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Elongation Affinity, Activation Barrier, and Stability of A β 42 Oligomers/Fibrils in Physiological Saline

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Abstract

Amyloid-beta (A β) peptides, A β 40 and the more neurotoxic A β 42, have been the subject of many research efforts for Alzheimer's disease. In two recent independent investigations, the atomistic structure of A β 42 fibril has been clearly established in the S-shaped conformation consisting of three β -sheets stabilized by salt bridges formed between the Lys28 sidechain and the C-terminus of Ala42. This structure distinctively differs from the long-known structure of A β 40 in the β -hairpin shaped conformation consisting of two β -sheets. Recent *in silico* investigations based on all-atom models have reached closer agreement with the *in vitro* measurements of A β 40 thermodynamics. In this study, we present an *in silico* investigation of A β 42 thermodynamics. Using the established force field parameters in seven sets of all-atom simulations, we examined the stability of small A β 42 oligomers in physiological saline. We computed the elongation affinity of the S-shaped A β 42 fibril, reaching agreement with the experimental data. We also estimated the Arrhenius activation barrier along the elongation pathway (from the disordered conformation of a free A β 42 peptide to its S-shaped conformation on a fibril) that amounts to about 16 kcal/mol, which is consistent with the experimental data. Based on these quantitative agreements, we conclude that aggregation of A β 42 peptides into fibrils is thermodynamically slow without precipitation by extrinsic factors such as heparan sulfate proteoglycan and highlight the possibility to prevent A β 42 aggregation by eliminating some precipitation factors or by increasing competitive agents to capture and transport free A β 42 peptides from the cerebrospinal fluid.

Graphical abstract

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Keywords

Amyloid beta; Peptides; Affinity; Protein Interaction; Molecular Dynamics

Introduction

Formation of amyloid-beta ($A\beta$) fibrils has been identified as a hallmark of Alzheimer's disease (AD) pathology. The 42-residue peptide $A\beta_{1-42}$ and the 40-residue peptide $A\beta_{1-40}$ have both been subject to extensive investigations[1-3]. The β -hairpin shaped structures of the truncated $A\beta_{9-40}$ and $A\beta_{17-42}$ fibrils were resolved in multiple NMR experiments[4-6] and, based on the NMR structure coordinates, many *in silico* studies were accomplished (e.g. [7-15]). Through *in silico* experiments, peptides without N-terminal residues such as $A\beta_{9-40}$ were found capable of forming stable filaments/fibrils.[7, 8, 15] While all-atom models are crucial for elucidating atomistic details, one can probe much longer time scales with the coarse-grained models[16-18], implicit water[19, 20], or the hybrid models[21].

Through *in vitro* experiments of biochemical kinetics, the elongation processes of both $A\beta_{40}$ and $A\beta_{42}$ fibrils were carefully characterized.[22-30] The elongation affinities were found to correspond to the Gibbs free energies of -9 kcal/mol for $A\beta_{40}$ [25] and -11.8 kcal/mol for $A\beta_{42}$ [27] respectively. An earlier all-atom *in silico* study[9] of the $A\beta_{17-42}$ filament based on the β -hairpin structure[5] gave atomistic insights into the intra- and the inter-peptide interactions along with the relative free energies of elongation among various $A\beta$ mutants. The estimated absolute free energy of elongation was -50.5 kcal/mol for the wild type $A\beta$. A recent all-atom *in silico* investigation[13] of the $A\beta_{9-40}$ filament based on the structure of Ref.[6] gave atomistic details of the docking and locking mechanisms[21] and the free-energy landscape along the dissociation pathway of an $A\beta_{40}$ peptide from the β -hairpin shaped filament. This study also gave estimated free energies of elongation: -13.7 kcal/mol on the even end and -14.8 kcal/mol on the odd end. All these indicate that all-atom *in silico* studies can reach quantitative agreement with *in vitro* experiments when the statistical mechanics is implemented with sufficient accuracy.

Very recently, the $A\beta_{1-42}$ fibril structure has been resolved to atomistic resolution[31-33] that is distinctively different from the β -hairpin characteristics of $A\beta_{1-40}$ or $A\beta_{17-42}$. $A\beta_{1-42}$ fibril is characterized with an S-shaped conformation involving a salt bridge formed between the negatively charged terminal carboxyl group of Ala42 and the positively charged sidechain of Lys28. In this paper, we report an all-atom *in silico* study based on the S-shaped structure of $A\beta_{1-42}$. We computed the free energy of elongation, namely, the Gibbs free energy of adding a peptide onto a fibril. The result of -11.3 kcal/mol agrees with the *in vitro* data of -11.8 kcal/mol. We also examined the free-energy landscape along a path from the random-coil conformation of a free $A\beta_{42}$ peptide to the S-shaped conformation without a

“chaperon”. We estimated the barrier separating the two conformations to be around 16.2 kcal/mol which is consistent with the *in vitro* data of 23 kcal/mol[23] and 15.8 kcal/mol[29]. With this, we confirmed the quantitative accuracy of the CHARMM force field parameters for peptide-peptide interactions in physiological saline. In addition, we searched for the smallest oligomers that are stable by themselves for further growth into A β 42 fibrils by conducting four separate *in silico* experiments of A β _{11–42} monomer, dimer, trimer, and tetramer in physiological saline. Furthermore, we explored the possibility that an S-shaped filament facilitates the formation of new S-shaped oligomers by simulating a peptide monomer/dimer placed in the proximity of an S-shaped filament.

Methods

System setup

For the affinity study, we took the S-shaped fibril structure consisting of two filaments [31] (PDB code: 5KK3), rotated it so that the filament axis is along the z-axis of our coordinate system, placed the protein in the center of a 100Å×100Å×130Å box of water, neutralized the charge with 18 Na⁺ ions, and salinated the system into 150 mM of NaCl. The model system so constructed has 123,338 atoms in total. After equilibration under 1 bar of constant pressure, the system settled down to the size of 98.3Å×98.3Å×127.3Å. In similar manners, we constructed four model systems of small oligomers and two model systems for a monomer/dimer interacting with an S-shaped filament, all in 150 mM saline.

Simulation parameters

In all the equilibrium molecular dynamics (MD) and nonequilibrium steered molecular dynamics (SMD) runs, we used the CHARMM force field [34, 35] for all the intra- and inter-molecular interactions. We implemented the Langevin stochastic dynamics with NAMD[36] to simulate the systems at a constant temperature of 298 K and a constant pressure of 1 bar. We implemented full electrostatics by the means of particle mesh Ewald (PME)[37] at the grids of 128×128×128. The time step was 1 fs for short-range and 2 fs for long-range interactions. The PME was updated every 4 fs. The damping constant was 5/ps. Explicit solvent was represented with the TIP3P model. [38] In the SMD runs (pulling peptide P1 away from the fibril), the alpha carbons on three peptides of each filament (P7, P8, P8, P16, P17, and P18) were fixed to their coordinates of the fully equilibrated structure. The pulling velocity was chosen as $\mathbf{v_d} = (0, 0, \pm 2.5 \text{ Å/ns})$ along the z-axis.

Computation of elongation affinity

We followed the procedure of the hybrid SMD (hSMD) detailed in Refs. [39, 40]. Briefly, we conducted MD runs to sample the fluctuations of the peptides in the bound state and in the dissociated state. We conducted SMD runs to pull peptide P1 from the one chosen bound state (the SMD starting point) along a chosen dissociation path to the one corresponding dissociated state (the SMD endpoint), disallowing any fluctuations of the pulling centers along the pulling paths. In this way, we computed the difference in potential of mean force (PMF) $\text{PMF}_{0,\infty}$ between the one dissociated state and the corresponding one bound state via the Brownian dynamics fluctuation-dissipation theorem (BD-FDT) [41]. We computed the partial partitions in the bound state from the fluctuations around the one chosen bound

state which is the starting point of the SMD runs and the partial partition functions in the dissociated state from the fluctuations around the one dissociated state which is the endpoint of the SMD runs. The bound state partial partition Z_0 was evaluated with the Gaussian approximation. The unbound state partial partition Z_∞ involved multiple SMD runs between intermediate conformations and MD runs around those conformations of a free A β 42 peptide in physiological saline. The dissociation constant was obtained as $k_D = Z_\infty \exp[-\text{PMF}_{0,\infty} / k_B T] / Z_0$ or, equivalently, the free energy of elongation $G = \text{PMF}_{0,\infty} + k_B T \ln(Z_\infty / c_0 Z_0)$. Here $c_0 = 1 \text{ mol/L} = 6.02 \times 10^{-4} / \text{\AA}^3$ is the standard concentration. k_B is the Boltzmann constant. T is the absolute temperature.

Results and Discussion

Elongation affinity

In order to determine the free energy of elongation (namely, the free energy required to add a monomer A β peptide to the end of an S-shaped filament), we computed the PMF along a dissociation path from one chosen bound state (P1 on the filament) to the corresponding one unbound state (P1 away from the filament) by pulling the eight α -carbons on P1 away from the end of the fibril (Fig. 1). The PMF curve along the pulling path is shown in Fig. 2 (A). The PMF difference between the two ends of this dissociation path was found to be

$\text{PMF}_{0,\infty} = -65.7 \text{ kcal/mol}$. Along this dissociation path, the eight α -carbons on His13, Phe20, Asp23, Val24, Asn27, Leu34, Gly38, and Ala42 of P1 were not allowed to fluctuate. Their fluctuations were instead taken into account in the partial partitions Z_0 of the bound and Z_∞ of the unbound states, respectively. During the SMD pulling, the same set of eight α -carbons on peptide P2 were fixed, not allowed to fluctuate as well. Therefore, their fluctuations in the bound and the unbound states were also included in Z_0 and Z_∞ , respectively. The fluctuations of the two sets of α -carbons on P1 and P2 can be approximated as Gaussian in the bound state ensemble (shown in supplemental information (SI), Figs. S1 to S16). They altogether gave $Z_0 = 9.6 \times 10^3 \text{ \AA}^{48}$. In the unbound state ensemble, only the fluctuations of the second set of α -carbons (those on peptide P2) can be approximated as Gaussian (SI, Figs. S9 to S16), giving rise to $Z_{\infty P2} = 3.8 \times 10^6 \text{ \AA}^{24}$. In this case, however, the contributions to Z_∞ from P1 and P2 are separable. Namely, $Z_\infty = Z_{\infty P1} Z_{\infty P2}$. The partial partition $Z_{\infty P1}$ involves not only the fluctuations of the α -carbons but also the conformational changes from the S-shape peptide (in the mold of the fibril, Fig. 2 (E) inset) to the random coil of a free A β peptide in a saline solution (Fig. 2(B), insets). Combining the four sets of data shown in Fig. 2 (B) to (E), we obtained $Z_{\infty P1} = 3.9 \times 10^{33} \text{ \AA}^{21}$. Altogether, the free energy of elongation, namely, the free energy required in the molecular event of adding a monomer A β peptide onto the S-shaped fibril,

$$\Delta G = \Delta \text{PMF}_{0,\infty} + k_B T \ln(Z_{\infty P1} Z_{\infty P2} / c_0 Z_0) = -11.3 \text{ kcal/mol}.$$

Correspondingly, the dissociation constant $k_D = 6.9 \times 10^{-9} \text{ mol/L}$. This value is in close agreement with the experimental data of $k_{\text{off}} / k_+ = 3.3 \times 10^{-9} \text{ mol/L}$ derived from the on rate k_+ and the off rate k_{off} [27]. This agreement demonstrates that the all-atom force-field

parameters employed in our study are quantitatively accurate for protein-protein interactions in physiological saline.

Activation barrier for elongation

It should be noted that, in the all-atom studies of the β -hairpin shaped filaments of the truncated A β 40/42 peptides, the free-energy landscape along the one-dimensional (1D) dissociation path (inverse of the association path) was monotonic, suggesting the absence of an activation barrier along the association path of adding an A β peptide unto the end of an A β filament. In contrast, our PMF in Fig. 2(A) is a curve along the 24D phase space. The 24D PMF being monotonic does not indicate the absence of activation barriers in the pathway of adding an A β 42 peptide unto the S-shaped A β filament because along this path 24 degrees of freedom of the peptide were not allowed to fluctuate. The use of this fluctuation-less path is correct only in combination with fully sampling the fluctuations in the bound state and in the unbound state. While the fluctuations of the 24D degrees of freedom in the bound state are Gaussian and barrier-less, the fluctuations in the unbound state are highly nontrivial and non-Gaussian. They involve conformational changes of the A β 42 peptide from its S-shape in Fig. 2 (E) to the random coil in Fig. 2(B). Following the PMF curves from Point (4.0 Å, 0 kcal/mol) to Point (0 Å, -11.5 kcal/mol) in Fig. 2(E), then from Point (5.4 Å, 0 kcal/mol) to Point (0 Å, -2.4 kcal/mol) in Fig. 2(D), then from Point (18.3 Å, 0 kcal/mol) to Point (16.2 Å, -0.7 kcal/mol) in Fig. 2(C), and then from Point (21.6 Å, 0 kcal/mol) to Point (18.3 Å, -1.6 kcal/mol) in Fig. 2(B), the total change in PMF is approximately 16.2 kcal/mol. Altogether, the PMF of an A β 42 peptide in the S-shaped conformation (Fig. 2(E)) is 16.2 kcal/mol above that in the random coil conformation (Fig. 2(B)). Considering the barriers of 0.95 kcal/mol in Fig. 2(D) and 16 kcal/mol in Fig. 2(E), we estimate that the Arrhenius activation energy of the elongation process is about 16.2 kcal/mol but certainly less than 33.2 kcal/mol. This estimation is consistent with the *in vitro* data of 23 kcal/mol[23] and 15.8 kcal/mol[29].

Stability of oligomers in physiological saline

Conducting four *in silico* experiments of a monomer, dimer, trimer, or tetramer of A β 42 peptides (shown in SI, Fig. S17) in physiological saline, we examined the stability and conformations of small oligomers of A β ₁₁₋₄₂ peptides. (For convenience, here an “oligomer” means either a monomer, dimer, trimer or tetramer.) Starting from the S-shaped conformation, a monomer rapidly became a random coil (Fig. S17(A)); a dimer quickly lost its S-shape but approximately retained its dimeric form (Fig. S17(B)); a trimer deviated significantly from its initial S-shaped conformation (Fig. S17(C)); a tetramer, however, was essentially stable (Fig. S17(D)). Quantitatively, Fig. 3 shows the number of hydrogen bonds per peptide that are formed within an oligomer in contrast with the number of hydrogen bonds per peptide that are formed between the peptides and their aqueous environment. Fig. 4 shows the peptide root mean square deviation (RMSD) as a function of time. The number of hydrogen bonds per peptide that are formed between waters and an oligomer fluctuates at a level that is dependent on the size of the oligomer with a monomer having the greatest value. The number of hydrogen bonds per peptide that are formed within an oligomer also fluctuates at a size-dependent level. And the level is time dependent in the course when the conformation of an oligomer deviates from the initial structure. In particular, the number of

hydrogen bonds per peptide that are formed within a trimer dropped about 50% (Fig. 3, third panel). This decrease is consistent with increased fluctuations visible in the atomistic trajectories. In contrast, the same number within a tetramer remained unreduced. While a trimer is somewhat stable, a tetramer of the S-shaped A β peptides is very stable in physiological saline. In contrast to the β -hairpin shaped A β_{40} peptides that need a minimum of six peptides to be a stable aggregate [8], a tetramer of the S-shaped A β_{42} peptides is the minimal oligomer that is stable in physiological saline for subsequent elongation growth into a mature fibril-filament.

Fibrils facilitate nucleation of A β peptides

To explore how an A β_{42} fibril/filament might facilitate nucleation of A β peptides/formation of new oligomers, we removed all but one or two monomers from one filament of the A β fibril (PDB code: 5KK3) consisting of two filaments of S-shaped A β_{42} peptides. We placed the so-formed monomer-filament and dimer-filament complexes in a box of water salinated with 150 mM NaCl. We ran 50 ns of equilibrium MD for each model system and obtained the RMSDs of the monomer/dimer from its initial S-shaped conformation which are shown in Fig. 4. These results demonstrate that a monomer does not assume the S-shaped conformation even in the proximity of a long filament of S-shaped A β_{42} peptides. Remarkably, a dimer of A β_{42} peptides showed stability and retained its S-shaped conformation. This *in silico* observation of the stability of an A β_{42} dimer interacting with a long filament is consistent with the *in vitro* experimental elucidation that fibrils facilitate nucleation of A β_{42} peptides, simultaneously taking two monomers in the initial step of oligomer formation.[27]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- A β 42 fibril elongation affinity was computed with chemical accuracy, in agreement with *in vitro* data
- Small A β 42 oligomers were found to be stable in physiological saline
- A long A β 42 fibril-filament was shown to stabilize an A β dimer in its proximity

Fig. 1.

Ribbon representation of the S-shaped conformation of 18 Aβ₁₁₋₄₂ peptides (P1 to P18) assembled into fibril-filaments. The top view (from the z-axis) and the side view are in the left and the right panels respectively. Peptides P2 to P18 are colored by residue types (hydrophilic, green; hydrophobic, white; positively charged, blue, negatively charged, red). The P1 ribbon is colored in blue on which the α-carbons of His 13, Phe 20, Asp 23, Val 24, Asn 27, Leu 34, Gly 38, and Ala 42, represented as large spheres, are chosen as the pulling centers. In the right panel, bottom array, from right to left are P1 to P9 and in top array, from right to left are P10 to P18. All molecular graphics in this paper were generated with VMD. [42]

Fig. 2.

PMF curves. (A) PMF along the dissociation path of the outermost A β_{11-42} peptide monomer P1 from the end of the A β fibril. Inset: each A β peptide is represented as spheres colored distinctively. In particular, P1 is colored blue. (B)-(E) PMFs along the conformational changes of A β_{11-42} (represented as ribbons in insets) from the random coil state of the free A β peptide to the S-shaped monomer ready for adhesion onto the end of a fibril. (B) PMF as a function of distance between Ala42Ca and Leu34Ca while all other pulling centers are free to fluctuate. (C) PMF as a function of the distance between Asn27Ca and Leu34Ca while Ala42Ca is fixed and all other pulling centers are free to fluctuate. (D) PMF as a function of Phe20Ca displacement while Ala42Ca, Asn27Ca, and Leu34Ca are fixed and all other pulling centers are free to fluctuate. (E) PMF as a function of Asp23Ca-Val24Ca displacement while all other pulling centers are fixed. In (B), (C), and (E), A β_{11-42} peptide is colored by residue types as in Fig. 1. In (D), the peptide is colored respectively in cyan in the conformation of zero displacement and in blue in the conformation of displacement at 5.4 Å. The error bars represent standard errors among four sets of SMD runs.

Fig. 3.
Number of hydrogen bonds per peptide that are formed within an oligomer vs those formed with waters.

Fig 4. RMSD of Aβ₁₁₋₄₂ oligomers. Top panel, oligomers in saline (illustrated in Fig. S17). Bottom panel, a monomer/dimer placed beside and interacting with an S-shaped filament shown in the insets. The filaments are shown as yellow cartoons while the monomer/dimer peptides are represented as blue ribbons.