Biophysical Journal, Volume 99

Supporting Material

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

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

MATERIALS

All chemicals and solvents were of HPLC-grade or better and were obtained from Sigma-Aldrich (St. Louis, MO, USA). All synthetic peptides (purity > 98% checked by RP-HPLC) were purchased from Biopeptide Co., LLC (San Diego, CA, USA). The amino acid sequence of each peptide was confirmed on an ultra high resolution Fourier transform ion cyclotron resonance mass-spectrometer 7T Apex Qe BRUKER (Bruker Daltonics, Bellerica, MA, USA) using a de-novo sequencing approach based on the CID fragmentation technique. The lyophilized peptides were dissolved in buffer before each experiment. The final peptide concentrations were determined by UV absorption spectroscopy using the extinction coefficient of 1450 M^{-1} cm^{-1} at 276 nm (from Tyr 10 of Aβ) or gravimetrically.

In order to preserve the positive charge on amino acid in position 6 and at the same time exclude the possibility of zinc ion chelation by this amino acid, we selected the Aβ(1-16)H6R mutant. The Aβ(1-16)E11A mutant was selected because small and inert methyl functional group of alanine cannot chelate the zinc ion and is not as hydrophobic as other non-charged amino acids.

ISOTHERMAL TITRATION CALORIMETRY

The thermodynamic parameters of zinc binding to peptides were measured using a iTC_{200} instrument (MicroCal, Northampton, MA, USA) as described previously (1). Experiments were carried out at 25°C in 50 mM Tris buffer, pH 7.3. 2-μl aliquots of $ZnCl_2$ solution were injected into the 0.2 mL cell containing the peptide solution to achieve a complete binding isotherm. Peptide concentration in the cell ranged from 0.1 to 1 mM and $ZnCl_2$ concentration in the syringe ranged from 5 to 15 mM. The heat of dilution was measured by injecting the ligand into the buffer solution or by additional injections of ligand after saturation; the values obtained were subtracted from the heat of reaction to obtain the effective heat of binding. The resulting titration curves were fitted using MicroCal Origin software. Affinity constants (KA), binding stoichiometry (N) and enthalpy (ΔH) were determined by a non-linear regression fitting procedure. ITC measurements for each peptide have been repeated at least three times at different peptide concentration and yielded similar thermodynamic parameters.

QM/MM SIMULATION METHODOLOGY

The binding of metal ions by protein can cause strong polarization, charge transfer effects and coordination geometries that are not easily described within standard force fields. In many cases, an adequate simulation can only be performed with an explicit quantum mechanical electronic-structure calculation. In addition, the problem of metal binding sites in proteins necessitates the use of an approach that takes into account the entire protein environment and incorporates finite temperature effects that are known to be crucial for biological function (2). In order to fulfill all these conditions, we applied the QM/MM Car–Parrinello simulation approach (3, 4) that turned out to be successful in the simulation of a number of metal-containing systems (5-7). Furthermore, a recent study of conformational stability of Zn^{2+}-Aβ(1-16) complexes using the QM/MM method was in good agreement with the NMR data (8).

The starting conformation of Aβ(1-16) peptide in complex with zinc cation was taken from PDB databank, PDB: 1ZE9. Conformations for Aβ(11-14) and Aβ(6-14) peptides in complex with Zn^{2+} were extracted from the structure of the whole Aβ(1-16) peptide by removing excessive amino acids. The molecular mechanics approach was applied using the parameters from parm99 force filed with corrections by Duan et al. (9) The QM system was described with density functional theory in the generalized gradient approximation (GGA) using the PW91 functional. The interactions between valence electrons and ionic cores were described by ultrasoft VDB pseudopetential (10, 11). Side chain atoms of His6, Glu11, His13, His14 were included in the QM system. Total size of the QM system was 38 atoms. In case of Aβ(11-14) peptide, seven water molecules closest to zinc were included in QM system as well. Van der Waals interactions, which are poorly described by default DFT, were corrected with Grimme analytical potential (12). Each simulation system was filled with water molecules presented by tip4p model, and total charge was neutralized with Na^+ or Cl^- ions. Water and ions were equilibrated around the peptide-ion complexes by carrying out a 100ps MD simulation with restrained position of peptide and zinc.

The prepared systems were subjected to QM/MM simulation with GROMACS/CPMD package (3). The time step used was 0.12 fs (~5 a.u.) and the fictitious electronic mass 500. Temperature coupling with Noose-Hover (13, 14) scheme allowed to observe behavior of systems at body temperature. Since we applied ultrasoft pseudopotentials, the basis set for the valence electrons consists of plane waves expanded up to a cutoff of 30 Ry. The QM subcell has cubic shape with 40 Ry side length, and as the result we obtained about 90.000 plane waves for wavefunction.

REFERENCES

1. Hauryliuk, V., V. A. Mitkevich, N. A. Eliseeva, I. Y. Petrushanko, M. Ehrenberg, and A. A. Makarov. 2008. The pretranslocation ribosome is targeted by GTP-bound EF-G in partially activated form. Proc Natl Acad Sci U S A 105:15678-15683.
2. Karplus, M., and G. A. Petsko. 1990. Molecular dynamics simulations in biology. Nature 347:631-639.
3. Biswas, P. K., and V. Gogonea. 2005. A regularized and renormalized electrostatic coupling Hamiltonian for hybrid quantum-mechanical-molecular-mechanical calculations. J Chem Phys 123:164114.
4. Laio, A., J. VandeVondele, and U. Rothlisberger. 2002. A Hamiltonian electrostatic coupling scheme for hybrid Car--Parrinello molecular dynamics simulations. The Journal of Chemical Physics 116:6941-6947.
5. Dal Peraro, M., L. I. Llarrull, U. Rothlisberger, A. J. Vila, and P. Carloni. 2004. Water-assisted reaction mechanism of monozinc beta-lactamases. J Am Chem Soc 126:12661-12668.
6. Guidoni, L., K. Spiegel, M. Zumstein, and U. Rothlisberger. 2004. Green oxidation catalysts: computational design of high-efficiency models of galactose oxidase. Angew Chem Int Ed Engl 43:3286-3289.
7. Spiegel, K., U. Rothlisberger, and P. Carloni. 2004. Cisplatin Binding to DNA Oligomers from Hybrid Car-Parrinello/Molecular Dynamics Simulations. The Journal of Physical Chemistry B 108:2699-2707.
8. Furlan, S., and G. La Penna. 2009. Modeling of the Zn2+ binding in the 1-16 region of the amyloid beta peptide involved in Alzheimer's disease. Phys. Chem. Chem. Phys. 11:6468-6481.
9. Duan, Y., C. Wu, S. Chowdhury, M. C. Lee, G. Xiong, W. Zhang, R. Yang, P. Cieplak, R. Luo, T. Lee, J. Caldwell, J. Wang, and P. Kollman. 2003. A point-charge force field for molecular mechanics simulations of proteins based on condensed-phase quantum mechanical calculations. J Comput Chem 24:1999-2012.
10. Laasonen, K., R. Car, C. Lee, and D. Vanderbilt. 1991. Implementation of ultrasoft pseudopotentials in ab initio molecular dynamics. Phys Rev B Condens Matter 43:6796-6799.
11. Laasonen, K., A. Pasquarello, R. Car, C. Lee, and D. Vanderbilt. 1993. Car-Parrinello molecular dynamics with Vanderbilt ultrasoft pseudopotentials. Phys Rev B Condens Matter 47:10142-10153.
12. Grimme, S. 2004. Accurate description of van der Waals complexes by density functional theory including empirical corrections. J Comput Chem 25:1463-1473.
13. Hoover, W. G. 1985. Canonical dynamics: Equilibrium phase-space distributions. Phys Rev A 31:1695-1697.
14. Nose, S. 1984. A unified formulation of the constant temperature molecular dynamics methods. The Journal of Chemical Physics 81:511-519.

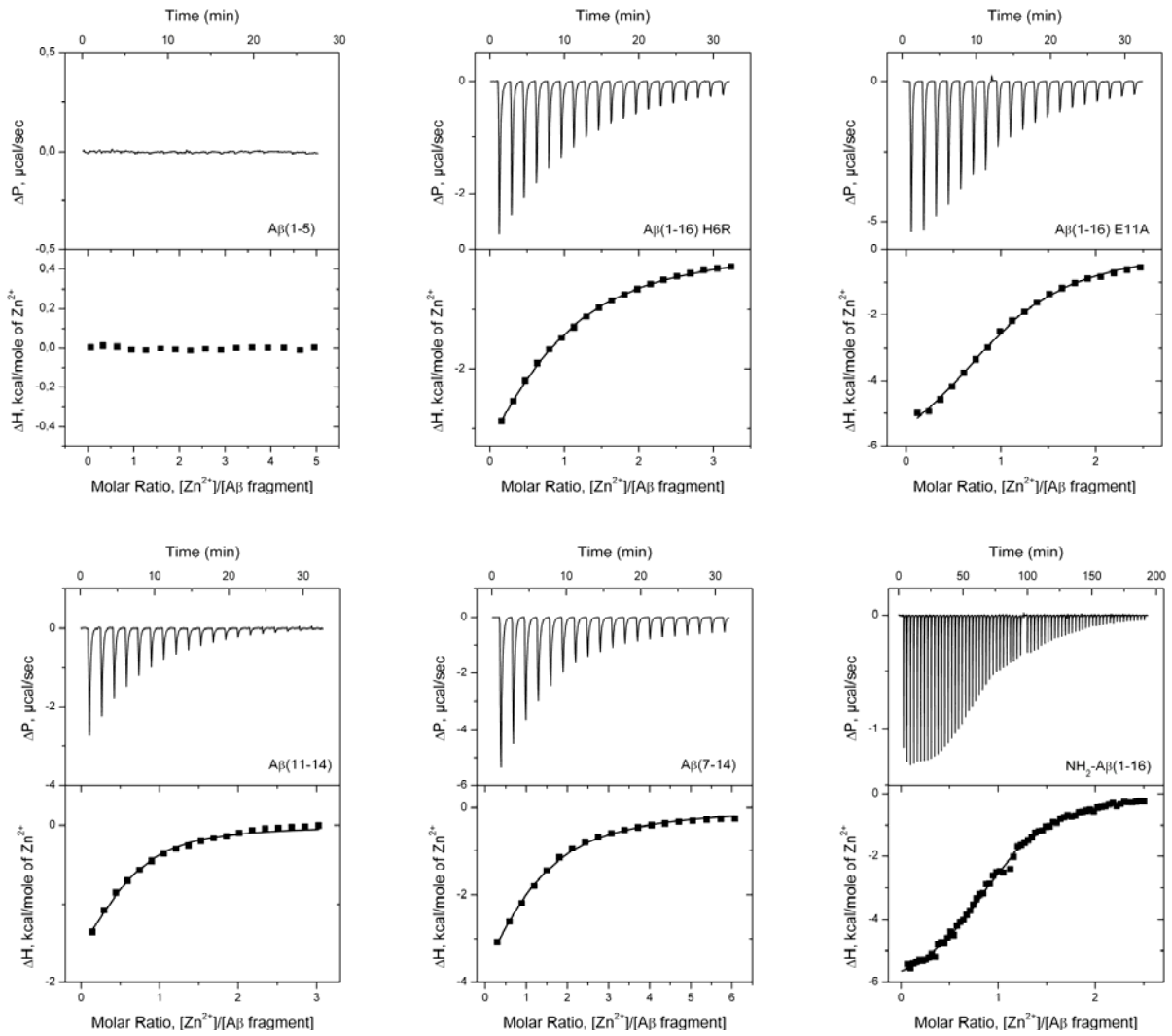

FIGURE S1. ITC titration curves (upper panel) and binding isotherms (lower pannel) for zinc interactions with Aβ(1-5), Aβ(1-16) H6R, Aβ(1-16) E11A, Aβ(11-14), Aβ(7-14) and NH_2-Aβ(1-16) at 25°C in 50 mM Tris buffer, pH 7.3. All experiments were carried out on iTC_{200} instrument (MicroCal, Northampton, MA, USA). All peptides were purchased from Biopeptide Co., LLC (San Diego, CA, USA). The N- and C-termini of each peptide were protected with acetyl and amide, respectively; peptide NH_2-Aβ(1-16) was protected at C-terminus with amide.

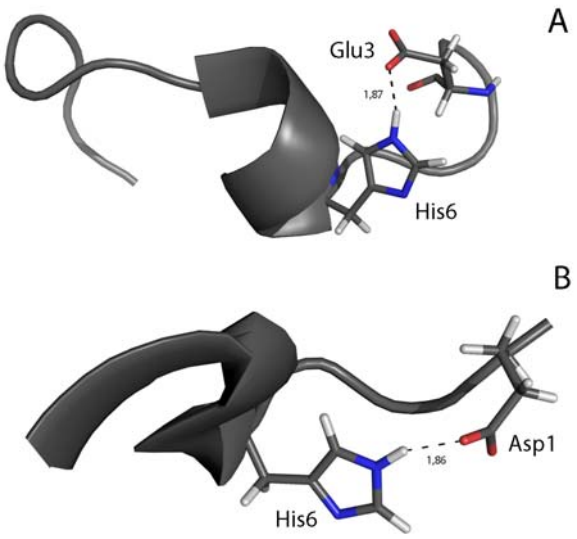

Figure S2. Fragments of Aβ(1-16) structure based on PDB ID: 1ZE7 which demonstrate hydrogen bonds between: His6 and Glu3 , 7th model in PDB file (A), and His6 and Asp1, 4th model in PDB file (B).

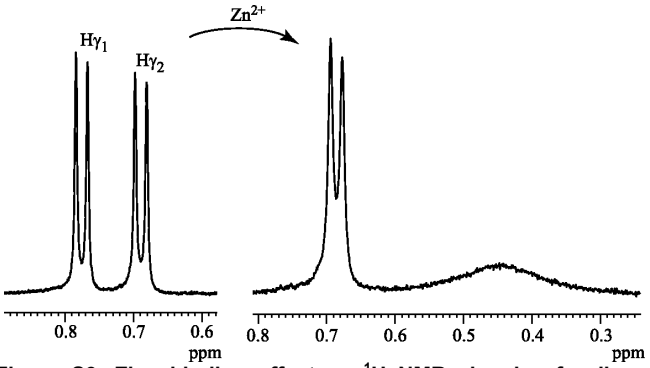

Figure S3. Zinc binding effect on ^{1}H NMR signals of valine methyl protons in Aβ(11-14) tetrapeptide. Methyl groups of Val12 are diastereotopic and are revealed in the spectrum of the free tetrapeptide as two doublets with different chemical shifts. Addition of Zn^{2+} ions to the sample results in a significant shift of these signals towards higher field, while one of them also broadens. This change indicates that the corresponding methyl group is close to the Zn^{2+} ion and therefore is oriented inward the complex. In addition, this confirms the ITC data that the hydrophobic surface of the peptide created primarily by the side chains of Val12 is not exposed to the solvent when the complex is formed. Spectra were recorded with Bruker AMXIII spectrometer in D_2O (pD = 7.3) at 400.13 MHz and 305 K.