Published in final edited form as:

Proteins. 2009 October ; 77(1): 1–13. doi:10.1002/prot.22406.

Interpeptide interactions induce helix to strand structural transition in A β peptides

Takako Takeda and Dmitri K. Klimov*

Department of Bioinformatics and Computational Biology, George Mason University, Manassas, VA 20110

Abstract

Replica exchange molecular dynamics and all-atom implicit solvent model are used to compute the structural propensities in A β monomers, dimers, and A β peptides bound to the edge of amyloid fibril. These systems represent, on an approximate level, different stages in A β aggregation. A β monomers are shown to form helical structure in the N-terminal (residues 13 to 21). Interpeptide interactions in A β dimers and, especially, in the peptides bound to the fibril induce a dramatic shift in the secondary structure, from helical states toward β -strand conformations. The sequence region 10–23 in A β peptide is found to form most of interpeptide interactions upon aggregation. Simulation results are tested by comparing the chemical shifts in A β monomers computed from simulations and obtained experimentally. Possible implications of our simulations for designing aggregation-resistant variants of A β are discussed.

Keywords

A β peptides; A β dimers; amyloid fibril; replica exchange molecular dynamics; secondary structure

Introduction

Alzheimer's disease (AD) belongs a growing list of disorders caused by aberrant aggregation of polypeptide chains¹. There is a compelling evidence accumulated over years of experimental work that the onset of AD is related to extracellular aggregation of A β peptides², which are produced by natural cleavage of the transmembrane amyloid precursor protein. These peptides are released in a variety of lengths, among which the 40- and 42-residue species, A β _{1–40} and A β _{1–42}, are most abundant. Aggregation pathway of A β peptides represents a complex cascade of structural transitions, which involves oligomerization of individual chains and formation of amyloid fibrils³. Although A β amyloid fibrils show cytotoxic properties⁴, recent findings suggest that A β oligomers are the primary cytotoxic species in AD^{5–8}. It has been recently reported that synaptic structure and function can be impaired even by the smallest A β oligomers, dimers⁹. However, even if amyloid fibrils are not the leading causative agents in AD, they still represent a reservoir of A β monomers and in certain cases may play a protective role¹⁰.

*corresponding author: dklimov@gmu.edu, phone (703)993-8395.

Under normal physiological conditions A β aggregation, which leads to the formation of amyloid fibrils, is irreversible. Nevertheless, on a molecular level there appears to be a dynamic equilibrium between soluble oligomeric species and amyloid fibrils^{11,12}. Similarly, solution-state NMR experiments indicate that there is an equilibrium between A β monomers and oligomers¹³. Therefore, it is conceivable that a single A β peptide may be recycled through different aggregated states.

A β monomers, which are the starting species in amyloidogenic pathway, have been a focus of numerous experimental studies. It has been shown that A β monomers adopt significant helical structure in the presence of chemical solvents or in a micelle-like environment^{14,15}. However, in aqueous solutions A β monomers appear to be largely unstructured lacking stable secondary or tertiary structures^{16,17}. Despite overall dominance of random coil structures, several regions of A β adopt weak β -strand and α -helix conformations¹⁸. The endproduct of amyloid assembly, A β fibrils, is characterized by extensive and remarkably homogeneous β -sheet structure^{19–24}. In contrast, relatively little is known about the structural organization of A β oligomers. Several experimental studies suggested the development of α -helical structure in A β oligomers^{13,25}. Whether α -helical conformers represent kinetic intermediates formed along amyloidogenic pathway or they are one of the states in the equilibrium ensemble, it is clear that A β amyloid assembly is associated with dramatic changes in secondary structure.

Molecular dynamics (MD) simulations have a potential to reveal microscopic details of A β amyloid formation²⁶. Several studies have explored the conformational properties of A β _{1–40} monomers^{27–30}. Garcia and coworkers have performed explicit water replica exchange molecular dynamics (REMD) simulations and found the existence of several structured regions in generally disordered A β monomer²⁸. Implicit solvent REMD simulations performed by Yang and Teplow also pointed out to random coil-like structure of A β monomer containing independently folded subunits³⁰. Interestingly, their simulations indicated that α -helix conformations in A β monomers have lower free energies than β -strand structures. A useful alternative to all-atom MD in exploring amyloid assembly are the coarse grained peptide models^{31–33}.

In this paper, we use all-atom REMD simulations to investigate the changes in the secondary structure in N-terminal truncated A β _{10–40} peptides upon aggregation. To this end, we study three A β systems - monomers, dimers, and the A β peptides interacting with preformed amyloid fibril (Fig. 1). The first two systems are taken to represent, on an approximate level, the initial (monomers) and intermediate (dimers) stages of A β aggregation. The system, in which A β peptides are bound to the edges of amyloid fibril, is considered as the final stage of aggregation. We term these peptides in the third system as edge peptides because of their location on the edge of amyloid fibril³⁴. Although the three A β systems do not exhaust all A β species, they do allow us to trace the changes in A β structure upon aggregation. We show that interpeptide interactions induce a shift in the structural equilibrium from helical toward β -strand conformations. The helix $\rightarrow$ strand conversion is moderate in A β dimers, but becomes very pronounced in the edge A β peptides. We also identify the regions in A β sequence, which exhibit the helix $\rightarrow$ strand conversion and are engaged in interpeptide interactions. To test our REMD simulations and support the choice of N-terminal truncated

peptide A β _{10–40} as a model for A β _{1–40} we computed the *in silico* chemical shifts in A β monomers and compared them with the experimental values¹⁸.

Materials and Methods

Molecular dynamics simulations

To perform simulations of A β peptides we used CHARMM MD program³⁵ and all-atom force field CHARMM19 coupled with the SASA implicit solvent model³⁶. Because we investigate the changes in A β secondary structure, it is important to choose the force field not known for structural bias. Previous simulations have indicated that CHARMM19+SASA force field does not favor particular protein secondary structure. It has been used to fold polypeptides, which contained α -helices or β -sheets^{37,38}. CHARMM19+SASA simulations were also employed for studying oligomerization of amyloidogenic peptides^{39,40}.

In this work, we use the peptide A β _{10–40}, which is the N-terminal truncated fragment of A β _{1–40}. Several reasons dictated our selection of A β _{10–40}. First, solid-state NMR resolved the fibril structure of A β _{1–40} excluding the first nine N-terminal residues²¹. Subsequent studies have shown that the fibril structures of A β _{1–40} and A β _{10–40} are almost identical⁴¹. Second, it is known that the size distributions for A β _{10–40} and A β _{1–40} oligomers are similar⁴². These findings suggest that the N-terminal play a minor role A β aggregation.

Three A β _{10–40} systems were considered: monomer, dimer, and the peptides interacting with A β fibril (Fig. 1). Each of these systems was subject to spherical boundary condition with the radius $R_s = 90\text{\AA}$ and the force constant $k_s = 10\text{kcal}/(\text{mol}\text{\AA}^2)$. The concentration of A β peptides is therefore of an order of *mM*. The system of A β peptides interacting with amyloid fibril was introduced in our previous studies^{34,40} (Fig. 1c). Briefly, the system consists of four A β peptides forming a fibril fragment and two incoming (edge) peptides. The fibril structure is modeled using the coordinates of backbone atoms determined from the solid-state NMR measurements²¹. To emulate the stability of large fibril sample, the backbones of fibril peptides (Fig. 1c) were constrained to their experimental positions using soft harmonic potentials with the constant $k_c = 0.6\text{kcal}/(\text{mol}\text{\AA}^2)$ ⁴⁰. The harmonic constraints permit backbone fluctuations with the amplitude of about $0.6\text{\AA}$ at 350K, which are comparable with the fluctuations of atoms on the surface of folded proteins⁴³. Constraints were not applied to the side chains of fibril peptides nor to the edge peptides. The latter were free to dissociate and reassociate with the fibril. The constraints capture the rigidity of amyloid fibril and eliminate the necessity to simulate large fibril systems to achieve their stability. More generally, three A β systems allowed us to consider structural changes in A β peptides upon aggregation.

Replica exchange simulations

To achieve exhaustive conformational sampling we used replica exchange molecular dynamics (REMD)⁴⁴. This method has shown its efficiency in sampling rugged free energy landscapes and has been applied to study protein folding and aggregation^{39,40,45–50}. To simulate A β monomers and dimers we used 24 replicas distributed linearly with the increment of 10K in the temperature range from 300 to 530K. The exchanges were

attempted every 40 ps in all neighboring replica pairs. The average acceptance rates were 67% (monomer) and 54% (dimer). In all, we produced four (monomer) and seven (dimer) REMD trajectories of the length 0.4 μ s each (per replica). Therefore, the cumulative simulation time for all replicas was 76 μ s (monomer) and 134 μ s (dimer). The structures were saved every 40 ps. Between replica exchanges the system was evolved using NVT underdamped Langevin dynamics with the damping coefficient $\gamma = 0.15\text{ps}^{-1}$ and the integration step of 2fs. The REMD simulations of A β peptides interacting with the fibril were described previously⁴⁰.

In each REMD trajectory we determined the initial equilibration interval τ_{eq} by monitoring the onset of equilibrium regime in the total effective energies E_{eff} (the sum of potential and solvation energies). As a result, the initial parts of REMD trajectories of the lengths up to 80 ns were excluded. Consequently, the cumulative equilibrium simulation times were $\tau_{sim} = 72\mu\text{s}$ (monomer) and 113 μs (dimer).

Computation of structural probes

To characterize intra- and interpeptide interactions we computed the number of side chain contacts as described in our previous studies⁵¹. Backbone hydrogen bonds between NH and CO groups were assigned according to Kabsch and Sander⁵². Secondary structure in A β peptides was assigned by evaluating their dihedral angles (ϕ , ψ)^{53,54}. To this end, the grid with the spacing of 18° was superimposed on the Ramachandran plot. The β -strand conformations are enclosed by the vertices of the polygon (−180°,180°), (−180°,126°), (−162°,126°), (−162°,108°), (−144°,108°), (−144°,90°), (−50°,90°), (−50°,180°); helix structure is confined to the polygon (−90°,0°), (−90°,−54°), (−72°,−54°), (−72°,−72°), (−36°,−72°), (−36°,−18°), (−54°,−18°), (−54°,0°). (Note that these definitions do not distinguish α -helix, 3_{10} -helix, or π -helix.) Using these definitions the fractions of residues in helix and β -strand conformations, H and S , can be computed in any structure.

We have also used the program STRIDE for secondary structure assignment⁵⁵. Although the assignments of helices based on (ϕ , ψ) angles and STRIDE are very similar, the correspondence between β -structure is not straightforward. Because STRIDE defines β -sheet structure using HBs, its fraction is low in disordered systems such as A β dimers and monomers. As a result STRIDE underestimates extended β -strand-like structures by assigning them to turns and coils. Consequently, in STRIDE about one-third of turn structure and half of coil structure correspond to β -strand conformations defined on the basis of (ϕ , ψ) angles. Because our aim is to monitor structural changes in A β peptides upon aggregation, we chose to assign secondary structure using (ϕ , ψ) angles. However, for completeness we list respectively the values of extended (β -sheet), α -helical, and β -turn structure contents obtained from STRIDE: monomer – 0.01, 0.38, 0.41; dimer – 0.06, 0.19, 0.49; edge peptides – 0.30, 0.06, 0.35. Because the amount of bridge structure is negligible, the remaining fractions correspond to coil.

Throughout the paper angular brackets $\langle \dots \rangle$ indicate thermodynamic averages. Because dimer and fibril systems include two indistinguishable peptides, we report averages over two peptides. The distributions of states produced by REMD were analyzed using multiple histogram method⁵⁶.

Convergence of REMD simulations

To evaluate the quality of REMD sampling we consider the number N_s of unique states (E_{eff} , N_{mhb}), which were sampled in the course of simulations at least once. Each state (E_{eff} , N_{mhb}) is defined by the effective energy of the A β system E_{eff} and the number of intrapeptide HBs, N_{mhb} . Fig. 2 shows N_s for A β dimer as a function of the cumulative equilibrium simulation time τ_{sim} . At $\tau_{sim} \gtrsim 10\mu s$ N_s approximately levels off suggesting approximate convergence of REMD. To further test the reliability of REMD we divided the dimer simulations into two equal subsets and analyzed them independently. The resulting errors in the intrapeptide and interpeptide HBs, $\langle N_{mhb} \rangle$ and $\langle N_{dhh} \rangle$, were 2% and 4%, respectively. In Fig. 2 (inset) we show the REMD error analysis for the secondary structure content. The average errors for the helix and strand fractions at individual sequence positions were 8% and 7%. The errors in monomer system are smaller than those in A β dimer. The error analysis for the fibril system was reported earlier⁴⁰.

Results

Using REMD we investigated the distribution of structures sampled by A β_{10-40} monomers, dimers, and the A β_{10-40} peptides bound to amyloid fibrils (Fig. 1). We have previously showed that A β peptides docked to amyloid fibrils assume ordered β -sheet conformations at the locking temperature $T_l \approx 360K$ ⁴⁰. Consequently, to compare different A β species on equal basis, we report their structural properties at the temperature $T_s = 350K < T_l$. Following the allocation of β -structure in A β_{1-40} amyloid fibril²¹, we distinguish three sequence regions in A β_{10-40} peptide - N-terminal (residues 10 to 23), which corresponds to the first fibril β -strand, C-terminal (residues 29 to 39), which corresponds to the second fibril β -strand, and the turn region (residues 24 to 28) (Fig. 1d).

Conformational propensities of A β monomers

With the decrease in temperature A β_{10-40} monomer collapses to the structures with the average radius of gyration $\langle R_g \rangle \approx 14.0\text{\AA}$. The collapse of A β_{10-40} is very gradual and spans the entire REMD temperature range. This observation is likely to be the consequence of the lack of A β stable tertiary fold. However, if the monomer melting temperature is attributed to the maximum in $d\langle R_g(T) \rangle / dT$, then it is about 470 K.

To probe the interactions in A β monomers we computed the number of side chain hydrophobic contacts $\langle C_{mHH} \rangle$ and the number of backbone hydrogen bonds (HB) $\langle N_{mhb} \rangle$. The collapsed conformations are stabilized by hydrophobic interactions ($\langle C_{mHH} \rangle \approx 7.6$) and intrachain HBs ($\langle N_{mhb} \rangle \approx 15.2$). Fig. 3a shows the thermal averages of the number of HBs $\langle N_{mhb}(i) \rangle$ formed by NH and CO groups of the residues i in A β monomer. Visual inspection of Fig. 3a suggests that N-terminal should be more structured than the C-terminal. Indeed, the average numbers of HBs per residue in the N- and C-terminals are 1.2 and 0.9, respectively.

To further investigate structural propensities we computed the distribution of secondary structure. The average fractions of residues in β -strand and helix conformations are $\langle S \rangle \approx 0.22$ and $\langle H \rangle \approx 0.34$, respectively. The random coil fraction is about 0.44. Fig. 4a displays

the distributions $\langle S(i) \rangle$ and $\langle H(i) \rangle$, which report the fractions of β -strand and helix structure formed by individual residues i . The average fractions of residues in β -strand and helix states in the N-terminal are $\langle S_N \rangle \approx 0.15$ and $\langle H_N \rangle \approx 0.53$, respectively. Within the C-terminal a weak preference for β -structure is observed ($\langle S_C \rangle \approx 0.27$ for β -strand and $\langle H_C \rangle \approx 0.16$ for helix). Thus, helix structure in A β monomers is observed more frequently than β -strand states.

Within the N-terminal the distributions of $\langle H(i) \rangle$ and $\langle S(i) \rangle$ show gradual variations (Fig. 4a). In contrast, due to structural flexibility of four Gly residues (Gly29, Gly33, Gly37, Gly38) pronounced variations in β -strand and helix contents are observed in the C-terminal. At these four Gly positions the fraction of random coil structure reaches 0.9. In contrast, the N-terminal (Fig. 1d) lacks Gly residues. It is also worth noting that the concentration of Gly residues in the C-terminal is consistent with the dominance of β -turns in this region. According to STRIDE⁵⁵ all residues within the C-terminal have the probabilities to form β -turn $\langle T(i) \rangle$ in excess of 0.5. For comparison, the average probability to form β -turns in the N-terminal is only 0.29.

Conformational propensities of A β dimers

What is the impact of interpeptide interactions on the structural propensities of A β peptides? To answer this question we first consider the distribution of intrapeptide HBs $\langle N_{mhb}(i) \rangle$ in A β dimers (Fig. 3b). Similar to the distribution computed for A β monomer in Fig. 3a, N-terminal appears to have elevated number of HBs. The average numbers of HBs per residue in the N- and C-terminals are 0.8 and 0.6, which are lower than in A β monomer. The thermal average of the total number of intrapeptide HBs $\langle N_{mhb} \rangle$ is ≈ 10.2 , whereas the number of intrapeptide hydrophobic contacts is $\langle C_{mHH} \rangle \approx 6.3$. Thus, compared to A β monomers the number of intrapeptide HBs in dimers is decreased by one-third. Despite the decrease in intrapeptide interactions, A β peptides in dimers have approximately the same dimensions ($\langle R_g \rangle \approx 14.2\text{\AA}$) as monomeric species.

Similar to the analysis of A β monomers our next step is to probe the distribution of secondary structure in A β dimers. From REMD data we obtained the average β -strand and helix contents in A β peptides to be $\langle S \rangle \approx 0.35$ and $\langle H \rangle \approx 0.23$, respectively. The random coil fraction is about 0.42. To get better insight in the secondary structure propensities we plot in Fig. 4b the fractions of helix $\langle H(i) \rangle$ and β -strand $\langle S(i) \rangle$ structure formed by individual residues i . Although the profiles of $\langle H(i) \rangle$ and $\langle S(i) \rangle$ remain qualitatively similar to those computed for A β monomers (Fig. 4a), a considerable redistribution of secondary structure is evident. For example, the average fractions of residues in helix and β -strand states in the N-terminal become equal in dimers ($\langle H_N \rangle \approx \langle S_N \rangle \approx 0.34$), whereas a strong preference for β -conformations is observed in the C-terminal ($\langle S_C \rangle \approx 0.35$ versus $\langle H_C \rangle \approx 0.12$). As in A β monomer a considerable drop in β -strand content is observed at four Gly residues (Gly29, Gly33, Gly37, Gly38) in the C-terminal. Hence, in contrast to A β monomers β -structure in dimers is observed more frequently than helical states.

Interpeptide interactions are quantified by the numbers of interpeptide HBs $\langle N_{dhh} \rangle$ and hydrophobic side chain contacts $\langle C_{dHH} \rangle$. Using REMD we found that $\langle N_{dhh} \rangle \approx 3.8$ and $\langle C_{dHH} \rangle \approx 5.5$. The probability of associated state, i.e., the probability of forming any

interpeptide HB, is 0.86. To obtain residue-specific information about interpeptide interactions we computed the thermal contact map $\langle C(i, j) \rangle$, which displays the probabilities of forming interpeptide contacts between amino acids i and j (Fig. 5a). Two observations follow from this plot. First, N-terminal of A β tends to form more interpeptide interactions than the C-terminal. From the contact map $\langle C(i, j) \rangle$ we calculated that the numbers of interpeptide side chain contacts formed by the N- and C-terminals are 16.6 and 8.8, respectively. Therefore, in A β dimers the interpeptide interface primarily involves the N-terminal.

The orientation of A β peptides in the dimer was probed using the vectors $\vec{R}(k) = \vec{r}_{C_\alpha}(23, k) - \vec{r}_{C_\alpha}(10, k)$, where $\vec{r}_{C_\alpha}(23, k)$ and $\vec{r}_{C_\alpha}(10, k)$ are the radius-vectors of C_α atoms of the residues Asp23 and Tyr10 in the peptide k . Because the fraction of ordered structure (helix or strand) in the N-terminal is high (~ 0.7) and, consequently, this region is relatively rigid, $\vec{R}(k)$ approximately describes the orientation of the N-terminal. Fig. 5b presents the distribution $P(\cos\phi)$, where ϕ is an angle between $\vec{R}(1)$ and $\vec{R}(2)$. The distribution $P(\cos\phi)$ is weakly bimodal, reflecting a tendency of A β peptides to form antiparallel docking interface as shown in Fig. 1b. Consequently, the probability of antiparallel orientation ($\cos\phi < 0$) is about 0.6.

Second, although none of the probabilities $\langle C(i, j) \rangle$ are larger than 0.1, there are several side chain contacts, which occur with elevated probabilities, namely Tyr10-Phe20, Tyr10-Asp23, Glu11-Glu11, Glu11-Asp23, His13-Phe20, His13-Asp23. Most of these contacts are involved in the formation of antiparallel dimer interface in the N-terminal. The probability of forming intermolecular salt bridge Asp23-Lys28, which is important for the stability of A β fibrils²¹, is low (0.05).

Finally, we evaluate the change in A β peptide structure by computing the difference in intrapeptide side chain contact probabilities in A β dimers relative to monomers. The difference contact map $\langle C(i, j) \rangle$ in Fig. 5a shows that the most notable change is related to the destabilization of the helix-like contacts $(i, i + 4)$, where $i = 15, \dots, 21$ and $i = 27, \dots, 33$. With the exception of very few side chain pairs the intrapeptide contacts tend to become less stable in A β dimer. Indeed, the average value of $\langle C(i, j) \rangle$ is about -0.1 . This observation is consistent with partial unraveling of helix structure in A β dimers. The probability of intrapeptide salt bridge Asp23-Lys28 is reduced from 0.31 (monomer) to 0.22 (dimer).

Conformational propensities of the A β peptides interacting with amyloid fibril

We now analyze the secondary structure and interactions in the A β peptides bound to the edge of amyloid fibril⁴⁰. The numbers of intrapeptide HBs $\langle N_{mhb} \rangle$ and hydrophobic side chain contacts $\langle C_{mHH} \rangle$ are reduced to 4.5 and 5.5, respectively. Therefore, compared to A β monomer there is a three-fold decrease in $\langle N_{mhb} \rangle$. There is also a significant extension of the edge A β peptides as their radius of gyration $\langle R_g \rangle$ increases from 14.0 (A β monomers) to 18.7Å. The loss in intrapeptide interactions is compensated by the dramatic rise in peptide-fibril interactions. The numbers of HBs $\langle N_{ehb} \rangle$ and hydrophobic side chain contacts $\langle C_{eHH} \rangle$ linking edge A β peptide with the fibril are increased to 10.8 and 10.0. Similar to A β dimers most of such interpeptide side chain contacts are formed by the N-

terminal. From the thermal contact map $\langle C(i, j) \rangle$ (data not shown) we obtained that the numbers of peptide-fibril side chain contacts formed by the N- and C-terminals are 21.8 and 12.0, respectively.

In the edge A β peptides the average β -strand $\langle S \rangle$ and helix $\langle H \rangle$ contents are 0.52 and 0.11, whereas the random coil fraction is 0.37⁴⁰. Fig. 4c shows the distributions of helix $\langle H(i) \rangle$ and β -strand $\langle S(i) \rangle$ structure formed by individual residues i in A β peptides bound to the fibril. This plot demonstrates that the probability of β -strand states exceeds that of helix for all residues. The average fractions $\langle S \rangle$ in the N- and C-terminals are $\langle S_N \rangle \approx 0.55$ and $\langle S_C \rangle \approx 0.50$, respectively. For comparison, the helical fractions in these regions are 0.16 and 0.05. Fig. 4c also suggests that as in A β monomers and dimers four Gly residues (Gly29, Gly33, Gly37, Gly38) may impart considerable flexibility to the C-terminal. Thus, in the edge A β peptides there is a strong dominance of β -strand structure and most of intrapeptide interactions are replaced with peptide-fibril HBs and side chain contacts.

Discussion

Interpeptide interactions drive a shift in secondary structure

We performed REMD simulations to sample conformational states of A β monomers, dimers, and the edge peptides bound to amyloid fibril. The main result of this investigation is that a significant shift in secondary structure distribution is observed in dimeric and edge A β species compared to A β monomers. Fig. 6a summarizes the changes in helix and β -strand populations at different stages of A β aggregation. A β monomer tends to adopt generally random coil structure augmented by significant helix ($\langle H \rangle = 0.34$) and, to a lesser extent, β -strand ($\langle S \rangle = 0.22$) populations. Large helix fraction is primarily due to a strong propensity to form this structure in the N-terminal (Fig. 3a). Formation of dimeric structures triggers partial conversion of helix into β -strand structure as the β -strand content $\langle S \rangle$ increases by $\approx 50\%$ from 0.22 to 0.35. The decrease in helix population from 0.34 to 0.23 matches in reverse manner the increase in $\langle S \rangle$. The association of A β peptides with large aggregates (amyloid fibrils) causes further helix $\rightarrow$ strand conversion. Compared to A β dimers β strand content increases by a factor 1.5 from 0.35 to 0.52, while the helix population decreases in half (from 0.23 to 0.11). The difference contact map in Fig. 5a is consistent with the destabilization of helical structure in A β dimers. Note also that the helix $\rightarrow$ strand conversion is in line with a dramatic extension of edge peptides compared to monomers or dimers (from $\langle R_g \rangle \approx 14.0$ to 18.7 Å).

It is natural to suggest that the helix $\rightarrow$ strand conversion is driven by interpeptide interactions. With respect to A β monomer the number of intrapeptide HBs $\langle N_{mhb} \rangle$ is reduced by 30% in dimers and three-fold in edge peptides (from 15.2 to 10.2 and 4.5, respectively). Simultaneously, in edge peptides compared to dimers the number of interpeptide HBs is increased almost by a factor of three (from 3.8 to 10.8). Thermal contact maps $\langle C(i, j) \rangle$ for dimers (Fig. 5a) and edge peptides suggest that about two-third of interpeptide interactions are formed by A β N-terminals. It is therefore indicative that the most significant change in secondary structure occurs within the N-terminal, in which the

fraction of β -strand $\langle S \rangle$ almost quadruples (from 0.15 to 0.55). For comparison, the change in $\langle S \rangle$ in the C-terminal is less than two-fold.

The helix $\rightarrow$ strand conversion occurring upon A β aggregation can be further illustrated by free energy computations. Fig. 6b shows free energies $F(H, S)$ as a function of the helix H and β -strand S fractions. In monomers, a compact free energy minimum is located at $H \sim 0.35$ and $S \sim 0.2$. In dimers, the minimum becomes stretched to include a broad range of H (0.05 to 0.4) and S (0.1 to 0.6) states. For the edge peptides, $F(H, S)$ reveals a well-defined minimum centered at $H \lesssim 0.1$ and $S \sim 0.6$. It is worth noting that the plot of $F(H, S)$ for dimers suggests that large (compared to other A β species) fluctuations in secondary structure should be observed. It follows from one-dimensional free energy profiles $F(H)$ shown in Fig. 6c that with respect to monomers the helix state is destabilized in dimers by $2.8RT$ and in edge peptides by $F_H = 4.0RT$. In contrast, compared to monomers the free energy of β -strand structure decreases by $-1.5RT$ in dimers and by $F_S = -5.3RT$ in edge peptides. Taken together, our data indicate that upon aggregation a dramatic helix $\rightarrow$ strand conversion occurs in A β peptides.

Comparison of experiments and simulations: A β monomers

To test our simulations of A β monomers we computed the chemical shifts $\delta_{sim}(i)$ for C_α and C_β carbons in residues i using the program SHIFTS⁵⁷. To compute $\delta_{sim}(i)$ we used the lowest REMD temperature ($T = 300K$). We choose to analyze C_α and C_β chemical shifts because of their sensitivity to α -helix and β -strand structures¹⁸. In Fig. 7 the distributions of $\delta_{sim}(i)$ are compared with the corresponding experimental chemical shifts $\delta_{exp}(i)$ measured by Zagorski and coworkers¹⁸. The experimental chemical shifts were obtained for A β_{1-40} -Met^{red} monomers at 278K. Several important observations follow from Fig. 7. First, despite a 20K difference in experimental and simulation temperatures, an excellent agreement between C_β $\delta_{exp}(i)$ and $\delta_{sim}(i)$ is observed (Fig. 7a). The corresponding correlation factor is $r = 0.9995$. The agreement between C_α $\delta_{exp}(i)$ and $\delta_{sim}(i)$ is also reasonably good, although some discrepancy can be seen in the N-terminal (Fig. 7b). However, the overall correlation between C_α chemical shifts is still very strong ($r = 0.984$, inset to Fig. 7b). Second, the experiments studied the full-length A β_{1-40} and in our simulations we used the truncated peptide A β_{10-40} . A good agreement between the distributions of $\delta_{exp}(i)$ and $\delta_{sim}(i)$ suggests that the truncation of the residues 1–9 does not lead to qualitative changes in the structural properties of A β monomers. This conclusion is in line with the arguments for selecting A β_{10-40} presented in Methods^{21,41,42}. It is also interesting to note that the recent study has also demonstrated that C_β chemical shifts are in better agreement with simulation data than those obtained for C_α ⁵⁸.

Because our simulations suggest the formation of the helix in the N-terminal of A β monomers (Fig. 4a), it is important to evaluate the reliability of this prediction. Three lines of evidence support helix structure. First, it is instructive to compare C_α $\delta_{exp}(i)$ in the N-terminal with the reference values of chemical shifts for α -helix δ_α , β -strand δ_β , and random coil structures δ_{RC} reported in the literature⁵⁹. For all residues in the N-terminal the reference chemical shifts are ordered as $\delta_\beta < \delta_{RC} < \delta_\alpha$. For the positions Val12, His14, Gln15, Leu17, Val18, Phe19, and Phe20 experimental δ_{exp} fall between random coil and α -

helix values, i.e., $\delta_{RC} < \delta_{exp} < \delta_{\alpha}$ (Table 1). At other positions, $\delta_{exp} \lesssim \delta_{RC}$ and the presence of helix is less likely. Therefore, the data in Table 1 are consistent with the formation of helix at seven out of 13 positions in the N-terminal. Recent NMR study of A β_{1-40} monomer determined eight J-coupling constants in the sequence region 10 to 23²⁸. Because the coverage of measurements is not continuous, it is difficult to make reliable identification of helical structure, although three constants are within the helical range ($^3J_{HNH\alpha} < 6\text{Hz}$)⁶⁰. Furthermore, out of eight measured J-coupling constants only two exceeded the value of $^3J_{HNH\alpha} = 7\text{Hz}$ normally attributed to β -structure (with the same accuracy as for single helical J-couplings)⁶⁰.

Second, the support for helix structure comes from simulations. Recent MD study, which used different force field and implicit solvent model (AMBER and Generalized Born model), demonstrated that the propensity to form α -helix at the positions 14 to 22 is larger than that for β -strand³⁰. For the residues Leu17, Val18, and Phe20 the combined probability of all types of helices reached about 0.3. The simulations have also shown that the free energy of α -helix conformers is lower than of any other conformational state. In another study, which employed explicit solvent and GROMACS force field, the probability of helix conformers in A β_{1-40} monomer exceeded that of β -structure about three-fold²⁷. Interestingly, helical conformations persisted within the sequence positions 14 to 21 after being melted in other sequence regions. (It is important to note that the memory of initial helix-favoring conditions taken from micellular environment could have biased the sampling, which was unlikely to reach equilibrium on ~ 100 ns time scale.)

Third, secondary structure analysis programs do not give consensus prediction concerning the N-terminal of A β_{10-40} . Predator⁶¹ predicts α -helix conformations at the A β_{10-40} positions 13 to 17 (Fig. 1d), whereas PHD⁶² assigns extended structure in that region. It is worth noting that the truncation of A β residues 1 to 9 could elevate the helical content in the N-terminal of A β^{10-40} . Our preliminary simulations of A β_{1-40} monomer⁶³ indicated that the fraction of helix structure $\langle H \rangle$ is reduced from 0.34 (A β_{10-40}) to 0.29 (A β_{1-40}), whereas the strand content rises from 0.22 to 0.28, respectively. Therefore, the dominant structure in A β_{1-40} is random coil (0.43), whereas the rest is about equally divided between helix and beta conformers. Consequently, the amount of helix in A β_{1-40} may be somewhat smaller than reported in our study for A β_{10-40} .

Taken together, the arguments cited above and overall good agreement between simulation and experimental chemical shifts support the formation of helical conformations in A β monomers.

Solution NMR studies are in general agreement that A β monomers in aqueous solutions lack stable tertiary structure¹⁶⁻¹⁸. In particular, NMR data do not reveal the presence of stable long-range interactions in A β monomer. This observation is consistent with the low probability of long-range side chain contacts in A β monomers in Fig. 5a. Experimental studies have also demonstrated that several regions in A β monomer are relatively structured. For example, NMR dynamics in A β_{1-40} monomer revealed higher mobility of the C-terminal compared to the N-terminal⁶⁴. Similar conclusion has been reached in all-atom explicit water simulations²⁸. Garcia and coworkers established that the N-terminal (residues

1 to 18) samples restricted conformational space compared to the C-terminal (25 to 40). These findings are consistent with the formation of helix structure in the N-terminal in our simulations.

To provide direct evaluation of A β monomer rigidity, we computed using REMD the standard deviations $\delta\phi(i)$ and $\delta\psi(i)$ of the backbone angles ϕ and ψ for the residues i . Fig. 8 shows that the amplitude of fluctuations in the N-terminal is generally lower than in the C-terminal. Indeed, the average of the combined set of $\delta\phi(i)$ and $\delta\psi(i)$ values in the N-terminal is 42, but it grows to 67 in the C-terminal. The increase in backbone flexibility is mainly caused by four Gly residues, which show dramatically higher values of $\delta\phi(i)$ than other residues. It is conceivable that large structural fluctuations caused by Gly residues destabilize interpeptide interactions originating in the A β C-terminal. Consequently, as shown in our previous studies^{34,40} and in this work A β peptides tend to aggregate through their N-terminals. A similar suggestion that A β C-terminal may not represent the primary aggregation interface was put forward by Hou et al.¹⁸ and by Melquiond et al.⁶⁵.

Comparison of experiments and simulations: A β oligomers

Experimental studies of A β _{1–40} oligomers have revealed significant population of α -helix states^{13,25}. α -Helix states may represent kinetic intermediates in amyloid assembly or these states may occur at equilibrium with other conformations¹³. These results appear to support our observation of significant helix content in A β dimers. Furthermore, solution-state NMR has been used to identify amino acids involved in interpeptide interactions in A β _{1–40} dimers¹³. Although unambiguous assignment of interpeptide interactions to specific amino acids is difficult, it has been concluded that the sequence positions 15 to 24 contribute most to dimer stability. This region of A β chain approximately coincides with the N-terminal of A β _{10–40}, which form most of interpeptide interactions. Interestingly, our simulations suggest that the largest number of interpeptide contacts is formed by Tyr10 (1.7 contacts, on an average). In solution-state NMR this residue also provides large contribution to interpeptide interactions¹³.

In summary, by taking into account the *in silico* distributions of chemical shifts and interpeptide interactions, overall dominance of random coil conformations, and flexibility of the C-terminal it appears that our simulations are in reasonable agreement with the experiments and previous computational studies.

Conclusions

In this paper, we used REMD simulations to compute the structural propensities of A β monomers, dimers, and A β peptides bound to the amyloid fibril edge. In a crude way, these three systems represent initial, intermediate, and final stages in A β aggregation. We showed that A β _{10–40} monomers form helical structure in the N-terminal. Upon aggregation interpeptide interactions induce a dramatic shift in the structural equilibrium, away from helical states toward β -strand conformations. This helix $\rightarrow$ strand conversion is relatively moderate in A β dimers, but is very pronounced in the A β peptides interacting with amyloid fibrils. We also determined that the N-terminal of A β _{10–40} peptide forms most of interpeptide interactions. To test our REMD simulations we computed *in silico* chemical

shifts for A β monomers and compared them with the experimental data¹⁸. Their good agreement suggest that our study fairly well represents the distribution of states sampled by A β peptides.

Our result that the N-terminal of A β peptides forms helical structures and is predominantly involved in interpeptide interactions may have implications for controlling A β amyloidogenesis. By site-directed mutations one may enhance α -helical propensity in A β peptides and therefore block helix $\rightarrow$ strand conversion occurring upon aggregation. Hence, it is of interest that the consensus secondary structure prediction⁶⁶ demonstrates that substituting Val18 and Phe19 with Glu triggers uniform assignment of α -helical structure in the A β _{10–40} N-terminal (positions 14 to 23) by all prediction algorithms. This mutation may be considered in the design of A β variants resistant to aggregation.

Acknowledgments

This work was supported by the grant R01 AG028191 from the National Institute on Aging (NIH). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute on Aging or NIH. We thank Prof. Michael Zagorski for sharing with us their chemical shift data.

References

1. Selkoe DJ. Folding proteins in fatal ways. *Nature*. 2003; 426:900–904. [PubMed: 14685251]
2. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science*. 2002; 297:353–356. [PubMed: 12130773]
3. Dobson CM. Protein folding and misfolding. *Nature*. 2003; 426:884–890. [PubMed: 14685248]
4. Yoshiike Y, Akagi T, Takashima A. Surface structure of amyloid- β fibrils contributes to cytotoxicity. *Biochemistry*. 2007; 46:9805–9812. [PubMed: 17676931]
5. Kirkitadze MD, Bitan G, Teplow DB. Paradigm shifts in Alzheimer's disease and other neurodegenerative disorders: The emerging role of oligomeric assemblies. *J. Neurosci. Res.* 2002; 69:567–577. [PubMed: 12210822]
6. Kaye R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, Glabe CG. Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science*. 2003; 300:486–489. [PubMed: 12702875]
7. Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: Lessons from the Alzheimer's amyloid β -peptide. *Nature Rev. Mol. Cell. Biol.* 2007; 8:101–112. [PubMed: 17245412]
8. Pastor MT, Kummer N, Schubert V, Esteras-Chopo A, Dotti CG, de la Paz ML, Serrano L. Amyloid toxicity is independent of polypeptide sequence, length and chirality. *J. Mol. Biol.* 2008; 375:695–707. [PubMed: 18036611]
9. Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, Brett FM, Farrell MA, Rowan MJ, Lemere CA, Regan CM, Walsh DM, Sabatini BL, Selkoe DJ. Amyloid- β protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nature Medicine*. 2008; 14:837–842.
10. Cheng IH, Scarce-Levie K, Legleiter J, Palop JJ, Gerstein H, Bien-Ly N, Puolivali J, Lesne S, Ashe KH, Muchowski PJ, Mucke L. Accelerating amyloid- β fibrillization reduces oligomer levels and functional deficits in Alzheimer disease mouse models. *J. Biol. Chem.* 2007; 282:23818–23828. [PubMed: 17548355]
11. Murphy RM, Pallitto MM. Probing the kinetics of β -amyloid self-association. *J. Struct. Biol.* 2000; 130:109–122. [PubMed: 10940219]
12. Carulla N, Caddy GL, Hall DR, Zurdo J, Gair M, Feliz M, Giralt E, Robinson CV, Dobson CM. Molecular recycling within amyloid fibrils. *Nature*. 2005; 436:554–558. [PubMed: 16049488]

13. Narayanan S, Reif B. Characterization of chemical exchange between soluble and aggregated states of β -amyloid by solution-state NMR upon variation of salt conditions. *Biochemistry*. 2005; 44:1444–1452. [PubMed: 15683229]
14. Barrow CJ, Zagorski MG. Solution structure of the β -peptide and its constituent fragments: Relation to amyloid deposition. *Science*. 1991; 253:179–182. [PubMed: 1853202]
15. Shao H, Jao S, Ma K, Zagorski MG. Solution structures of micelle-bound amyloid β -(1–40) and β -(1–42) peptides of Alzheimer's disease. *J. Mol. Biol.* 1999; 285:755–773. [PubMed: 9878442]
16. Esler WP, Stimson ER, Lachenmann MJ, Ghilardi JR, Lu Y, Vinters HV, Mantyh PW, Lee JP, Maggio JE. Activation barriers to structural transition determine deposition rates of Alzheimer's disease. *J. Struct. Biol.* 2000; 130:174–183. [PubMed: 10940224]
17. Riek R, Guntert P, Dobeli H, Wipf B, Wuthrich K. NMR studies in aqueous solution fail to identify significant conformational differences between the monomeric forms of two Alzheimer peptides with widely different plaque-competence, A β 1–40ox and A β 1–42ox. *Eur. J. Biochem.* 2001; 268:5930–5936. [PubMed: 11722581]
18. Hou L, Shao H, Zhang Y, Li H, Menon NK, Neuhaus EB, Brewer JM, Byeon I-JL, Ray DG, Vitek MP, Iwashita T, Makula RA, Przybyla AB, Zagorski MG. Solution NMR studies of the A β (1–40) and A β (1–42) peptides establish that the Met35 oxidation state affects the mechanism of amyloid formation. *J. Amer. Chem. Soc.* 2004; 126:1992–2005. [PubMed: 14971932]
19. Serpell LC. Alzheimer's amyloid fibrils: Structure and assembly. *Biochim Biophys. Acta.* 2000; 1502:16–30. [PubMed: 10899428]
20. Burkoth TS, Benzinger T, Urban V, Morgan DM, Gregory DM, Thiagarajan P, Botto RE, Meredith SC, Lynn DG. Structure of the β -amyloid(10–35) fibril. *J. Am. Chem. Soc.* 2000; 122:7883–7889.
21. Petkova AT, Yau W-M, Tycko R. Experimental constraints on quaternary structure in Alzheimer's β -amyloid fibrils. *Biochemistry*. 2006; 45:498–512. [PubMed: 16401079]
22. Luhrs T, Ritter C, Adrian M, Loher B, Bohrmann DR, Dobeli H, Schubert D, Riek R. 3D structure of Alzheimers amyloid- β (1–42) fibrils. *Proc. Natl. Acad. Sci. USA.* 2005; 102:17342–17347. [PubMed: 16293696]
23. Nelson R, Sawaya MR, Balbirnie M, Madsen AO, Riek C, Grothe R, Eisenberg D. Structure of the cross- β spine of amyloid-like fibrils. *Nature*. 2005; 435:773–778. [PubMed: 15944695]
24. Makin OS, Atkins E, Sikorski P, Johansson J, Serpell LC. Molecular basis for amyloid fibril formation and stability. *Proc. Natl. Acad. Sci. USA.* 2005; 102:315–320. [PubMed: 15630094]
25. Kirkitadze MD, Condron MM, Teplow DB. Identification and characterization of key kinetic intermediates in amyloid β -protein fibrillogenesis. *J. Mol. Biol.* 2001; 312:1103–1119. [PubMed: 11580253]
26. Ma B, Nussinov R. Simulations as analytical tools to understand protein aggregation and predict amyloid conformation. *Curr. Opin. Struct. Biol.* 2006; 10:445–452.
27. Xu Y, Shen J, Luo X, Zhu W, Chen K, Ma J, Jiang H. Conformational transition of amyloid β -peptide. *Proc. Natl. Acad. Sci. USA.* 2005; 102:5403–5407. [PubMed: 15800039]
28. Sgourakis NG, Yan Y, McCallum SA, Wang C, Garcia AE. The Alzheimers peptides A β 40 and 42 adopt distinct conformations in water: A combined MD / NMR study. *J. Mol. Biol.* 2007; 368:1448–1457. [PubMed: 17397862]
29. Triguero L, Singh R, Prabhakar R. Comparative molecular dynamics studies of wild-type and oxidized forms of full-length Alzheimer's amyloid β -peptide A β (1–40) and A β (1–42). *J. Phys. Chem. B.* 2008; 112:7123–7131. [PubMed: 18476733]
30. Yang M, Teplow DB. Amyloid β -protein monomer folding: Free energy surfaces reveal alloform specific differences. *J. Mol. Biol.* 2008 xx:xx–xx.
31. Nguyen HD, Hall CK. Molecular dynamics simulations of spontaneous fibril formation by random-coil peptides. *Proc. Natl. Acad. Sci. USA.* 2004; 101:16180–16185. [PubMed: 15534217]
32. Pellarin R, Guarniera E, Caisch A. Pathways and intermediates of amyloid fibril formation. *J. Mol. Biol.* 2007; 374:917–924. [PubMed: 18028943]
33. Chebaro Y, Derreumaux P. Targeting the early steps of A β 16–22 protofibril disassembly by n-methylated inhibitors: A numerical study. *Proteins Struct. Funct. Bioinform.* 2008

34. Takeda T, Klimov DK. Temperature-induced dissociation of A β monomers from amyloid fibril. *Biophys J*. 2008; 95:1758–1772. [PubMed: 18502791]
35. Brooks BR, Bruccoleri RE, Olafson BD, States DJ, Swaminathan S, Karplus M. CHARMM: A program for macromolecular energy, minimization, and dynamics calculations. *J. Comp. Chem*. 1982; 4:187–217.
36. Ferrara P, Apostolakis J, Caisch A. Evaluation of a fast implicit solvent model for molecular dynamics simulations. *Proteins Struct. Funct. Bioinform*. 2002; 46:24–33.
37. Ferrara P, Caisch A. Folding simulations of a three-stranded antiparallel β -sheet peptide. *Proc. Natl. Acad. Sci. USA*. 2000; 97:10780–10785. [PubMed: 10984515]
38. Hiltbold A, Ferrara P, Gsponer J, Caisch A. Free energy surface of the helical peptide Y(MEARA)6. *J. Phys. Chem. B*. 2000; 104:10080–10086.
39. Cecchini M, Rao F, Seeber M, Caisch A. Replica exchange molecular dynamics simulations of amyloid peptide aggregation. *J. Chem. Phys*. 2004; 121:10748–10756. [PubMed: 15549960]
40. Takeda T, Klimov DK. Replica exchange simulations of the thermodynamics of A β fibril growth. *Biophys. J*. 2009
41. Paravastu AK, Petkova AT, Tycko R. Polymorphic fibril formation by residues 10–40 of the Alzheimer's beta-amyloid peptide. *Biophys J*. 2006; 90:4618–4629. [PubMed: 16565054]
42. Bitan G, Vollers SS, Teplow DB. Elucidation of primary structure elements controlling early amyloid β -protein oligomerization. *J. Biol. Chem*. 2003; 278:34882–34889. [PubMed: 12840029]
43. Zhou Y, Vitkup D, Karplus M. Native proteins are surface-molten solids: Application of the Lindemann criterion for the solid versus liquid state. *J. Mol. Biol*. 1999; 285:1371–1375. [PubMed: 9917381]
44. Sugita Y, Okamoto Y. Replica-exchange molecular dynamics method for protein folding. *Chem. Phys. Lett*. 1999; 114:141–151.
45. Garcia AE, Onuchic JN. Folding a protein in a computer: An atomic description of the folding/unfolding of protein A. *Proc. Natl. Acad. Sci. USA*. 2003; 100:13898–13893. [PubMed: 14623983]
46. Tsai H-H, Reches M, Tsai C-J, Gunasekaran K, Gazit E, Nussinov R. Energy landscape of amyloidogenic peptide oligomerization by parallel-tempering molecular dynamics simulation: Significant role of Asn ladder. *Proc. Natl. Acad. Sci. USA*. 2005; 102:8174–8179. [PubMed: 15923262]
47. Baumketner A, Shea J-E. Folding landscapes of the Alzheimer amyloid- β (12–28) peptide. *J. Mol. Biol*. 2006; 362:567–579. [PubMed: 16930617]
48. Baumketner A, Shea J-E. The structure of Alzheimer amyloid beta 10–35 peptide probed through replica exchange molecular dynamics simulations in explicit solvent. *J. Mol. Biol*. 2007; 366:275–285. [PubMed: 17166516]
49. Zheng W, Andrec M, Gallicchio E, Levy RM. Simulating replica exchange simulations of protein folding with a kinetic network model. *Proc. Natl. Acad. Sci. USA*. 2007; 104:15340–15345. [PubMed: 17878309]
50. Jang S, Shin S. Computational study on the structural diversity of amyloid beta peptide (A β 10–35) oligomers. *J. Phys. Chem. B*. 2008; 112:3479–3484. [PubMed: 18303879]
51. Klimov DK, Thirumalai D. Dissecting the assembly of A β _{16–22} amyloid peptides into antiparallel β -sheets. *Structure*. 2003; 11:295–307. [PubMed: 12623017]
52. Kabsch W, Sander C. Dictionary of protein secondary structure: Pattern recognition of hydrogen-bonded and geometrical features. *Biopolymers*. 1983; 22:2577–2637. [PubMed: 6667333]
53. Munoz V, Serrano L. Intrinsic secondary structure propensities of the amino acids, using statistical $\phi - \psi$ matrices: Comparison with experimental scales. *Proteins: Struct. Funct. Genet*. 1994; 20:301–311. [PubMed: 7731949]
54. Street AG, Mayo SL. Intrinsic β -sheet propensities result from van der waals interactions between side chains and the local backbone. *Proc. Natl. Acad. Sci. USA*. 1999; 96:9074–9076. [PubMed: 10430897]
55. Frishman D, Argos P. Knowledge-based protein secondary structure assignment. *Proteins Struct. Funct. Gen*. 1995; 23:566–579.

56. Ferrenberg AM, Swendsen RH. Optimized Monte Carlo data analysis. *Phys. Rev. Lett.* 1989; 63:1195–1198. [PubMed: 10040500]
57. Xu XP, Case DA. Automated prediction of ^{15}N , $^{13}\text{C}_\alpha$, $^{13}\text{C}_\beta$ and $^{13}\text{C}'$ chemical shifts in proteins using a density functional database. *J. Biomol. NMR.* 2001; 21:321–333. [PubMed: 11824752]
58. Fawzi NL, Phillips AH, Ruscio JZ, Doucleff M, Wemmer DE, Head-Gordon T. Structure and dynamics of the A β 21–30 peptide from the interplay of NMR experiments and molecular simulations. *J. Amer. Chem. Soc.* 2008; 130:6145–6158. [PubMed: 18412346]
59. Wang Y, Jardetzky O. Probability-based protein secondary structure identification using combined NMR chemical-shift data. *Prot. Sci.* 2002; 11:852–861.
60. Pardi A, Billeter M, Wuthrich K. Calibration of the angular dependence of the amide protein- C^α proton coupling constants, $^3J_{\text{HN}^\alpha}$, in a globular protein. *J. Mol. Biol.* 1984; 180:741–751. [PubMed: 6084720]
61. Frishman D, Argos P. Incorporation of non-local interactions in protein secondary structure prediction from the amino acid sequence. *Protein Eng.* 1996; 9:133–142. [PubMed: 9005434]
62. Rost B, Sander C. Prediction of protein secondary structure at better than 70% accuracy. *J. Mol. Biol.* 1993; 232:584–599. [PubMed: 8345525]
63. Takeda T, Klimov DK. Role of the N-terminal in A β _{1–40} structural ensemble. 2008 unpublished.
64. Yan Y, Wang C. A β 42 is more rigid than A β 40 at the C terminus: Implications for A β aggregation and toxicity. *J. Mol. Biol.* 2006; 364:853–862. [PubMed: 17046788]
65. Melquiond A, Dong X, Mousseau N, Derreumaux P. Role of the region 23–28 in A β fibril formation: Insights from simulations of the monomers and dimers of Alzheimer's peptides A β 40 and A β 42. *Curr. Alzh. Res.* 2008; 5:244–250.
66. Combet C, Blanchet C, Geourjon C, Deleage G. NPS: Network protein sequence analysis. *Trends in Biol. Sci.* 2000; 25:147–150.

Fig. 1.

Three $\text{A}\beta_{10-40}$ systems studied in our simulations: (a) monomer, (b) dimer, (c) the peptides bound to the edge of $\text{A}\beta$ amyloid fibril. N- and C-terminals are colored in purple and green. The peptides forming amyloid fibril in (c) are in grey. We use $\text{A}\beta$ systems in (a)–(c) to represent, on a basic level, initial, intermediate, and final stages of $\text{A}\beta$ aggregation. $\text{A}\beta$ monomer in (a) and, to a lesser degree, dimer in (b) reveal the formation of helix structure in the N-terminal. Upon aggregation helical structure is replaced with β -strand conformations. This process is clearly manifested in $\text{A}\beta$ peptides bound to the amyloid fibrils in (c), where β

strands are shown in cartoon representation. (d) Sequence of A β_{10-40} peptide. The residues in red are predicted to form helical structure by Predator algorithm⁶¹.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 2.

The number N_s of the new states (E_{eff} , N_{mhb}) in A β dimer not previously sampled in REMD as a function of the cumulative equilibrium simulation time τ_{sim} . Continuous and dotted lines indicate N_s for each of the two peptides in a dimer. The inset shows the fractions of helix $\langle H(i) \rangle$ and β -strand $\langle S(i) \rangle$ structure formed by individual residues i in the dimer. The data in grey refer to the first and second half-sets of REMD trajectories, the data in black are for the full trajectory set. The errors in $\langle H(i) \rangle$ and $\langle S(i) \rangle$ are increased in the N-terminal due to interpeptide interactions.

Fig. 3.

Thermal averages of the number of intrapeptide HBs $\langle N_{mhb}(i) \rangle$ formed by the backbone NH and CO groups of the residues i in A β monomer (a) and dimer (b). The data in grey and black are for NH and CO groups, respectively. The allocation of N- and C-terminals is shown by boxes in this figure and in Figs. 4,5,7,8. Large number of HBs formed in the N-terminal of A β peptide is indicative of helix formation.

Fig. 4.

Thermal distributions of the fractions of helix $\langle H(i) \rangle$ and β -strand $\langle S(i) \rangle$ structure formed by individual residues i in A β monomer (a), dimer (b), and the edge A β peptides bound to amyloid fibril (c). Compared to A β monomers there is a uniform decrease in helix content and simultaneous increase in β -population in dimers, and, especially, in edge A β peptides.

Fig. 5.

(a) The thermal contact map $\langle C(i, j) \rangle$ (lower right corner), which displays the probabilities of forming interpeptide contacts between amino acids i and j in A β dimer. Difference contact map $\Delta \langle C(i, j) \rangle$, which reflects the change in intrapeptide contact probabilities in A β dimers relative to monomers, is displayed in the upper left corner. $\langle C(i, j) \rangle$ and $\Delta \langle C(i, j) \rangle$ are color coded according to the scales shown. Areas in white correspond to the intrapeptide contacts, which occur with the probability < 0.1 in monomers. The contact map $\langle C(i, j) \rangle$ indicates that most interpeptide interactions in A β dimer are formed by the N-

terminals. The difference contact map $\langle C(i, j) \rangle$ shows that the intrapeptide contacts destabilized most in the dimer are those involved in helix formation in monomers. (b) Probability distribution $P(\cos\varphi)$ for the cosine of the angle φ , which describes the orientation of A β N-terminals in dimer. Antiparallel docking of A β peptides in the dimer is weakly preferred.

Fig. 6.

(a) Probabilities of helix, β -strand, and random coil states in A β monomers, dimers, and edge peptides. The plot summarizes the conversion of helix into β -strand structure upon aggregation. (b) Two-dimensional projections of the free energy $F(H, S)$ as a function of the helix and strand contents, H and S . The minima in free energy are set to zero. The values of $F(H, S)$ are color coded according to the scale. (c) One-dimensional profiles of the free energies $F(H)$ and $F(S)$. The data for monomer, dimer, and edge peptides are shown by squares, open and filled circles, respectively. The states with zero helix or strand contents

are set to have zero free energy. The plots in (b) and (c) demonstrate that upon aggregation free energy minimum shifts from helix to β -strand region. F_S and F_H are the total changes in free energy of strand and helix states occurring between A β monomers and the peptides bound to amyloid fibril. The free energies in (b,c) are computed at $T_s = 350K$.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 7. Experimental $\delta_{exp}(i)$ (open circles) and simulation $\delta_{sim}(i)$ (full triangles) chemical shifts in A β monomer as a function of residue number i : (a) C_{β} atoms, (b) C_{α} atoms. The inset to (b) shows $\delta_{sim}(i)$ vs $\delta_{exp}(i)$ for C_{α} atoms. Chemical shifts of Gly C_{β} atoms are set to zero.

Fig. 8.

Standard deviations $\delta\phi(i) = (\langle \phi^2(i) \rangle - \langle \phi(i) \rangle^2)^{1/2}$ and $\delta\psi(i) = (\langle \psi^2(i) \rangle - \langle \psi(i) \rangle^2)^{1/2}$ of the backbone angles $\phi(i)$ and $\psi(i)$ for the residues i in A β monomer. The data for $\delta\phi$ and $\delta\psi$ are shown in grey and black, respectively. Due to backbone steric constraints $\delta\phi(i)$ are smaller than $\delta\psi(i)$ for all residues except Gly. The distributions of $\delta\phi(i)$ and $\delta\psi(i)$ suggest that the C-terminal is more exible than the N-terminal.

Table IExperimental and Reference C_{α} Chemical Shifts for N-Terminal Residues in A β Monomer

Residue	δ_{β}^a (ppm)	δ_{RC}^a (ppm)	δ_{α}^a (ppm)	δ_{exp}^b (ppm)
Glu11	55.55	56.39	59.30	56.38
Val12	60.72	61.80	65.96	62.80
His13	54.80	55.78	59.62	55.79
His14	54.80	55.78	59.62	56.03
Gln15	54.33	55.94	58.61	56.20
Lys16	55.01	56.40	59.11	56.35
Leu17	53.94	54.85	57.54	55.04
Val18	60.72	61.80	65.96	61.87
Phe19	56.33	56.94	60.74	57.31
Phe20	56.33	56.94	60.74	57.23
Ala21	50.86	52.67	54.86	52.51
Glu22	55.55	56.39	59.30	56.29
Asp23	53.41	54.09	57.04	53.97

^aData from Ref.60.^bChemical shifts from Ref.18.