Zinc-induced heterodimer formation between metal-binding domains of intact and naturally modified amyloid-beta species: implication to amyloid seeding in Alzheimer’s disease?

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Zinc ions and modified amyloid-beta peptides (Aβ) play a critical role in the pathological aggregation of endogenous Aβ in Alzheimer’s disease (AD). Zinc-induced Aβ oligomerization is mediated by the metal-binding domain (MBD) which includes N-terminal residues 1–16 (Aβ1–16). Earlier, it has been shown that Aβ1–16 as well as some of its naturally occurring variants undergoes zinc-induced homodimerization via the interface in which zinc ion is coordinated by Glu11 and His14 of the interacting subunits. In this study using surface plasmon resonance technique, we have found that in the presence of zinc ions Aβ1–16 forms heterodimers with MBVs of two Aβ species linked to AD: Aβ containing isoAsp7 (isoAβ) and Aβ containing phosphorylated Ser8 (pS8-Aβ). The heterodimers appear to possess the same interface as the homodimers. Simulation of 200 ns molecular dynamic trajectories in two constructed models of dimers ([Aβ1–16/Zn/Aβ1–16] and [isoAβ1–16/Zn/Aβ1–16]), has shown that conformational flexibility of the N-terminal fragments of the dimer subunits is controlled by the structure of corresponding sites 6–8. The data suggest that isoAβ and pS8-Aβ can be involved in the AD pathogenesis by means of their zinc-dependent interactions with endogenous Aβ resulting in the formation of heterodimeric seeds for amyloid aggregation.

Keywords: Alzheimer’s disease; amyloid-beta; zinc; chemical modifications; oligomerization; surface plasmon resonance; molecular modeling; molecular dynamics

1. Introduction

The key event in pathogenesis of Alzheimer’s disease (AD) is associated with amyloid-beta (Aβ) polypeptide, which undergoes transition from a soluble (physiologically normal) monomeric form into neurotoxic dimers and oligomers; this finally results in the accumulation of Aβ aggregates with characteristic supramolecular structure (amyloid plaques) in specific brain regions (Masters & Selkoe, 2012). Zinc ions play a critical role in the pathological aggregation of Aβ in vivo (Bush, 2013). Although intact Aβ spontaneously aggregates in vitro in the presence of zinc ions (Alies, Hureau, & Faller, 2013), the resultant Aβ aggregates are non-toxic in vivo (Meyer-Luehmann et al., 2006). In contrast to intact Aβ, its chemically and/or structurally modified variants found in the amyloid plaques of AD patients may act as seeds for the formation of pathological aggregates of Aβ (Meyer-Luehmann et al., 2006). However, the molecular mechanism responsible for conversion of the native conformation of endogenous Aβ into the pathological one (induced by the modified Aβ variants) remains unknown (Cummings, 2004).

Recently, it has been demonstrated that Aβ phosphorylated at Ser8 (pS8-Aβ) promotes toxic aggregates formation during the pathogenesis of AD (Kumar et al., 2011, 2012). We have found that in vivo administration of Aβ containing isoAsp7 (isoAβ), the most common modified Aβ variant detected in the amyloid plaques (Roher et al., 1993), accelerates progression of cerebral amyloidosis in transgenic mice (Kozin et al., 2013). IsoAβ was also neurotoxic for neuronal cell cultures (Mitkevich et al., 2013). In this context, it is important that both the pathogenically relevant modifications of Aβ (Ser8 phosphorylation and Asp7 isomerization) as well as so-called the ‘English’ familial mutation (H6R) associated with the inherited form of AD (Hori et al., 2007; Janssen et al., 2003; Ono, Condon, & Teplow, 2010) are localized in the metal-binding domain (MBD) of Aβ. This domain comprising the N-terminal residues 1–16 (Kozin, Zirah, Rebuffat, Hoa, & Debey, 2010; Zirah et al., 2006) is a good model for the analysis of interactions between zinc ions and Aβ: in contrast to the full-length Aβ, MBD does not aggregate in aqueous solutions at physiological pH (Faller et al., 2012; Kozin et al., 2001; Zirah et al., 2006).

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In this study using a surface plasmon resonance (SPR) technique we have demonstrated for the first time that in the presence of zinc ions intact Aβ MBD (Aβ1-16) forms stable heterodimers with Aβ MBD containing isoAsp7 (isoAβ1-16) and also with Aβ MBD containing phosphorylated Ser8 (pS8-Aβ1-16). Using an available structure of the Aβ MBD homodimer carrying the English mutation H6R [H6R-Aβ1-16/Zn/H6R-Aβ1-16] (PDB code 2MGT) as a prototype we have generated spatial models of the homodimer [Aβ1-16/Zn/Aβ1-16] and the heterodimer [isoAβ1-16/Zn/Aβ1-16]. Simulation of 200 ns molecular dynamic trajectories in two constructed models of the dimers have shown that conformational flexibility of the N-terminal fragments of the dimer subunits is controlled by the structure of corresponding sites 6–8.

2. Materials and methods

2.1. Materials

Synthetic peptides (purity > 98% by RP-HPLC) corresponding to human Aβ MBD, its isoforms, and rat Aβ MBD (ratAβ1-16) were purchased from ‘Biopeptide Co., LLC’ (USA) (Table 1). All synthesized peptides used as ligands for immobilization contained the C-terminal tetraglycylcysteine tag. Various paired combinations of these peptides have been used in the SPR-based study in which one peptide was immobilized on the optical chip as a ligand, while the other was added as a soluble analyte interacting with the immobilized ligand. Standard optical chips CM5 and special reagents were purchased from GE Healthcare (USA). These included: HBS buffer (150-mM NaCl, 3-mM EDTA, .005% surfactant P20, 10-mM HEPES, pH 7.4); 10-mM acetate buffer (pH 4.5); a thiol coupling kit. Solution containing 10-mM HEPES (pH 6.8) and 100-μM ZnCl2 was used as a working buffer for SPR analysis of zinc-dependent dimerization of MBDs of various Aβ isoforms.

2.2. SPR analysis

All SPR measurements were carried out using an optical biosensor Biacore T200 (GE Healthcare, USA) operated under ‘Biacore T200 Control Software’ and BiaEvaluation v.4.1 software. Covalent immobilization of the peptide ligands (Table 1) on the surface of optical chip was performed using the sulphydryl group of the N-terminal tetraglycylcysteine tag. Briefly, carboxyl groups on the chip surface were activated by serial injections of mixture of 2 M EDC/.05 M NHS at a flow rate of 5 μL/min for 2 min and solution of 80-mM PDEA in 50-mM borate buffer (pH 8.5) for 4 min. Solutions of 20 μM peptides in the immobilization buffer (10 mM acetate buffer, pH 4.5) were injected for 7 min at a flow rate of 5 μL/min. Unreacted activated groups on the chip were blocked by further injection of solution containing 50-mM cysteine, 1-M NaCl, .1-M sodium acetate (pH 4.0) for 4 min at a flow rate of 5 μL/min, followed by washing with HBS buffer for 5 min. Possible nonspecific analyte binding to the chip surface was evaluated using a free (control) channel of the biosensor exposed to the same treatments as the working channel except peptides. SPR signals were recorded in real time in resonance units (RU; 1 RU corresponds to 1 pg of analyte) and were presented in the form of sensorgrams showing time-dependent signal changes. Series of sensorgrams representing the difference of SPR-signals from working and control channels were obtained by serial injections

### Table 1. Synthetic peptides and fragments of MBD of different variants of Aβ used in this study.

| No | Designation | Amino acid sequence | Description |
|----|-------------|---------------------|-------------|
| 1  | Aβ1-16      | Acetyl-DAEFRHDSGYEVHHQK-Amide | MBD of intact Aβ |
| 2  | ratAβ1-16   | Acetyl-DAEFRHIDSGFYEHQK-Amide | MBD of rat Aβ |
| 3  | isoAβ1-16   | Acetyl-DAEFRH[isoD]SGYEVHHQK-Amide | MBD of Aβ with isomerized Asp7 |
| 4  | pS8-Aβ1-16  | Acetyl-DAEFRHdpSGYEVHHQK-Amide | MBD of Aβ with phosphorylated Ser8 |
| 5  | isoAβ1-16-G4-C | Acetyl-DAEFRH[isoD]SGYEVHHQKGGGGC-Amide | MBD of Aβ with phosphorylated Asp7 and modification of C-terminus by tetraglycylcysteine |
| 6  | pS8-Aβ1-16-G4-C | Acetyl-DAEFRHdpSGYEVHHQKGGGGC-Amide | MBD of Aβ with phosphorylated Ser8 and modification of C-terminus by tetraglycylcysteine |
| 7  | pS8-Aβ6-9   | Acetyl-DpSG-Amide | Aβ 7–9 fragment with phosphorylated Ser8 |
| 8  | pS8-Aβ6-10  | Acetyl-DpEFHRHDSGY-Amide | Aβ 7–10 fragment with phosphorylated Ser8 |
| 9  | Aβ11-16     | Acetyl-LEHHIEK-Amide | Aβ 11–16 fragment |
| 10 | Aβ11-14     | Acetyl-LEHHH-Amide | Aβ 11–14 fragment |
| 11 | Aβ1-5       | Acetyl-DAEFR-Amide | Aβ 1–5 fragment |
| 12 | Aβ6-13      | Acetyl-HDSGYEVH-Amide | Aβ 6–13 fragment |
| 13 | Aβ6-14      | Acetyl-HDSGYEVHII-Amide | Aβ 6–14 fragment |

Notes: N- and C-termini of the peptides are chemically protected by acetyl and amide groups, respectively. Three amino acid residues that differ in human (Aβ1-16) (no. 1) and rat Aβ (ratAβ1-16) (no. 2) MBDs and are shown in bold.
of analyte solutions (Figure 1(A)) through the working and control channels at a flow rate of 5 μL/min for 5 min. During each experiment, at least five solutions with different analyte concentrations were used. All SPR measurements were repeated three times. After each measurement, the optical chip surface was regenerated by injecting HBS buffer for 30 s. Analyte samples in the concentration range from 2 to 20 μM were prepared in the working buffer containing 100-μM ZnCl₂. In accordance with results of previous studies (Kozin et al., 2001, 2011), a series of pilot experiments without zinc ions in the working buffer or zinc ion replacement for other divalent ions (Ca²⁺, Mg²⁺, Cu²⁺ and Fe²⁺) confirmed a specific role of zinc ions for Aβ MBD oligomerization.

The obtained sensograms were analyzed by means of the standard software ‘BIAevaluation v.4.1’ employing different mathematical kinetic models based on global fitting of theoretical curves to the experimental sensograms.

2.3. Modeling

Calculations have been performed for two models: (1) homodimer [Aβ1-16/Zn/Aβ1-16], and (2) heterodimer [isoAβ1-16/Zn/Aβ1-16]. Initial models have been constructed using the representative NMR structure of the homodimer of Aβ1-16 with the English mutation H6R (PDB code 2MGT). Virtual mutations in the Aβ structure were performed using the Chimera software (Pettersen et al., 2004). Partial charges have been assigned with ‘Add charges’ module of Chimera. ‘Amber ff99SB’ (Hornak et al., 2006) values of partial charges have been used for standard residues. Partial charges for the non-standard residue, isoAsp7, have been calculated using the AM1-BCC method (Jakalian, Jack, & Bayly, 2002). Conformations of the fragments H6-D7-S8 and H6-isoD7-S8 of the obtained charged models have been minimized using 200 steps of the steepest descent minimization.

2.4. Molecular dynamic simulation of the dimers

Molecular dynamic simulation has been performed using the ‘GROMACS 4.6.5’ software package (Van Der Spoel et al., 2005) and the ‘Amber ff99SB-ILDN’ force field (Lindorff-Larsen et al., 2010). This force field has an improved parameterization for the side-chain torsion angle potential of the aspartate residue, which is particularly important for our problem (Lindorff-Larsen et al., 2010). Implementation of the non-standard residue iso-aspartate did not introduce new atom types for ‘Amber ff99SB-ILDN’. All parameters for the bonded and non-bonded interactions needed for describing non-standard residue, except partial charges, have been already defined in the force field and used without modifications (Spitaleri et al., 2008). Partial charges for the new residue type have been calculated in Chimera as described above (Supplementary Table 1).

The starting model of the dimer has been placed in the cubic cell with a minimum distance between the protein and the box of .8 nm. TIP3P water molecules have

![Figure 1](image.png)

Figure 1. A set of sensograms illustrating paired interaction of various concentrations of soluble Aβ1-16 with immobilized pS8-Aβ1-16. Notes: Panel A shows the SPR response after addition of soluble Aβ1-16 in the working buffer without of zinc ions (w/o), and either in the presence of zinc ions or other divalent cations calcium, magnesium, copper or iron ions. In the case of experiments without zinc ions in the presence of other divalent cations only the maximal concentration of Aβ1-16 (20 μM) was used. Panel B shows superposition of simulated curves based on the Langmuir theoretical model of the 1:1 interaction (dotted line) on the same set of sensograms in the presence of zinc ions in the working buffer. Arrows indicate the beginning and the end of the analyte injection.
been added to the system and Na\(^+\) ions have been used to neutralize total charge (Jorgensen, Chandrasekhar, Madura, Impey, & Klein, 1983). The system was minimized using a steepest descent algorithm. Positions for the peptide atoms were restrained and the system was equilibrated with 100 ps of constant volume (NVT) molecular dynamic followed by 100 ps of constant pressure (NPT) molecular dynamic. Molecular dynamic trajectory of 200 ns was calculated using the NPT ensemble. Positions of the atoms for the \(\alpha\) and 14 together with the coordinated zinc ion were restrained at all steps of simulation using corresponding harmonic potential implemented in ‘GROMACS 4.6.5’ and a force constant of 9999 KJ/(mol nm\(^2\)) for each of three dimensions. Calculations have been performed at temperature 300 K, pressure 1 bar, with 2 fs integration step using the Berendsen barostat and the velocity rescale method for the thermostat. The particle-mesh Ewald method (Darden, York, & Pedersen, 1993) has been implemented to treat long-range electrostatic interactions and the LINCS algorithm controlled lengths of covalent bonds (Hess, Bekker, Berendsen, & Fraaije, 1997).

Clustering analysis of the similarity of the conformations from the trajectory has been accomplished using g_cluster tool of ‘GROMACS 4.6.5’ (Van Der Spoel et al., 2005). Clustering has been performed separately for each peptide chain of the dimer using single linkage algorithm with the RMSD cut-off 1.5 Å. Conformations from molecular dynamic trajectories have been sampled with 100 ps step. RMSD have been calculated over all atoms of the peptide chain.

3. Results

3.1. Biosensor analysis of the interactions between MBDs of different variants of \(\alpha\) in the presence of zinc ions: SPR-based evidence for heterodimer formation

Zinc-dependent interactions have been studied between the immobilized ligands (iso\(\alpha\)\(\beta\)\(_{1–16}\) or pS8-\(\alpha\)\(\beta\)\(_{1–16}\)), and the soluble analytes (iso\(\alpha\)\(\beta\)\(_{1–16}\), pS8-\(\alpha\)\(\beta\)\(_{1–16}\) and \(\alpha\)\(\beta\)\(_{1–16}\)). Analysis of resultant sets of sensograms performed by means of the BIAevaluation software revealed the best correspondence with the Langmuir theoretical model of the 1:1 interaction (Figure 1(B), Table 2). The closeness of the fit is confirmed by the statistical value \(\chi^2\), which represents the deviation of experimental sensogram from the fitted curve (Biacore Life Sciences, 2004). The lower the \(\chi^2\) value the higher the correspondence to the theoretical curve is. Among five theoretical models used for description of the most evident types of molecular interactions between immobilized ligand and added (free) analyte, the lowest \(\chi^2\) value was obtained only in the case of the Langmuir theoretical model of the 1:1 (Table 2).

Table 2. Comparison of correspondence of the experimental curves obtained using immobilized pS8-\(\alpha\)\(\beta\)\(_{1–16}\) and various concentrations of soluble \(\alpha\)\(\beta\)\(_{1–16}\) as the analyte with various kinetic models.

| Kinetic models                                      | \(\chi^2\) |
|----------------------------------------------------|-----------|
| 1:1 (Langmuir) binding                             | 16.5      |
| 1:1 binding with mass transfer                      | 119       |
| Two state reaction (conformation change)           | 81        |
| Bivalent analyte                                    | 48.8      |
| Heterogeneous ligand – Parallel reactions          | 60.9      |

Note: Explanations are given in the text (Section 3.1).

Although the SPR technology can detect the fact of intermolecular interactions, stoichiometry of ligand–analyte interactions can be evaluated not only by the best fitting to the theoretical model, but also by the \(R_{\text{max}}/R_{\text{im}}\) ratio. \(R_{\text{im}}\) is the total amount of the immobilized ligand and \(R_{\text{max}}\) is a maximum analyte binding capacity of the chip with the immobilized ligand. The \(R_{\text{im}}\) value (in RU) is obtained from immobilization sensogram and the \(R_{\text{max}}\) value (in RU) is calculated by the BIA evaluation software from global fitting and extrapolation of steady-state biosensor signals to total saturation of the chip. Table 3 shows that in all investigated pairs of ligand–analyte interactions the \(R_{\text{max}}/R_{\text{im}}\) ratio did not exceed 1. Lower values observed for some examined ligand–analyte pairs may be attributed to steric limitations for analyte binding.

Thus, taking together all these results may be interpreted as indications for heterodimer formations between various \(\alpha\)\(\beta\) MBDs.

Table 4 shows that the complexes formed were characterized by reasonably high affinity with \(K_D\) values ranged from 1 to 5 \(\mu\)M. The high affinity of the complexes [pS8-\(\alpha\)\(\beta\)\(_{1–16}\)/\(\alpha\)\(\beta\)\(_{1–16}\) and \(\alpha\)\(\beta\)\(_{1–16}\)] was mainly determined by the high rate of complex formation (\(k_{\text{on}}\) of \(10^4–10^5\) M\(^{-1}\)s\(^{-1}\)) (Table 2).

Table 3. The \(R_{\text{im}}\) and \(R_{\text{max}}\) values (in RU) and their ratio reflecting stoichiometry of the analyzed paired interactions.

| Interacting pairs | \(R_{\text{max}}/R_{\text{im}}\) |
|-------------------|--------------------------------|
| [iso\(\alpha\)\(\beta\)\(_{1–16}\)/\(\alpha\)\(\beta\)\(_{1–16}\)]   | 553/770 \(\sim\) 0.7 |
| [pS8-\(\alpha\)\(\beta\)\(_{1–16}\)/\(\alpha\)\(\beta\)\(_{1–16}\)]   | 800/832 \(\sim\) 1.0 |
| [pS8-\(\alpha\)\(\beta\)\(_{1–16}\)/iso\(\alpha\)\(\beta\)\(_{1–16}\)] | 728/817 \(\sim\) 0.9 |
| [iso\(\alpha\)\(\beta\)\(_{1–16}\)/\(\alpha\)\(\beta\)\(_{1–16}\)]   | 309/751 \(\sim\) 4   |
| [pS8-\(\alpha\)\(\beta\)\(_{1–16}\)/\(\alpha\)\(\beta\)\(_{1–16}\)]   | 356/570 \(\sim\) 0.6 |

\(^{1}\)\(R_{\text{max}}\) is the maximum analyte binding capacity of the chip with the immobilized ligand.

\(^{2}\)\(R_{\text{im}}\) is the total amount of the immobilized ligand.
These results clearly demonstrate high stability of the formed heterodimers.

### 3.2. Identification of the key MBD segment involved in formation of the zinc-induced heterodimers

Importance of various MBD sites for zinc-induced dimerization has been evaluated during analysis of interaction of immobilized pS8-Aβ<sub>1-16</sub> with added pS8-Aβ<sub>1-16</sub> or Aβ<sub>1-16</sub> or their fragments (Figure 2). Figure 2 shows that the immobilized pS8-Aβ<sub>1-16</sub> formed complexes only with peptides containing the fragment Aβ<sub>11-14</sub> (EVHH). This fragment was previously identified as the critical one for the formation of soluble zinc-bound pS8-Aβ<sub>1-16</sub> homodimers (Kulikova et al., 2014).

For independent validation of the key role of the Aβ<sub>11-14</sub> site, we have also analyzed zinc-induced interaction between the immobilized Aβ<sub>1-16</sub> and the synthetic peptide corresponding to the rat Aβ MBD (ratAβ<sub>1-16</sub>). It is well documented that rat Aβ MBD and human Aβ MBD differ by three amino acid residues (Istrate et al., 2012), one of which is located just in the segment 11–14 (Table 1). Figure 3 shows that ratAβ<sub>1-16</sub> did not interact with the immobilized Aβ<sub>1-16</sub>. This obviously means that replacement of only one amino acid residue in the 11–14 segment dramatically impairs (or even abolishes) heterodimer formation.

### 3.3. Molecular dynamics of the heterodimers

Earlier, we have shown that peripherally applied synthetic isoAβ<sub>1-42</sub> accelerated pathological cerebral

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**Table 4. Affinity and kinetic parameters of interactions between different variants of Aβ MBD.**

| Complexes                          | $k_{on}$, M<sup>-1</sup>s<sup>-1</sup> | $k_{off}$, s<sup>-1</sup> | $K_D$, M<sup>b</sup> | $\chi^2$<sup>c</sup> |
|------------------------------------|------------------------------------|---------------------------|----------------------|----------------------|
| [isoAβ<sub>1-16</sub>/Zn/isoAβ<sub>1-16</sub>] | (1.39 ± .03) × 10<sup>5</sup> | (1.3 ± .3) × 10<sup>-2</sup> | (9 ± .2) × 10<sup>-7</sup> | 3.7                  |
| [pS8-Aβ<sub>1-16</sub>/Zn/pS8-Aβ<sub>1-16</sub>] | 11.0 ± 2                      | (2.9 ± .1) × 10<sup>-5</sup> | (2.5 ± .1) × 10<sup>-6</sup> | 12.1                |
| [pS8-Aβ<sub>1-16</sub>/Zn/isoAβ<sub>1-16</sub>] | 9.8 ± 5                      | (3.2 ± .3) × 10<sup>-5</sup> | (3.3 ± .5) × 10<sup>-6</sup> | 11.4                |
| [isoAβ<sub>1-16</sub>/Zn/Aβ<sub>1-16</sub>] | (4.6 ± .2) × 10<sup>4</sup> | (10.1 ± .5) × 10<sup>-2</sup> | (2.2 ± .2) × 10<sup>-6</sup> | 17.2                |
| [pS8-Aβ<sub>1-16</sub>/Zn/Aβ<sub>1-16</sub>] | (8.3 ± .2) × 10<sup>3</sup> | (1.9 ± .1) × 10<sup>-2</sup> | (2.3 ± .2) × 10<sup>-6</sup> | 16.5                |

Notes: All the parameters have been calculated using sets of sensograms obtained during serial injections of the analytes with different concentrations.

<sup>a</sup>Association ($k_{on}$) and dissociation ($k_{off}$) rate constants were calculated using the Langmuir binding model (1:1 complex formation) with fitting of model and experimental curves.

<sup>b</sup>Equilibrium dissociation ($K_D$) constants for the complexes were calculated as the ratio: $K_D = k_{off}/k_{on}$.

<sup>c</sup>Quality of superimposed curves was evaluated using $\chi^2$. If the square root of $\chi^2$ has the same value as the noise of the SPR signal, it indicates a good fitting.

Data represent mean ± SD of three independent experiments.

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![Figure 2](image-url)

**Figure 2.** Interaction of immobilized pS8-Aβ<sub>1-16</sub> with added pS8-Aβ<sub>1-16</sub> and its fragments (A) or with added Aβ<sub>1-16</sub> and its fragments (B).

Notes: The concentration of all analytes in the samples was 20 μM. The ordinates axis represents the biosensor signal at the end of the analyte injection (after 300 s). All measurements were repeated in triplicates. Standard deviation of the measurements did not exceed 5%.
amyloidosis in a transgenic AD model (Kozin et al., 2013). Probable mechanism of such pathogenic action of isoAβ\(_{1-42}\) is obviously associated with its ability to act as an aggregation seed for endogenous Aβ molecules due to zinc-inducible intermolecular interactions (Miller, Ma, & Nussinov, 2010; Tsvetkov et al., 2010). In contrast to intact Aβ, isoAβ MBD containing isoAsp7 undergoes more pronounced zinc-dependent dimerization (Kozin et al., 2015; Tsvetkov et al., 2008). However, despite these differences, conformation of the 10–15 segment remains basically unaltered in MBDS of both intact and isomerized Aβ (Nisbet et al., 2013; Zirah et al., 2006). In order to investigate conformational behavior of the Aβ N-terminal fragments 1–10, we have accomplished simulation of 200 ns restrained molecular dynamic trajectories for two model dimers: homodimer [Aβ\(_{1-16}/\text{Zn}/Aβ\(_{1-16}\)] and heterodimers [isoAβ\(_{1-16}/\text{Zn}/Aβ\(_{1-16}\)]. The structures of zinc-bound fragments 11–14 were kept invariant for all dimers and corresponded to that in the NMR structure of the English mutant (PDB code 2MGT), while motions of the residues 1–10 were not restrained.

Analysis of the molecular dynamic trajectories revealed that fragments 1–10 are remarkably flexible in both intact Aβ\(_{1-16}\) and isoAβ\(_{1-16}\) peptide chains, however,

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**Figure 3.** Sensograms of Aβ\(_{1-16}\) (1) and ratAβ\(_{1-16}\) (2) interaction with the immobilized Aβ\(_{1-16}\).

Note: The analyte concentration was 5 μM.

**Figure 4.** RMSD from the initial structure for the conformations sampled from the molecular dynamic trajectory with the 100 ps step: (A) Homodimer [Aβ\(_{1-16}/\text{Zn}/Aβ\(_{1-16}\)]; (B) Heterodimer [isoAβ\(_{1-16}/\text{Zn}/Aβ\(_{1-16}\)].

Note: RMSD values are calculated over all back-bone heavy atoms (N, Ca and C) separately for each peptide chain of the dimer.
the normal Aβ₁₋₁₆ chain is more mobile (Figure 4, Supplementary Figure 1). Dimer structures relax during the first nanosecond of simulation, as reflected by the initial rapid increase of the RMSD (Figure 4). During the rest part of the trajectories RMSD fluctuates near the average values (average RMSD over the 2–200 ns part of the trajectory for the homodimer: .35 nm for chain A and .43 nm for chain B; for the heterodimer: .51 nm for the isoAβ₁₋₁₆ chain and .41 nm for the Aβ₁₋₁₆ chain). These fluctuations correspond to the conformational transition of the 1–10 fragment of the peptide chains (Figure 5). Fluctuations have higher amplitude and are more frequent for the intact chains of the dimers indicating that the normal Aβ₁₋₁₆ chain is more flexible than the isoAβ₁₋₁₆ chain. This finding is supported by the cluster analysis. In the case of the isoAβ₁₋₁₆ chain 54.5% of all conformations sampled from the trajectory with 100 ps step are grouped in three large clusters, while for the intact chain of homodimer overall cluster heterogeneity is higher (Supplementary Figure 2).

Structured bended fragments stable along the trajectory have been found in the region of residues 6–8 of the different Aβ peptides. Two hundred conformations of the fragment 7–10 for one peptide chain of each dimer are shown. The structures are superimposed over N, Ca and C atoms of the residues 6, 7 and 8. (A) homodimer [Aβ₁₋₁₆/Zn/Aβ₁₋₁₆]; (B) heterodimer [isoAβ₁₋₁₆/Zn/Aβ₁₋₁₆].

Notes: The area of hydrogen bond formation between side chains of D7 and S8 is pointed by dashed line. Snapshots are taken with 1 ns step. Side chains are shown for the residues 7 and 8 for all peptides.
Elongation of the isoAβ1–16 backbone with the extra CH2 group disrupted hydrogen bond between side chains of the residues 7 and 8. The corresponding fragment 6–8 of the isoAβ1–16 demonstrates a strong propensity toward extended conformations (Figure 6(B)) and is more flexible (Table 5).

4. Discussion

This SPR-based study provided the first experimental evidence that synthetic peptides corresponding to MBD of two Aβ variants associated with the development of AD, pS8-Aβ, and isoAβ, form zinc-induced heterodimers with ‘normal’ Aβ MBD. These heterodimers, [isoAβ1–16/Zn/Aβ1–16] and [pS8-Aβ1–16/Zn/Aβ1–16], are characterized by very similar Kd values of about 10^-6 M, suggesting reasonable stability of the complexes under physiologically relevant conditions. Results of this study (Figures 2 and 3) suggest that [isoAβ1–16/Zn/Aβ1–16] and [pS8-Aβ1–16/Zn/Aβ1–16] have the same intermolecular interface, in which one zinc ion is coordinated by Glu11 and His14 of the interacting subunits.

Earlier, it was demonstrated that the fragment including residues 11–14 is a primary site for recognition and binding of zinc ion by the intact Aβ (Tsvetkov et al., 2010; Zirah et al., 2006). This site is critically important for the formation of the zinc-bound interface in the homodimers formed by Aβ1–16 (Kozin et al., 2011), pS8-Aβ1–16 (Kulikova et al., 2014), and H6R-Aβ1–16 (Kozin et al., 2015). The same fragment constitutes the zinc-induced interface in the homodimers formed by the peptides corresponding to intact Aβ residues 11–18 (Alies et al., 2012). Taking into consideration all these data, it is reasonable to conclude that the fragment 11–14 plays a key role not only in the molecular mechanism of zinc-dependent homodimerization but also in heterodimerization of different human Aβ variants.

Simulation of 200 ns molecular dynamic trajectories for the homodimer [Aβ1–16/Zn/Aβ1–16] and the heterodimer [isoAβ1–16/Zn/Aβ1–16] demonstrated some differences in conformational behavior of the fragments 1–10. The region including residues 6–8 of the intact peptide adopted stable (along the trajectory) conformation of the bend, while corresponding fragment of isoAβ1–16 existed in the extended state (Figure 6(A), (B)). Our analysis of molecular dynamic trajectories suggests that the structure of the conformational motif 6–8 modulates motions of the peptides. Bended conformation of the 6–8 site in Aβ1–16 yields higher flexibility for the intact peptide chain (Figure 4(A)), while extended conformation of the same site in isoAβ1–16 makes peptide chain more rigid (Figure 4(B)). These variations in the protein chain dynamic properties can significantly influence the zinc-mediated oligomerization process for different Aβ forms: more rigid structures are more susceptible for oligomerization (Parker et al., 1999).

Driving forces responsible for the formation of neurotoxic Aβ dimers and oligomers in AD still remain unknown (Jucker & Walker, 2013; Prusiner, 2012). On the one hand, Aβ oligomers isolated from both soluble and insoluble fractions of AD postmortem brain tissue (Larson & Lesné, 2012) are detergent resistant; on the other hand, synthetic analogs of intact Aβ are not amyloidogenic in vivo (Meyer-Luehmann et al., 2006). Assuming that the behavior of isolated MBDs may be extrapolated to the full-length Aβ, it is reasonable to propose the following scenario in AD: occasionally appeared pathologically altered Aβ molecule forms zinc-induced heterodimer with a native Aβ molecule, which adopts altered conformation. Acting as pathogenic seeds, these heterodimers cause further propagation of the pathological processes resulting in Aβ-induced neurotoxicity and/or initiation of AD. Chemically modified Aβ variants (Meyer-Luehmann et al., 2006), particularly, isoAβ (Kozin et al., 2013), may well serve as putative candidates responsible for such amyloid seeding in this disease.

Table 5. Backbone RMSD for the residues 6, 7, and 8 of one of the dimer chains (Aβ1–16 chain and isoAβ1–16 chain).

| Dimer          | RMSD for the fragment including residues 6–8 [Å] |
|---------------|-----------------------------------------------|
| [Aβ1–16/Zn/Aβ1–16] | .36                                           |
| [isoAβ1–16/Zn/Aβ1–16] | .61                                           |

Notes: RMSD has been calculated over the family of 200 conformations from the molecular dynamic trajectory taken with 1 ns step. Structures of each family have been superimposed over N, Cα and C atoms of the same residues 6, 7 and 8.

Abbreviations

AD: Alzheimer’s disease
Aβ: amyloid-beta
MBD: metal-binding domain
pS8-Aβ: amyloid beta with the phosphorylated Ser8 residue
isoAβ: amyloid-beta with the isomerized Asp7 residue
SPR: surface plasmon resonance

Supplementary material

The supplementary material for this paper is available online at http://dx.doi.org/10.1080/07391102.2015.1113890.
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