Spontaneous β-barrel formation: An all-atom Monte Carlo study of Aβ$_{16–22}$ oligomerization

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ABSTRACT

Using all-atom Monte Carlo simulations with implicit water, combined with a cluster size analysis, we study the aggregation of Aβ$_{16–22}$, a peptide capable of forming amyloid fibrils. We consider a system of six initially randomly oriented Aβ$_{16–22}$ peptides, and investigate the thermodynamics and structural properties of aggregates formed by this system. The system is unaggregated without ordered secondary structure at high temperature, and forms β-sheet rich aggregates at low temperature. At the crossover between these two regimes, we find that clusters of all sizes occur, whereas the β-strand content is low. In one of several runs, we observe the spontaneous formation of a β-barrel with six antiparallel strands. The β-barrel stands out as the by far most long-lived aggregate seen in our simulations.

INTRODUCTION

Amyloid fibril formation is a symptom of several human neurodegenerative diseases, but increasing evidence suggests that the neurotoxic agent is not the fibrils themselves. Much current research is directed at characterizing small soluble oligomers of amyloid proteins, in order to identify the major toxic species. For example, in a study of Alzheimer’s amyloid-β protein, Aβ, it was found that 56-kDa Aβ assemblies could be linked to loss of memory function in mouse and rat.

While atomic-level structural models have now emerged for some amyloid fibrils, less is known about the detailed structure of the oligomeric states. These states are difficult to characterize because of their transient nature—they can transform into other classes of oligomers, break up into monomers, or move onto fibril formation (which is probably an irreversible process so that there is no equilibrium distribution). On the other hand, there are some common classes of oligomeric states that have been observed for several amyloid proteins such as spherical, chain-like, and annular species. One possible explanation of neurotoxicity is that annular pore-like aggregates cause membrane permeabilization.

Here, we explore the structure and stability of small Aβ$_{16–22}$ oligomers by all-atom Monte Carlo (MC) simulations for a system of six Aβ$_{16–22}$ peptides. This seven-residue fragment of Aβ is known to be able to make amyloid fibrils. Furthermore, it has been demonstrated, by solid-state NMR, that the β-strand organization is antiparallel in Aβ$_{16–22}$ fibrils. A recent study of Aβ$_{16–22}$ by infrared spectroscopy (IR) found evidence for antiparallel β-sheet structure also in solution.

The availability of experimental data, its small size, and the fact that it spans an aggregation-prone region of Aβ, make the Aβ$_{16–22}$ peptide a suitable model system for computational studies, and simulations of Aβ$_{16–22}$ aggregation have been reported by several groups. Here, we study Aβ$_{16–22}$ oligomerization by unbiased thermodynamic simulations started from random initial conformations. We use a simple but novel procedure to identify which clusters are formed in a given multichain conformation. Another novelty is that we observe the spontaneous formation of a β-barrel. The formation of annular, β-barrel-like structures and open high-curvature β-sheets has previously been seen in simulations based on coarse-grained models, but as far as we know, not in atomic-level simulations for Aβ$_{16–22}$ or any other sequence. Reviews of computational aggregation studies of amyloid peptides can be found in two recent articles.

Calculations similar to those presented here, but without any cluster size analysis, have been reported earlier. In that study no β-barrel was observed. The fact that we do so here could, in part, simply be due to improved statistics (by a factor 6). Another

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difference is that we here use a slightly modified energy function that incorporates attractions and repulsions between side-chain charges (see Materials and Methods), which increase the stability of the β-barrel (see Results and Discussion).

MATERIALS AND METHODS

Model

The system we study consists of six Aβ\textsubscript{16–22} peptides (acetyl-Lys-Leu-Val-Phe-Phe-Ala-Glu-NH\textsubscript{2}) contained in a periodic box of size (50.4 Å\textsuperscript{3}), with implicit water. All atoms of the peptide chains are included in our calculations, but we assume fixed bond lengths, bond angles, and peptide torsion angles (180°), so that each residue only has the Ramachandran torsion angles φ, ψ and a number of side-chain torsion angles as its degrees of freedom. Numerical values of the geometrical parameters held constant can be found elsewhere.\textsuperscript{30}

The energy function we use is a close variant of an energy function\textsuperscript{30,31} that has been used to study the folding of several peptides with about 20 residues,\textsuperscript{31} the aggregation of Aβ\textsubscript{16–22},\textsuperscript{16} and the mechanical and thermal unfolding of ubiquitin.\textsuperscript{32,33} It is composed of four terms,

\[ E = E_{\text{loc}} + E_{\text{ev}} + E_{\text{hb}} + E_{\text{sc}}. \]  

The term \( E_{\text{loc}} \) is an intrachain potential and is local in sequence. It represents an electrostatic interaction between adjacent peptide units along the chain. The other three terms are both intra- and interchain potentials and are nonlocal in sequence. The excluded volume term \( E_{\text{ev}} \) is a 1/r\textsuperscript{12} repulsion between pairs of atoms. \( E_{\text{hb}} \) represents two kinds of H bonds: backbone–backbone bonds and bonds between charged side chains and the backbone. The last term \( E_{\text{sc}} \) represents interactions between pairs of side chains. It is a simple pairwise additive potential based on the degree of contact between two side chains.

There is one major difference between the energy function used here and that used in the previous studies of this model.\textsuperscript{16,31–33} The difference is in the term \( E_{\text{sc}} \), which in the previous studies represented an effective hydrophobic attraction between pairs of nonpolar side chains. In the present study, we have incorporated attraction and repulsion between charged side chains into this term, while leaving the hydrophobicity part unchanged. The interaction between two charged side chains is assumed to be of short range because of screening by water, and is, for simplicity, taken to have the same functional form as the hydrophobic interaction between two nonpolar side chains.\textsuperscript{31} Specifically, the new part is of the form

\[ E_{\text{sc}}^{(q)} = e_q \sum_{i < j} q_i q_j C_{ij}, \]  

where \( I \) and \( J \) denote charged residues, \( q_I \) and \( q_J \) are charges (±1), and \( e_q \) sets the strength of the interaction (≈2.0 kcal/mol). \( C_{ij} \) is a measure of the degree of contact between two amino acids

\[ C_{ij} = \frac{1}{N_I + N_J} \left[ \sum_{i \in A_I} f(\min r_{ij}^2) + \sum_{j \in A_J} f(\min r_{ij}^2) \right]. \]  

\( A_I \) denotes a predefined set of atoms: for Glu it is the two side-chain oxygens and for Lys the hydrogens in the NH\textsubscript{3}-group (there are no Asp or Arg residues in Aβ\textsubscript{16–22}). \( N_I \) and \( N_J \) are the number of atoms in the sets \( A_I \) and \( A_J \). The function \( f(x) \) is given by \( f(x) = 1 \) if \( x < A \), \( f(x) = 0 \) if \( x > B \), and \( f(x) = (B - x)/(B - A) \) otherwise \( [A = (3.5 \text{ Å})^2, B = (4.5 \text{ Å})^2] \). The form of the other energy terms has been described elsewhere.\textsuperscript{31}

Having modified the energy function, we also recalibrated the energy scale of the model by folding simulations for the Trp cage peptide. The energy scale of the model is determined by using the model prediction for the melting temperature of Trp cage and the experimental value\textsuperscript{34} for the same (315 K). On the internal scale of the model, the melting temperature changed by 2% (from 0.470 to 0.479) with the new energy function.

Simulation methods

The thermodynamics of aggregation for this system is investigated by using simulated tempering,\textsuperscript{35–37} in which the temperature is a dynamical variable. This method is closely related to the replica exchange- or parallel tempering method.\textsuperscript{38–40} The main difference is that simulated tempering works with only one copy of the system, whereas the replica exchange method simulates several copies of the system in parallel (which exchange temperatures with each other). Our simulations are carried out using the software package PROFASI.\textsuperscript{41} A total of 30 independent simulated-tempering runs is collected. Ten of the runs span six temperatures from 293 to 362 K, whereas the other 20 runs span five temperatures from 306 K to 362 K. The six-temperature runs each comprise \( 10^{10} \) elementary MC steps. The length of each five-temperature run is \( 6 \times 10^7 \) elementary MC steps. All the runs are started from random conformations.

For the backbone degrees of freedom, we use two different elementary moves: single-variable updates of individual torsion angles, which is a nonlocal method, and biased Gaussian steps,\textsuperscript{42} a semilocal move that simultaneously updates up to eight angles. Side-chain angles are updated one by one. In addition to these updates, for computational efficiency, we also include rigid-body translations and rotations of whole chains. Every update
involves a Metropolis accept/reject step, thus ensuring detailed balance.

**Measurements**

To monitor the aggregation state of the system, we use a cluster-size analysis. Two chains $I$ and $J$ are said to be in the same cluster if the sum of their interchain side-chain interactions, $E_{sc}(I,J)$, and interchain backbone–backbone H bond energy, $E_{bb}(I,J)$, is lower than a cutoff, $E_{c}(I,J) + E_{bb}(I,J) < -1.5 \ v_{bb}^{0}$ corresponding to 2–3 H bonds ($v_{bb}^{0}$ sets the strength of backbone–backbone H bonds $^{31}$). The cutoff is chosen to exclude brief random contacts, without being too restrictive. The size of the largest cluster in a given conformation is denoted by $\Lambda$. The clusters obtained by this definition are referred to as “general” clusters, or simply clusters, and may completely lack ordered secondary structure. We also use a stricter cluster definition. These clusters are referred to as “ordered” clusters. An ordered cluster is formed by pairs $I,J$ of chains with $E_{bb}(I,J) < -1.5 \ v_{bb}^{0}$ and a $\beta$-strand content, as defined below, higher than 0.3 for both chains. An ordered cluster is thus always part of a general cluster. The size of the largest ordered cluster is denoted by $\Lambda_o$.

For a chain with $N$ amino acids, we define the $\alpha$-helix and $\beta$-strand contents as the fractions of the $N - 2$ inner amino acids with their $(\phi, \psi)$ pair in the $\alpha$-helix and $\beta$-strand regions of the Ramachandran space. We assume that $\alpha$-helix corresponds to $-90^\circ < \phi < -30^\circ$, $-77^\circ < \psi < -17^\circ$ and that $\beta$-strand corresponds to $-150^\circ < \phi < -90^\circ$, $90^\circ < \psi < 150^\circ$. The average $\alpha$-helix and $\beta$-strand contents, over all the chains of the system, are denoted by $n_{\alpha}$ and $n_{\beta}$, respectively.

To determine the amounts of parallel and antiparallel $\beta$-sheet structure in a given multichain conformation, we consider all possible pairs of chains. We first identify all chain pairs $I,J$ such that their interchain backbone–backbone H bond energy satisfies $E_{bb}(I,J) < -1.5 \ v_{bb}^{0}$ and both chains $I$ and $J$ have a $\beta$-strand content higher than 0.5. For each such pair of chains, we then calculate the scalar product of their normalized end-to-end vectors. If this scalar product is greater than 0.7 (less than  −0.7), we say that the two chains are parallel (antiparallel). The number of parallel and antiparallel pairs of chains is denoted by $n_{++}$ and $n_{--}$, respectively.

The simulation data are analyzed using multihistogram techniques.$^{43}$ All statistical uncertainties quoted are $1\sigma$ errors obtained by the jackknife method.$^{44}$

Figures of 3D structures were prepared using PyMOL.$^{45}$

**RESULTS AND DISCUSSION**

**Thermodynamics**

Using the methods described earlier, we study the system of six $\alpha\beta_{16-22}$ peptides in the temperature range $293$ K $< T < 362$ K. Figure 1(a) shows the calculated specific heat curve, which exhibits a sharp peak centered at $T = T_{\text{max}} \approx 335$ K. Our cluster size analysis (see Materials and Methods) reveals that large aggregated structures start to form around this temperature. Figure 1(b) shows the probability of having a cluster with five or six chains, $P(\Lambda \geq 5)$, against temperature. This probability increases from close to 0 at high temperature to more than 0.9 at low temperature, through a sigmoid-like transition centered in the vicinity of $T_{\text{max}}$. There is also a clear increase in $\beta$-strand content, $\langle n_{\beta} \rangle$, as the temperature decreases [see Fig. 1(c)]. The $\alpha$-helix content, $\langle n_{\alpha} \rangle$, is, by contrast, small throughout the temperature range studied (data not shown). We thus find that the chains lack ordered secondary structure at high temperature, but form $\beta$-sheet structure at low temperature.

An interesting detail in Figure 1 is that $P(\Lambda \geq 5)$ starts to increase slightly before $\langle n_{\beta} \rangle$, as the temperature is decreased. At $T = T_{\text{max}}$, clusters with five or six chains occur with a significant frequency, whereas the $\beta$-strand content is small.

Figure 2(a) shows the probability that the largest cluster is of size $n$, $P(\Lambda = n)$, against temperature, for different $n$. The maximum of $P(\Lambda = 2)$ is at 357 K, which is well above the specific-heat maximum $T_{\text{max}}$. As $n$ is increased, the maximum of $P(\Lambda = n)$ shifts toward lower temperature. Near $T_{\text{max}}$ all $\Lambda$ are roughly equally probable, showing that clusters of all sizes occur. At low temperature, $\Lambda = 6$ is by far the most common value, so all the chains tend to form a single cluster.

Figure 2(b) shows an analysis similar to that in Figure 2(a) but for ordered clusters. Since an ordered cluster is always part of a general cluster, the size of the largest ordered cluster, $\Lambda_o$, cannot exceed $\Lambda$. Large ordered clusters are, in contrast to large general clusters, very rare at $T = $
$T_{\text{max}}$, where $P(A_5 = 5)$ and $P(A_6 = 6)$ both are close to 0. The absence of large ordered clusters is consistent with the finding that the β-sheet content is small at $T = T_{\text{max}}$ [see Fig. 1(c)]. Large ordered clusters are, by contrast, common at the lowest temperatures studied, where $P(A_5 \geq 5) > 0.5$.

The ordered aggregates seen at low temperature contain β-sheets, which can be either parallel, antiparallel, or mixed parallel/antiparallel. All these three kinds of β-sheet structure occur in our simulations. To find out whether there is a preference for parallel or antiparallel organization, we examine the probability distribution of the variables $n_+$ and $n_-$ (see Materials and Methods), $P(n_+, n_-)$. Figure 3 shows $P(n,0)$ and $P(0,n)$ as functions of $n$ at a fixed temperature of 306 K. $P(n,0)$ is markedly smaller than $P(0,n)$ for all $n$, implying that antiparallel structures are more common than parallel ones. For comparison, Figure 3 also shows the corresponding results from simulations with the interactions between side-chain charges switched off. As previously reported,16 we find a clear preference for the antiparallel organization in this case too, although slightly weaker. This finding suggests that the interactions between side-chain charges alone are not responsible for the antiparallel organization seen in Aβ16–22 experiments10–12.

Figure 4 shows the MC evolution of the energy $E$ in two of our runs, along with some snapshots of long-lived aggregated structures. The simulation time is long enough for aggregated structures to both form and dissolve in the course of the runs, but simulating this system with >900 atoms is nevertheless a challenge. To test the convergence of our results, we computed the specific heat with statistical errors using two different data sets, the five- and six-temperature runs, respectively (see Figure 1).

Figure 1

Temperature dependence of (a) the specific heat $C_V = \langle (E^2) - (E)^2 \rangle / k_B T^2$ (in mK), (b) the probability that the size of the largest (general) cluster is 5 or 6, $P(A \geq 5)$, and (c) the β-strand content $\langle n_\beta \rangle$.
Materials and Methods). The results of the two analyses were in perfect agreement. This agreement suggests that the relative weights of high- and low-energy states are properly sampled, so that quantities like the specific heat can be reliably estimated.

Longer simulations would, by contrast, be needed in order to determine the relative weights of different low-energy states. Nevertheless, we next take a closer look at one particular low-energy state, the β-barrel. The β-barrel occurs in only one of our runs, the one shown in Figure 4(b), although several runs contain curved, almost closed β-sheets. The β-barrel has lower energy than any other observed state and stays intact over a very long period, about $5 \times 10^9$ MC steps [see Fig. 4(b)]. It is by far the most long-lived state seen in any of our simulations.

Another caveat of the analysis presented above is the small number of chains used. To make testable predictions for thermodynamic quantities like the temperature at which aggregation sets in, it would be necessary to study larger systems. New techniques for encapsulating peptides are, however, being developed,51 which have the potential to facilitate future comparisons of experimental and computational studies.

**Structure and stability of the β-barrel**

The geometry of regular β-barrel structures can be classified by the number of strands, $n$, and the shear number, $S$ (see Materials and Methods).46–50 It has been argued that regular β-barrels with good β-sheet geometries and well-packed interiors can be obtained only for a limited set of 10 different $(n,S)$ pairs, namely $(n,8)$ with $4 \leq n \leq 8$, $(n,10)$ with $5 \leq n \leq 8$, and with $(n,S) = (6,12).48,49$ For $n = 6$, the preferred values of $S$ are 8, 10, and 12. The corresponding tilt angles are in the range $45°$–$56°$.

Figure 5 shows a schematic snapshot of the β-barrel observed in our simulations. It is (right-) twisted, as it should, and composed of six antiparallel strands that are tilted relative to the barrel axis. A closer inspection reveals that among the six pairs of adjacent strands, the alignment is in register for one pair and out of register by two residue units for the other five pairs, leading to a shear number of $S = 10$. The observed barrel thus has one of the three preferred $S$ values for regular six-stranded barrels. In fact, $S = 10$ is expected to be optimal with respect to β-sheet geometry, given $n = 6.48$ The $(n,S)$ classification of this barrel is thus perfectly consistent with its high apparent stability [see Fig. 4(b)].

At first glance, a β-barrel may seem inconsistent with IR experiments on Aβ16–22 in solution,12 which found evidence for an antiparallel in-register β-sheet structure, corresponding to $S = 0$. However, this behavior was observed after a relaxation period, during which the
spectra changed with time. A nonnegligible \( \beta \)-barrel population might have been present in the early stages.

Our calculations aim at exploring thermodynamically relevant states of the system, rather than the kinetics of the aggregation process. Nevertheless, they can elucidate possible pathways for the formation of A\( \beta \)\(_{16-22} \) oligomers. Figure 6 illustrates how the configuration of ordered clusters evolves with MC time in two runs. The first run [Fig. 6(a)] is the one containing the \( \beta \)-barrel. The barrel is present over a period extending roughly from \( 2 \times 10^9 \) MC steps to \( 7 \times 10^9 \) MC steps, during which the chains tend to form an ordered cluster of size 6. Immediately before the formation of the barrel, there is a brief phase in which the largest ordered cluster is typically of size 5. This period is, in turn, preceded by a stage dominated by ‘3 + 3’ conformations, which have two ordered clusters of size 3. During the whole duration of the intermediate peak for ordered clusters of size 5, it turns out that the probability of having a general cluster of size 6 is above 0.8 (data not shown). So, all chains are in contact most of this time, but one chain forms less ordered contacts with the other chains. The emerging picture is that of two three-stranded \( \beta \)-sheets merging to form the barrel, although it is not a simple docking of two rigid structures.

An ordered cluster of size 6 occurs in the second run as well [Fig. 6(b)]. It is present approximately between \( 1.0 \times 10^9 \) MC steps and \( 1.8 \times 10^9 \) MC steps. Inspection of snapshots from the run shows that this cluster corresponds to a six-stranded \( \beta \)-sheet that is curved but not closed. The formation of this state occurs through addition of chains one by one to a growing \( \beta \)-sheet, rather than through fusion of two smaller \( \beta \)-sheets. Growth by monomer addition has been experimentally verified as a viable mechanism of fibril formation.\(^{52}\)

The A\( \beta \)\(_{16-22} \) peptide has two charged side chains, which are those of the end residues Lys16 and Glu22. The formation of the \( \beta \)-barrel, with antiparallel strands, brings oppositely charged side chains close to each other. The interactions between these charges might be crucial for the stability of the \( \beta \)-barrel, especially since the number of chains is even, so that a closed structure can be created without getting any conflicting pair of nearby like charges. To test the importance of the interactions between side-chain charges, we study the stability of the \( \beta \)-barrel both with and without these interactions in the model. For each of the two cases, 100 runs are performed at a constant temperature of 334 K, which is near the
specific-heat maximum $T_{\text{max}}$. In these stability tests, we do not use the nonlocal single-variable update of backbone angles (see Materials and Methods). The runs are started with the β-barrel as the initial conformation. Figure 7 shows the MC evolution of the probability of having an ordered cluster of size 5 or 6, $P(L_o \geq 5)$, as obtained from these two sets of runs. Initially, this probability is 1, since $L_o = 6$ for the β-barrel conformation. The subsequent decay of $P(L_o \geq 5)$ is seen to be faster if the interactions between side-chain charges are removed from the model, which shows that the β-barrel indeed is less stable in this case. The difference is, however, quantitative rather than qualitative. The β-barrel remains a local free-energy minimum, although less pronounced, in the absence of these interactions.

**CONCLUSION**

To explore the nature of small oligomers of the fibril-forming Aβ$_{16–22}$ peptide, we have performed an all-atom study of six Aβ$_{16–22}$ peptides enclosed in a periodic box. A simple but useful cluster-size analysis was devised and employed to characterize multichain conformations with respect to aggregates. The analysis distinguishes between general and ordered clusters. At the onset of aggregation, where the specific heat has a sharp peak, general clusters of all sizes were found to occur. Large ordered clusters are, by contrast, rare at this temperature. This difference indicates that hydrophobic association tends to precede secondary-structure formation in the aggregation process. The formation of ordered aggregates may involve fusion of two smaller aggregates, or occur by monomer addition to a growing β-sheet. The runs discussed in Figure 6, although not kinetic simulations, illustrate these two types of behavior.

At low temperature, the aggregated structures were found to be β-sheet rich, with either parallel, antiparallel, or mixed parallel/antiparallel strands. While all these three possibilities occur in the simulations, a clear statistical preference was found for antiparallel over parallel alignment, which is consistent with Aβ$_{16–22}$ experiments.10–12 It is worth noting that the antiparallel preference persists, though slightly reduced, upon removal of the interactions between side-chain charges. This finding suggests that these interactions are not alone responsible for the antiparallel alignment, but other factors play a significant role, too.

In one of our runs, the six chains spontaneously self-assembled into a β-barrel. It occurred only once in 30 runs, but once formed, the β-barrel remained intact over an extraordinary long period, about $5 \times 10^9$ MC steps. This behavior suggests that the β-barrel represents a sharp but not easily accessible minimum on the free-energy landscape. Many other aggregated conformations were also seen in the simulations, indicating a rugged free-energy landscape with many distinct minima.

The observed β-barrel has six antiparallel strands and a shear number of $S = 10$. This value of $S$ is what one expects for a six-stranded barrel with optimal β-sheet geometry,48 which in part explains the high apparent stability of the state.

For peptides in the diverse class of amyloid-forming sequences, the β-barrel motif is a natural but not obvious candidate for a relatively stable oligomeric state. A β-barrel, indeed, is the most long-lived species found in our Aβ$_{16–22}$ simulations. To address the question of whether the ability to form β-barrels is a common property among amyloid-forming peptides, it would be interesting to extend these calculations to other sequences.

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**REFERENCES**

4. Jaroniec CP, MacPhee CE, Bajaj VS, McMahon MT, Dobson CM, Griffin RG. High-resolution molecular structure of a peptide in an


