

SUPPORTING INFORMATION

Zinc-induced dimerization of the amyloid- β metal-binding domain 1-16 is mediated by residues 11–14

Sergey A. Kozin,^{*a,b} Yuri V. Mezentsev,^b Alexandra A. Kulikova,^a Maria I. Indeykina,^{a,c} Andrey V. Golovin,^d Alexis S. Ivanov,^b Philipp O. Tsvetkov^{a,b} and Alexander A. Makarov^{*a}

Materials

All synthetic peptides (purity > 98% checked by RP-HPLC) used throughout the study were purchased from Biopeptide (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) by using a de-novo sequencing approach based on a CID fragmentation technique. The lyophilized peptides were dissolved in the appropriate 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 A β) or gravimetrically.

Research grade sensor chips CM5 carrying the hydrophilic carboxymethylated dextran matrix, HBS buffer (10mM HEPES, pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% surfactant P20), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), N-hydroxysuccinimide (NHS), 2-(2-pyridinyldithio)-ethaneamine (PDEA), and cysteine were purchased from BIAcore (GE, USA). All other chemicals and solvents were of HPLC grade or better and were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Surface Plasmon Resonance (SPR) Biosensing

Attachment of the synthetic peptide Acetyl-DAEFRHDSGYEVHHQKGGGGC-Amide (A β ₁₋₁₆-G₄-C), which will serve as the ligand in further assays, to the CM5 chip was done according to the thiol bond formation protocol described in the Sensor surface handbook (GE Healthcare, USA). The peptide was immobilized through thiol bond formation in 10 mM sodium acetate (pH 4.5). For the regeneration the HBS buffer containing 10 mM HEPES, 3 mM EDTA, 0.005 % surfactant P20 and 150 mM NaCl (pH 7.4) was used. The running buffer was 50 mM HEPES (pH 6.8) with 100 μ M of zinc ions. All buffers were filtered (0.45 μ m, nylon) prior to use. The flow rate used for all immobilization steps was 5 μ L/min. The carboxymethyl dextran matrix was activated by injection of a 1:1 mixture of EDC and NHS (30 μ L, 400 mM EDC, 100 mM NHS) with a following injection of a 80 mM PDEA solution in 0.1 M sodium borate (pH 8.5). The A β ₁₋₁₆-G₄-C solution was then injected into the activated flow cell (0.05 mg/ml peptide in sodium acetate buffer). Unreacted disulfide groups on the CM5 chip surface were capped with 50 mM cysteine solution in 0.1 M sodium acetate buffer (pH 4.0). The change corresponding to immobilization of A β ₁₋₁₆-G₄-C was 719

Table S1. Analyte concentrations

Analyte	Analyte concentrations, μ M
Acetyl-DAEFRHDSGYEVHHQK- Amide	2, 10, 15, 20
Acetyl-HDSGYEVHH- Amide	100, 120, 140, 150
Acetyl-EVHH- Amide	50, 100, 150, 200, 250
Acetyl-DSGYEVHH- Amide	100, 150, 200, 250
Acetyl-HDSGYEVH- Amide ^a	10,50, 100, 300
Acetyl-DAEFR- Amide ^a	10, 50,100, 300

^a Interaction of this analyte with the immobilized ligand was not observed.

response units (RU). In the control flow cell the reaction was carried using the same protocol, but where the surface activation step was lacking.

All SPR experiments were carried out on a BIAcore 3000 instrument (GE Healthcare, USA), thermostated at 25°C. The binding affinities of the immobilized ligand to the following peptide-analytes were measured: Acetyl-DAEFRHDSGYEVHHQK-Amide (A β ₁₋₁₆), Acetyl-HDSGYEVHH- Amide (A β ₆₋₁₄), Acetyl-EVHH- Amide (A β ₁₁₋₁₄), Acetyl-DSGYEVHH- Amide (A β ₇₋₁₄), Acetyl-HDSGYEVH- Amide (A β ₆₋₁₃), Acetyl-DAEFR- Amide (A β ₁₋₅). Samples of analytes were prepared by dilution of respective stock solutions in the running buffer (50 mM HEPES, pH 6.8) in the absence or presence of zinc (100 μ M) or copper (100 μ M) ions. Each analyte was diluted to different concentrations (Table 1) and injected in multichannel mode (volume 25 μ L and rate 5 μ L/min) during 300 s. Then, the chip surface was exposed to the running buffer without analyte for 300 s. The chip surface was regenerated by injecting the regeneration buffer (20 μ L). The control line data (the signal from the channel with carboxymethyl dextran without the immobilized ligand) was subtracted from the raw data, obtained from the flow cell with the immobilized ligand. The kinetic rate constants were calculated from the sensorgrams by globally fitting the response curves obtained at various analyte concentrations using the Langmuir model (1:1 binding) in the BIAevaluation 4.1 program. The association (k_{on}) and the dissociation (k_{off}) rate constants were fitted simultaneously:

$$dR/dt = k_{on} C (R_{max} - R) - k_{off} R,$$

where R stands for the biosensor response of the formed complex, C is the concentration of the analyte, and R_{max} is the maximal theoretical value of the binding response for a given analyte.

Using the obtained data association (K_a) and dissociation (K_d) constants were calculated from the ratios of the association (k_{on}) and dissociation (k_{off}) rate constants: $K_a = k_{on}/k_{off}$, $K_d = k_{off}/k_{on}$.

5 Mass-spectrometry

All of the experiments were carried out on a 7T Thermo Finnigan LTQ FT Ultra instrument. The following settings of the mass-spectrometer were used:

- Spray voltage 3.2-3.4kV,
- Capillary temperature 120°C, low temperatures were used to prevent in source dissociation of the zinc-ion complexes,
- Flow rate 1 μ L/min,
- AGC parameters (the amount of ions gathered): full MS 1×10^6 , narrow SIM 5×10^4 , wide SIM 5×10^5 ,
- The rest of the parameters were semi-automatically tuned for the following masses: 666, 687, 998 and 1030, which correspond to the triply and doubly charged molecular ions of the A β_{1-16} peptide and its zinc ion adducts.

The samples for mass-spectrometric analysis were prepared from stock solutions by diluting them in MeOH and water to their final ratio of 1:1 and addition of 2mM Zn(CH₃COO)₂ in MeOH, pH 6.3 in the desired amount. The stock solutions were prepared from lyophilized peptides in HPLC grade water. The concentrations of the stock solutions were determined gravimetrically. The final peptide concentration in the samples used for analysis was 1-200 μ M. And the final zinc concentration was 120-2000 μ M.

30 Isothermal Titration Calorimetry

The thermodynamic parameters of zinc binding to EVRH peptide were measured using an iTC₂₀₀ 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- μ l aliquots of ZnCl₂ solution were injected into the 0.2 mL cell containing the peptide solution to get a complete binding isotherm. Peptide concentration in the cell ranged from 1 to 2 mM and ZnCl₂ 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 the 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. The affinity constants (K_a), binding stoichiometry (N) and binding enthalpy (ΔH) were determined by a non-linear regression fitting procedure. Isothermal titration calorimetry measurements have been repeated at least three times at different peptide concentrations and yielded similar thermodynamic parameters.

Modeling

The earlier described model² of the structure of the peptide complex A β_{11-14} -Zn²⁺ was amplified to a complete dimer by rotating it around the axis, which connects the zinc atom to the OD1 Glu. For this structure two models were constructed

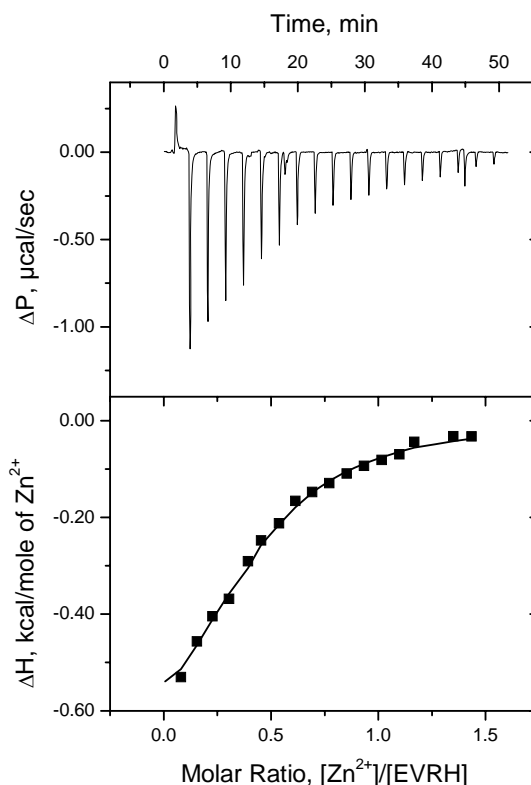


Fig. S1 ITC titration curve (upper panel) and binding isotherm (lower panel) for zinc interaction with EVRH peptide at 25°C in 50 mM Tris buffer, pH 7.3.

in the amber03 force field. The first model contained the parameters³ corresponding to the geometry of a complex in which the zinc atom is coordinated by the Glu11 and His14 residues of both peptides. This model is further referred as M1. The second model corresponds to the geometry for zinc coordination by residues His13 and His14 of both peptides, and is further referred as M2.

The GROMACS 4.0 software package was used for MD simulation and analysis of the trajectories.⁴ The simulations in explicit solvent were carried out at 300 K under the control of a velocity rescaling thermostat⁵ at constant pressure and using the PME (particle mesh Ewald) method⁶ to take into account the electrostatic interactions. The dimers were put into the center of a triclinic cell with the distance to the borders of 20 Å. The cell was filled by TIP4P water⁷ and the negative charge of the system was compensated by sodium ions. The concentration of monovalent ions was set to 0.15 M. The trajectory length for both of the models was 100 ns. The most symmetrical conformations over the last 10 ns of the trajectory were selected and their geometry was optimized using the QM/MM method as described in the work by Biswas and Gogonea.⁸ The QM system was described in terms of PW-DFT with a spin polarized formalism and PW91 functional.⁹ The interactions between valence electrons and the ionic cores are described by ultrasoft VDB pseudopotential.^{10, 11} Side-chain atoms from Glu11, His14 or His13, His14 pairs were

included in the QM system. Van der Waals interactions between heterocyclic bases, which are poorly described by default DFT, were corrected with the Grimme analytical potential.¹² QM/MM geometry optimization was performed with the GROMACS/CPMD package.⁸ 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 had a cubic shape with 40 Ry side length, in result we have got about 90.000 plane waves for wavefunction.

In order to calculate the energy gain from zinc binding the following equation was used: $\Delta E = \langle E_{M1} \rangle - \langle E_{M1 \text{ w/o Zn}} \rangle - E_{Zn}$, where E_x corresponds to the total energy of the corresponding quantum subsystem. To calculate the energy of the quantum system of the chelators in the absence of zinc, the geometry of the dimmer was also optimized without the cation. The energy values were averaged over the last 15 steps of the geometry optimization procedure.

Table S2. Energies of the quantum systems used for calculations of the energy gain from the $\text{A}\beta\text{11-14-Zn}^{2+}\text{-A}\beta\text{11-14}$ complex formation

System	$\langle E \rangle$, a.u.	ΔE , a.u.*
Zn^{+2}	-63.66	
M1	-234.45	-1.11
M1 w/o Zn^{+2}	-169.82	
M2	-222.75	-0.69
M2 w/o Zn^{+2}	-158.06	

* To calculate the energy gain from zinc binding the following equation was used: $\Delta E = \langle E_{M1} \rangle - \langle E_{M1 \text{ w/o Zn}} \rangle - E_{Zn}$

Notes and references

^a Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilov street 32, 119991 Moscow, Russia; E-mail: amakarov@eimb.ru, kozinsa@gmail.com

^b Orekhovich Institute of Biomedical Chemistry, Russian Academy of Medical Sciences, Pogodinskaya street 10, 119832 Moscow, Russia

^c Emanuel Institute of Biochemical Physics, Kosygina street 4, 119334 Moscow, Russia

^d Bioengineering and Bioinformatics Department, M. V. Lomonosov Moscow State University, Leninskie Gory 1, 119992 Moscow, Russia

- V. Hauryliuk, V. A. Mitkevich, N. A. Eliseeva, I. Y. Petrushanko, M. Ehrenberg and A. A. Makarov, *Proceedings of the National Academy of Sciences*, 2008, **105**, 15678-15683.
- P. O. Tsvetkov, A. A. Kulikova, A. V. Golovin, Y. V. Tkachev, A. I. Archakov, S. A. Kozin and A. A. Makarov, *Biophys J*, **99**, L84-86.
- F. Lin and R. Wang, *Journal of Chemical Theory and Computation*, **6**, 1852-1870.
- B. Hess, C. Kutzner, D. van der Spoel and E. Lindahl, *Journal of Chemical Theory and Computation*, 2008, **4**, 435-447.
- G. Bussi, D. Donadio and M. Parrinello, *The Journal of Chemical Physics*, 2007, **126**, 014101-014107.
- T. Darden, D. York and L. Pedersen, *The Journal of Chemical Physics*, 1993, **98**, 10089-10092.
- W. L. Jorgensen, J. Chandrasekhar, J. D. Madura, R. W. Impey and M. L. Klein, *The Journal of Chemical Physics*, 1983, **79**, 926-935.
- P. K. Biswas and V. Gogonea, *The Journal of Chemical Physics*, 2005, **123**, 164114-164119.

- J. P. Perdew, *Electronic Structure of Solids 1991*, Akademie Verlag, Berlin, 1991.
- K. Laasonen, R. Car, C. Lee and D. Vanderbilt, *Physical Review B*, 1991, **43**, 6796.
- K. Laasonen, A. Pasquarello, R. Car, C. Lee and D. Vanderbilt, *Physical Review B*, 1993, **47**, 10142.
- S. Grimme, *Journal of Computational Chemistry*, 2004, **25**, 1463-1473.