. I. Bush, …, Q. X. Li. 2005. Metals and amyloid-β in Alzheimer's disease. *Int. J. Exp. Pathol.* 86:147–159.
5. Tsvetkov, P. O., I. A. Popov, …, S. A. Kozin. 2008. Isomerization of the Asp^{7} residue results in zinc-induced oligomerization of Alzheimer's disease amyloid β (1–16) peptide. *ChemBioChem.* 9:1564–1567.
6. Faller, P., and C. Hureau. 2009. Bioinorganic chemistry of copper and zinc ions coordinated to amyloid-β peptide. *Dalton Trans.* 1080–1094.
7. Kozin, S. A., S. Zirah, …, P. Debey. 2001. Zinc binding to Alzheimer's Aβ (1–16) peptide results in stable soluble complex. *Biochem. Biophys. Res. Commun.* 285:959–964.
8. Zirah, S., S. A. Kozin, …, S. Rebuffat. 2006. Structural changes of region 1–16 of the Alzheimer disease amyloid β -peptide upon zinc binding and in vitro aging. *J. Biol. Chem.* 281:2151–2161.
9. Damante, C. A., K. Osz, …, I. Sovago. 2009. Metal loading capacity of Aβ N-terminus: a combined potentiometric and spectroscopic study of zinc (II) complexes with Aβ (1–16), its short or mutated peptide fragments and its polyethylene glycolated analogue. *Inorg. Chem.* 48:10405–10415.
10. Danielsson, J., R. Pierattelli, …, A. Graslund. 2007. High-resolution NMR studies of the zinc-binding site of the Alzheimer's amyloid β -peptide. *FEBS J.* 274:46–59.
11. Gaggelli, E., A. Janicka-Klos, …, E. Wieczerzak. 2008. NMR studies of the Zn^{2+} interactions with rat and human β-amyloid (1–28) peptides in water-micelle environment. *J. Phys. Chem. B.* 112:100–109.
12. Toropygin, I. Y., E. V. Kugaevskaya, …, S. A. Kozin. 2008. The N-domain of angiotensin-converting enzyme specifically hydrolyzes the Arg^{5}-His^{6} bond of Alzheimer's Aβ -(1–16) peptide and its $isoAsp^{7}$ analogue with different efficiency as evidenced by quantitative matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Rapid Commun. Mass Spectrom.* 22:231–239.
13. Talmard, C., L. Guilloreau, …, P. Faller. 2007. Amyloid-β peptide forms monomeric complexes with Cu(II) and Zn(II) prior to aggregation. *ChemBioChem.* 8:163–165.
14. Miller, Y., B. Ma, and R. Nussinov. 2010. Zinc ions promote Alzheimer Aβ aggregation via population shift of polymorphic states. *Proc. Natl. Acad. Sci. USA.* 107:9490–9495.
15. Furlan, S., and G. La Penna. 2009. Modeling of the Zn^{2+} binding in the 1–16 region of the amyloid β peptide involved in Alzheimer's disease. *Phys. Chem. Chem. Phys.* 11:6468–6481.