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Side chain interactions can impede amyloid fibril growth: Replica exchange simulations of A β peptide mutant

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Abstract

Using replica exchange molecular dynamics we study the effect of Asp23Tyr mutation on A β _{10–40} fibril growth. The effect of this mutation is revealed through the computation of free energy landscapes, the distributions of peptide-fibril interactions, and by comparison with the wild-type A β _{10–40} peptide. Asp23Tyr mutation has relatively minor influence on the docking of A β peptides to the fibril. However, it has strong impact on the locking stage due to profound stabilization of the parallel in-registry β -sheets formed by the peptides on the fibril edge. The enhanced stability of parallel β -sheets results from the deletion of side chain interactions formed by Asp23, which are incompatible with the fibril-like conformers. Consequently, Asp23Tyr mutation is expected to promote fibril growth. We argue that strong off-registry side chain interactions may slow down fibril assembly as it occurs for the wild-type A β peptide. The analysis of experimental data offers support to our *in silico* conclusions.

Introduction

A propensity to form amyloid supramolecular assemblies appears to be a common, if not a generic, property of polypeptide chains.¹ A large body of experimental evidence indicates that amyloid formation is associated with about 20 various disorders, including Alzheimer's, Parkinson's, type II diabetes, and Creutzfeldt-Jakob disease.² Although amyloid fibrils were traditionally assumed to be the causative agents in these diseases, recent data suggested that oligomeric species, in some cases as small as dimers,³ are responsible for cell toxicity.^{4–6} Irrespective of the precise pathological role of amyloid fibrils, these species are important because they serve as polypeptide “reservoirs” and participate in molecular recycling of monomers through different aggregation states.^{7–9}

Structural studies have uncovered a remarkable homogeneity of amyloid fibril cores formed of extensive β -sheet structure.^{10–14} Backbone hydrogen bonds (HBs) linking polypeptide chains in β -sheets as well as side chain interactions impart remarkable stability to amyloid fibrils against thermal, chemical, or mechanical denaturations.¹⁵ Amyloid formation, which involves multiple structural transitions, begins with oligomerization of monomers and progresses with the development of protofibrils and mature amyloid fibrils.^{1,16,17} Once fibrils emerge, their further growth occurs through the deposition of individual chains.¹⁸

Despite intensive experimental efforts the molecular aspects of fibril growth are still illusive. The lack of molecular level information on fibril elongation can be in part rectified by computer modeling and simulations.¹⁹ For example, all-atom molecular dynamics (MD) simulations

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Supporting Information is available free of charge via the Internet at <http://pubs.acs.org>.

investigated fibril elongation mechanism for various peptides.^{20–24} MD has been also used to explore the stability and energetics of fibril architectures.^{25–28} In addition, physicochemical propensities of polypeptides for aggregation can be mapped using computer simulations.²⁹

Among amyloidogenic species are the fragments of amyloid precursor protein, A β peptides, which are linked to the onset of Alzheimer's disease. Although A β peptides are found in a variety of lengths, the most common is 40-mer A β_{1-40} fragment. The structure of A β_{1-40} fibril protofilament has been recently derived from solid-state NMR experiments¹² (Fig. 1). In this structure A β peptides are organized into parallel in-registry β -sheets laminated into several layers.^{11–13} A β fibril elongation was proposed to proceed via two-stage “dock-lock” mechanism.^{18,30,31} The first stage involves docking of disordered A β monomers to the fibril without their integration into the fibril structure. The second stage locks a monomer in the fibril state through structural reorganization of bound peptides. In our recent study, we have probed the thermodynamics of A β fibril growth by computing its free energy landscape.²⁴ Our simulations suggested that docking and locking stages are fundamentally different. The former occurs without detectable free energy barriers and resembles polymer adsorption on attractive walls. In contrast, locking is governed by rugged free energy landscape and consequently bears some similarity to protein folding. Locking transition is associated with the formation of ordered β -sheets by A β peptides on the edges of amyloid fibrils.²⁴

Because both, side chain interactions and backbone HBs, are involved in the energetics of fibril growth, it is important to evaluate their contributions. Whereas backbone HBs are clearly crucial for fibril formation and growth, the role of side chain interactions is less obvious. Experimental data indicate that sequence mutations may have profound effect on the free energy landscape of fibrillogenesis. For example, single site mutations in A β_{1-40} could increase its amyloidogenic propensity and make it even more aggregation-prone than A β_{1-42} variant known for its ultra fast spontaneous aggregation.³² Different mutations at a single position Val18 in A β_{1-40} were shown to considerably effect the free energy of fibrillation.³³ Furthermore, bioinformatics approaches were successful in predicting the amyloidogenic propensities of polypeptide chains by taking into account the factors exclusively related to physicochemical properties of sidechains, such as hy-drophobicity, net charges, and residue patterns.^{34–36}

The relation between the strength of side chain interactions and amyloidogenic propensity may not be straightforward. It is generally assumed that sequence hydrophobicity is a factor accelerating amyloid assembly.^{37,38} However, in our previous study we used the free energy perturbation method to show that the stability of fibril state can be compromised by strong hydrophobic side chain contacts.²⁸ These interactions compete with the formation of regular fibril-like HBs and therefore reduce the free energy gap between the docked and locked states. However, it was not clear if these generic computational predictions²⁸ can be used to interpret or rationalize specific experimental data.

In this paper, we use replica exchange MD (REMD) to investigate the impact of single site mutation Asp23Tyr in A β sequence on the thermodynamics of fibril growth. Our goal is two-fold. First, substitution of aspartic acid at the position 23 with tyrosine is known to drastically accelerate amyloid formation. The importance of the position 23 also follows from the observations that its isomerization promotes fibril growth.³⁹ Thus, by performing REMD we seek to provide a microscopic explanation for these findings. Second, studying the fibril growth for the mutant A β offers a direct computational test to our proposal that side chain interactions may impede fibril growth.²⁸ This aspect of our study bears some general interest in the context of the role of sequence in amyloid formation. We show that Asp23Tyr mutation indeed decreases the free energy of locked states by promoting the formation of parallel fibril-like β -sheets on the fibril edges. We demonstrate that the impact of Asp23Tyr mutation can be

explained by destabilization of the interactions formed by Asp23 with the amino-terminal of incoming A β peptides. These interactions in the wild-type (WT) sequence are responsible for the formation of metastable docked states, which interfere with the A β fibril growth.

Model and Simulation Methods

Molecular dynamics simulations

Simulations of A β peptides were performed using CHARMM MD program⁴⁰ and all-atom force field CHARMM19 coupled with the SASA implicit solvent model.⁴¹ In this model the solvation free energy of an atom i scales linearly with the accessible surface area of atom A_i . The total solvation free energy is $G_{solv} = \sum_i \sigma_i A_i$, where σ_i is temperature-independent solvation parameter equal to $0.012 \text{ kcal}/(\text{mol}\text{\AA}^2)$ for carbon atoms and $-0.06 \text{ kcal}/(\text{mol}\text{\AA}^2)$ for oxygen and nitrogen atoms. The areas A_i are computed using approximate analytical expression.⁴² Dielectric constant ϵ is set to depend on the distance between charges r ($\epsilon = 2r$) and is assumed independent on the environment. To prevent excessive stability of salt bridges due to implicit treatment of water the ionizable side chains are neutralized. Combination of CHARMM19 force field and SASA model has been used to fold α -helical and β -sheet polypeptides to their native states^{43,44} and to study aggregation of amyloidogenic peptides.^{24,45}

Simulation system

We consider a hexamer system formed by A β_{10-40} peptides, which are the N-terminal truncated fragments of the full-length A β_{1-40} (Fig. 1). We have previously showed that amino-truncated A β_{10-40} aggregates via the pathway similar to that of the full-length peptide A β_{1-40} .⁴⁶ Furthermore, solid-state NMR studies have demonstrated that the fibril structures of A β_{1-40} and A β_{10-40} peptides are remarkably similar.^{12,47} Therefore, it appears that in aggregation studies A β_{10-40} can be used in lieu of A β_{1-40} .

In each A β_{10-40} peptide we introduced a single site mutation Asp23Tyr. This mutation was selected based on two considerations. First, experimental studies suggest that the mutations at the position 23 enhance, in some cases, dramatically, the aggregation propensity of A β peptide.^{32,48} Second, we have previously showed that the Asp23 side chain from the WT fibril forms the largest number of interactions with incoming peptides.²⁸ Therefore, one may expect Asp23Tyr mutation to have considerable impact on A β aggregation. In this study, we compare the fibril elongation thermodynamics for the Asp23Tyr mutant (MT) and WT A β_{10-40} peptides. In doing so we assume that the modifications of Asp23 do not significantly change the fibril structure. This assumption finds support in experimental studies.³⁹

Because the simulation system is similar to that used in our previous studies,²⁴ we provide here only its brief description. The system includes four peptides forming a fibril fragment and two incoming peptides interacting with the fibril (Fig. 1). The backbones of fibril peptides were constrained to the experimental positions determined from the solid-state NMR measurements.¹² The constraints were implemented using soft harmonic potentials with the constant $k_c = 0.6 \text{ kcal}/(\text{mol}\text{\AA}^2)$. The harmonic constraints permit backbone fluctuations with the amplitude of about $0.6\text{\AA}$ at 360K, which are comparable with the fluctuations of atoms on the surface of folded proteins.⁴⁹ The side chains of fibril peptides and all atoms in incoming peptides were unconstrained. Hence, 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. The hexameric system 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 about 3 mM . Throughout the paper the peptides in grey in Fig. 1 are referred to as fibril and the colored peptides are termed incoming.

Replica exchange simulations

To achieve exhaustive conformational sampling we used replica exchange molecular dynamics (REMD).⁵⁰ This method efficiently samples rugged free energy landscapes and has been applied to study protein folding and aggregation.^{24,45,51–54} Our REMD implementation is described in the previous studies.^{24,28} In all, 24 replicas were distributed linearly in the temperature range from 330 to 560K with the increment of 10K. The exchanges were attempted every 20 ps between all neighboring replicas with the average acceptance rate of 36%. Small temperature increment between replicas ensured relatively high exchange rate. For the MT we produced seven REMD trajectories resulting in a cumulative simulation time of 34 μ s. 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. By monitoring the hexamer effective energy E_{eff} , which includes the potential and solvation energies, we determined the equilibration intervals in REMD trajectories. These intervals of the lengths up to 30 ns were excluded from analysis. As a result the cumulative equilibrium simulation time was reduced to $\tau_{\text{sim}} = 30 \mu\text{s}$. In the starting REMD structures incoming peptides were placed randomly in the fibril vicinity.

Computation of structural probes

To probe the interactions between incoming peptide and the fibril, we computed the number of side chain contacts. A side chain contact was assumed formed, if the distance between the centers of mass of side chains is less than 6.5Å.⁵⁵ Backbone hydrogen bonds (HBs) between NH and CO groups were assigned according to Kabsch and Sander.⁵⁶ In all, we defined three classes of backbone HBs between incoming peptides and the fibril. The first includes any peptide-fibril HB. The second class corresponds to parallel (antiparallel) β -sheet HBs. A parallel HB (pHB) is formed between the residues i and j , if at least one other HB is also present between $i+2$ and j or $j+2$ (or between $i-2$ and j or $j-2$). An antiparallel HB (aHB) is formed between the residues i and j , if at least one other HB is also formed between either $i+2$ and $j-2$ or between $i-2$ and $j+2$. For any HB (or side chain contact) a registry offset $R = |j-i|$ can be defined, where j and i are the indices of the residues in the incoming and fibril peptides linked by HB (or contact). In general, pHBs may have arbitrary R . In-registry parallel alignment of peptides in A β fibril displayed in Fig. 1 corresponds to $R = 1$. Consequently, peptide-fibril HBs with $R = 1$ or 3 are termed fibril-like (fHB, a third HB class). Note that the HBs with $R = 2$ are excluded from fHBs, because such bonds would result in the peptide backbone adopting “flipped” conformation on the fibril edge. Bound states of incoming peptides with large number of pHBs are termed “locked” (see Results), whereas the states lacking pHBs are referred to as “docked”.²⁴ The secondary structure content was computed using the distribution of (ϕ, ψ) dihedral angles as described in our previous study.⁵⁷ The thickness D of the layer formed by incoming peptides bound to the fibril edges is computed using the approach introduced earlier.²⁴

Throughout the paper angular brackets $\langle \dots \rangle$ imply thermodynamic averages. Because hexamer system includes two indistinguishable incoming peptides, we report averages over two peptides. The distributions of states produced by REMD were analyzed using multiple histogram method.⁵⁸ The errors in computing thermodynamic quantities for the MT hexamer and the convergence of REMD simulations were similar to those obtained for the WT.²⁴ To assess the sampling errors we divided REMD simulations in two equal batches. The agreement in the numbers of peptide-fibril HBs and pHBs computed using two batches was within 1 and 4 %, respectively. The largest error (15%) is associated with the number of antiparallel HBs. Additional data suggesting approximate convergence of REMD are presented in Supporting Information.

Testing reliability of implicit solvent model

In our recent studies we have tested the accuracy of CHARMM19+SASA force field by comparing the *in silico* and experimental chemical shifts, $\delta_{sim}(i)$ and $\delta_{exp}(i)$, for A β monomer.⁵⁷ We chose to analyze C_α and C_β chemical shifts of the residues $10 \leq i \leq 40$ because of their sensitivity to α -helix and β -strand structures.⁵⁹ An excellent correlation was obtained between C_β $\delta_{sim}(i)$ and $\delta_{exp}(i)$ shifts (correlation coefficient $r = 0.9995$). The consistency between C_α $\delta_{sim}(i)$ and $\delta_{exp}(i)$ is also very good ($r = 0.987$). The overall agreement between experimental and *in silico* distributions of chemical shifts suggests that the implicit solvent model reproduces the conformational ensemble of A β_{10-40} peptides. Additional arguments on applicability of implicit solvent model can be found in Supporting Information.

Results

Using REMD we probed the mechanism of fibril growth for the mutant Asp23Tyr A β_{10-40} peptide (Fig. 1). Because we have previously investigated the fibril growth for the WT A β_{10-40} peptide,^{24,28} our findings are presented through the comparison of MT and WT. In experimental fibril structure,¹² A β_{1-40} peptide forms two β -strands separated by a turn. Accordingly, we distinguish three sequence regions in A β_{10-40} (Fig. 1) - the N-terminal (residues 10 to 23), which corresponds to the first fibril β -strand $\beta 1$; the C-terminal (residues 29 to 39), which corresponds to the second fibril β -strand $\beta 2$; and the turn region (residues 24 to 28). Unless otherwise stated the MT thermodynamic quantities are computed at 360K as those for the WT in our previous studies.^{24,28}

Thermodynamics of fibril growth: Docking of MT peptides

We first consider the temperature dependence of the deposition of incoming MT peptides onto the preformed fibril. The interactions between incoming peptides and the fibril were quantified by the thermal averages of the number of hydrophobic contacts $\langle C_{hh}(T) \rangle$, the number of HBs $\langle N_{hb}(T) \rangle$, and the number of parallel HBs $\langle N_{phb}(T) \rangle$ (see Methods). Fig. 2 shows that with the decrease in temperature T the number of peptide-fibril interactions increases. At $T = 360K$ the numbers of peptide-fibril hydrophobic contacts and HBs reach $\langle C_{hh} \rangle \approx 11.9$ and $\langle N_{hb} \rangle \approx 13.5$, respectively. At this temperature more than 70% of peptide-fibril HBs are classified as parallel ($\langle N_{phb} \rangle \approx 9.9 = 0.71 \langle N_{hb} \rangle$). Comparison with the WT reveals that the MT forms more peptide-fibril interactions. For example, at $T = 360K$ $\langle C_{hh}(T) \rangle$ increases 20% (from 9.8 (WT) to 11.9 (MT)), whereas $\langle N_{hb} \rangle$ shows a 30% increase (from 10.5 to 13.5). More importantly, the number of pHBs $\langle N_{phb} \rangle$ demonstrates a 70% increase (from 6.0 to 9.9). These data suggest that Asp23Tyr mutation has significant impact on the binding of A β peptides to the fibril.

Because the number of peptide-fibril HBs N_{hb} does not presume the formation of ordered structure by incoming peptide on the fibril edge, it can be used as a docking progress variable.²⁴ In Fig. 3a the free energy of the incoming peptide $\Delta F(N_{hb})$ shows a single minimum and no evidence of significant barriers*. It is also clear that apart from the location of minimum $\Delta F(N_{hb})$ for the MT and WT are similar. In our study of the WT we interpreted this behavior as an indication of continuous transition, which occurs without free energy barriers or intermediates.²⁴ To further test the continuous nature of MT docking we computed the temperature dependence of the hexamer free energy $\Delta F(T)$ (inset to Fig. 3a). As expected from the theory of continuous phase transitions⁶⁰ $\Delta F(T)$ displays an almost perfect quadratic dependence on temperature $\Delta F(T) \sim -(T - T_d)^2$, where $T_d \approx 370K$ is the docking temperature. The WT demonstrates similar docking behavior with $T_d \approx 380K$.²⁴

*The free energy profile remains qualitatively unchanged, if one considers as a progress variable the number of peptide-fibril hydrophobic contacts C_{hh} .

To illustrate that T_d approximately corresponds to the lower boundary of docking temperature interval, we consider the thickness D of the layer formed by the incoming peptides bound to the fibril edge. The temperature dependence $D(T)$ shown in Fig. 3b can be reasonably well fitted with the inverse temperature function $(T_u - T)^{-1}$. The relationship $D(T) \sim (T_u - T)^{-1}$, where T_u is unbinding temperature, follows from the theory of adsorption of polymers on attractive walls.⁶¹ Because polymer adsorption is analytically described by continuous phase transition, the inverse temperature dependence of $D(T)$ suggests that similar transition underlines A β binding to the fibril edge. The dependence $D(T)$ for the WT closely resembles the one shown in Fig. 3b.²⁴

The inset to Fig. 3b shows the probability distribution $P(z)$ of the position of incoming peptide's center of mass along the axis z , which coincides with the fibril axis (Fig. 1). This distribution demonstrates that incoming peptides are virtually always bound either to the concave or convex fibril edges and the probability of binding to the fibril side is negligible. The inset to Fig. 3b also suggests that the binding to the concave edge is strongly preferred. To investigate this finding further, we plot in Fig. 4 the probabilities of concave and convex edge binding as a function of temperature, $P_{CV}(T)$ and $P_{CX}(T)$. At $T \lesssim 420K$ the binding to the concave edge is favored and at $T = 360K$ $P_{CV} \approx 0.87$. At $T > 420K$ thermal fluctuations erase differences in the edge affinities and $P_{CV} \approx P_{CX}$. Fig. 4 indicates that the MT preference for CV binding is only marginally weaker than that for the WT.²⁴ To check this result we computed the free energy gap between the concave and convex bound states, $\Delta F_{CV-CX} = F_{CV} - F_{CX}$. For the MT, $\Delta F_{CV-CX} \approx -2.0RT$, whereas for the WT the free energy gap is somewhat larger ($-2.5RT$).

The impact of the Asp23Tyr mutation on the secondary structure is small. From the REMD sampling we obtained the MT fractions of β -strand $\langle S \rangle$ and helical $\langle H \rangle$ residues in incoming peptides to be 0.53 and 0.09 at $T = 360K$. These values are similar to those for the WT (0.52 and 0.11, respectively). Thus, bound peptides mostly sample extended β -strand states. Taken the results for the WT and MT together we surmise that their docking properties are qualitatively similar. Thus, the impact of Asp23Tyr mutation on docking to the fibril appears to be relatively minor. However, as shown below this mutation has a profound impact on the locking stage of binding.

Thermodynamics of fibril growth: Locking of MT peptides

It has been shown experimentally^{18,30,31} and computationally²⁴ that docking transition is followed by locking of incoming peptides in the fibril-like conformations. We have previously showed that locking is associated with the formation of parallel β -sheets by incoming peptides on the fibril edge.^{24,28} Consequently, the appropriate progress variable for locking is the number of peptide-fibril pHBs, N_{phb} . It is important to note that the formation of antiparallel peptide-fibril HBs might also lead to ordered binding. Therefore, to get better insight into the nature of locking transition we plot in Fig. 5 the free energy surface $\Delta F(N_{phb}, N_{apb})$ as a function of the numbers of parallel N_{phb} and antiparallel HBs N_{apb} . Due to existence of multiple basins, this rugged free energy landscape is fundamentally different from the barrierless free energy profile in Fig. 3a. Similar to the WT²⁴ Fig. 5 displays four basins - the docked (D, no parallel or antiparallel HBs), the locked (L, only parallel HBs are present), the antiparallel (AP, only antiparallel HBs are formed), and the mixed (M). The latter contains the mixture of parallel and antiparallel HBs formed by incoming peptide. The M state has high minimum free energy ($\Delta F_M = 3.3RT$) and is thermodynamically unstable thus disfavoring coexistence of parallel and antiparallel β -sheets. The antiparallel basin AP also has elevated minimum free energy $\Delta F_{AP} = 1.6RT$. In contrast, the minimum free energy of the locked state L, ΔF_L , is approximately equal to that of the docked state $\Delta F_D = 0$. The L state is separated from other basins by high ($\geq 3.9RT$) free energy barriers.*

Using the definition of the L state (see the caption to Fig. 5) we compute the probability of occupancy of L to be $P_L \approx 0.7$ at $T = 360K$. The corresponding free energy gap separating the L state from other conformers is $\Delta\Delta F_L = -RT\ln(P_L/(1 - P_L)) \approx -0.8RT$. For comparison, under the same conditions the WT free energy gap $\Delta\Delta F_L \approx 0$ and the probability of the L state is $P_L \approx 0.5$. As for the WT we associate the locking transition with the temperature T_l , at which $P_L(T_l) \approx 0.5$. Accordingly, for the MT $T_l \approx 390K$, whereas for the WT it is 30K lower ($T_l \approx 360K$). Hence, these findings indicate that the Asp23Tyr mutation enhances the stability of the locked state. This result is consistent with the increase in $\langle N_{phb} \rangle$ observed for the MT (Fig. 2c).

Maps of peptide-fibril interactions

To map the peptide-fibril aggregation interface we compute the distribution of interactions between incoming peptides and the fibril. Fig. 6a shows the thermal map $\langle C(i, j) \rangle$ of side chain contacts formed between the fibril residues i and the residues j from incoming peptide. Visual inspection of Fig. 6a suggests that many contacts are formed along the main diagonal ($R = |i - j| \leq 3$). These contacts are in approximate registry and are similar to those formed within β -sheets in the fibril interior. It follows from Fig. 6a that the total number of peptide-fibril side chain contacts $\langle C \rangle$ is 41.6, of which 21.0 (or 51%) correspond to those in approximate registry. In contrast, for the WT the fraction of such contacts is considerably lower (21%).

A further illustration of significant shift in the MT peptide-fibril interactions is provided in Table 1a. This table shows that the largest numbers of side chain contacts are formed by the pairs of β -strands $\beta 1 - \beta 1$ or $\beta 2 - \beta 2$ from incoming peptide and the fibril. It is also instructive to consider the difference contact map $\langle \Delta C(i, j) \rangle = \langle C(i, j) \rangle - \langle C(i, j) \rangle_{WT}$, where $\langle C(i, j) \rangle_{WT}$ is the WT contact map (Fig. 6b). $\langle \Delta C(i, j) \rangle$ reveals that the contacts stabilized by the mutation are almost exclusively aligned along the main diagonal. Indeed, Table 1a lists the values of $\langle \Delta C(s1, s2) \rangle$ integrated over the β -strands $s1$ and $s2$. It follows that the increase in the number of peptide-fibril contacts occurs *only* for the diagonal elements $\langle \Delta C(s1, s1) \rangle$, whereas the off-diagonal elements $\langle \Delta C(s1, s2) \rangle$ ($s1 \neq s2$) register fewer interactions for the MT compared to the WT. This observation also implies that in contrast to the MT some of the WT off-diagonal elements (such as $\langle C(\beta 1, \beta 2) \rangle_{WT}$) are larger than the diagonal ones.

The conclusions reached from the consideration of side chain contacts are consistent with the analysis of peptide-fibril HBs. Fig. 6c shows the thermal map $\langle N_{hb}(i, j) \rangle$ of HBs formed between the fibril residues i and the residues j from incoming peptide. Similar to the contact map $\langle C(i, j) \rangle$ the majority of peptide-fibril HBs are formed between the residues with small registry offsets R . Using $\langle N_{hb}(i, j) \rangle$ we find that the number of HBs being formed between the residues in approximate registry ($R \leq 3$) is 9.1 that constitutes two-thirds of all peptide-fibril HBs ($\langle N_{hb} \rangle = 13.5$). For comparison, in the WT the fraction of such HBs is only 17%. One may expect from these findings that the parallel β -structure formed by the MT should be in approximate registry. To check this we computed the average values of registry offsets $\langle R(s1, s2) \rangle$ for pHBs (Table 1b). The pHBs between the pair of $\beta 1$ strands or the pair of $\beta 2$ strands occur in almost perfect in-registry alignment. As expected for in-registry parallel β -sheets the offsets between the $\beta 1$ and $\beta 2$ strands are close to their sequence distance $|i_{\beta 1} - i_{\beta 2}| = 17.5$, where $i_{\beta 1}$ and $i_{\beta 2}$ are the sequence midpoints of $\beta 1$ and $\beta 2$, respectively. Finally, our result that the Asp23Tyr mutation promotes the formation of parallel in-registry β -sheets is supported by the direct computation of the average number of fibril-like HBs, $\langle N_{fhb} \rangle$. For the MT, $\langle N_{fhb} \rangle = 6.8$, which is profoundly larger than $\langle N_{fhb} \rangle = 1.0$ for the WT.

*The one-dimensional free energy profile $\Delta F(N_{phb})$ is also rugged, in which the docked ($N_{phb} = 0$) and locked ($N_{phb} > 3$) basins are separated by the barrier of $4.4RT$ at $T = 360K$.

To evaluate the distribution of pHBs along the sequence of incoming peptides we consider the fraction of pHBs $n_{phb}(j) = \langle N_{phb}(j) \rangle / \langle N_{hb}(j) \rangle$, where $\langle N_{phb}(j) \rangle$ and $\langle N_{hb}(j) \rangle$ are the numbers of pHBs and HBs formed by the amino acid j with the fibril. Fig. 6d shows that for the MT $n_{phb}(j) \gtrsim 0.8$ within the strands $\beta 1$ and $\beta 2$. In contrast, $n_{phb}(j)$ for the WT is generally lower and within the $\beta 2$ strand $n_{phb} < 0.55$. These observations suggest that the Asp23Tyr mutation facilitates the formation of parallel β -sheets by incoming peptides on the fibril edges that is in accord with the free energy computations above.

Discussion

Asp23Tyr mutation promotes fibril growth

In this study we investigated the impact of the single site mutation Asp23Tyr on the mechanism fibril elongation. The following findings indicate that this mutation does not significantly affect docking of incoming peptides to the fibril. First, although the MT forms somewhat larger number of peptide-fibril HBs and hydrophobic side chain contacts than the WT, the docking of the MT remains barrierless similar to the WT (Fig. 3a). The continuous nature of MT docking is consistent (i) with the quadratic temperature dependence of the hexamer free energy⁶⁰ (Fig. 3a) and (ii) with the inverse temperature dependence of the thickness of adsorbed peptide layer formed on the fibril edge⁶¹ (Fig. 3b). Similar observations have been made for the WT.²⁴ The mutation also causes a relatively small change in the docking temperature T_d by 10K (compared to the change in the locking temperature T_l , see below). Second, for the MT the binding affinity of the concave edge is about seven times stronger than of the convex edge (Fig. 4). Likewise, the WT demonstrates about 10:1 preference to bind to the concave edge over the convex one.²⁴ This observation raises the possibility of unidirectional growth of A β amyloid fibrils.^{62,63}

However, the Asp23Tyr mutation has considerable impact on the locking transition. From the analysis of free energy landscape (Fig. 5) it follows that the mutation increases the locking temperature T_l by about 30K. Due to mutation the free energy gap separating the locked L state from other conformers is increased by $\sim RT$ at 360K and the probability of L rises from ≈ 0.5 (WT) to ≈ 0.7 (MT). Consistent with these changes in free energy landscape the number of peptide-fibril parallel HBs increases 65% (from 6.0 in the WT to 9.9 in the MT). More importantly, the Asp23Tyr mutation causes profound changes in the peptide-fibril aggregation interface. The mutation selectively enhances the peptide-fibril interactions, which are in approximate registry. This observation follows from the analysis of (difference) contact maps and the maps of HBs (Fig. 6a–c). For example, the fraction of HBs formed between the residues in approximate registry ($R \leq 3$) grows four-fold (from 17% in the WT to 67% in the MT). Simultaneously, the number of fibril-like HBs $\langle N_{fhb} \rangle$ demonstrates almost seven-fold increase (from 1.0 to 6.8). If we assume that in the fibril-like state $6 \leq N_{fhb} \leq 22$, then the free energy gap separating this state from other conformers increases $2.7RT$ for the MT relative to the WT. (In these computations the docked states with $N_{fhb} = 0$ are set to have zero free energy.) As a result Asp23Tyr mutant forms on the fibril edge parallel β -sheets, which are almost in registry with the fibril ($\langle R \rangle \sim 1$, Table 1b).

Because the MT differs from the WT only by a single amino acid substitution, we can readily pinpoint the source of differences in their mechanisms of fibril growth. If so, then what is the molecular basis for the enhanced stability of the MT locked state and promotion of in-registry parallel β -sheets? The answer is provided by the difference contact map $\langle \Delta C(i, j) \rangle$. It follows from Fig. 6b that the contact between the fibril residue 23 and the residue 11 from incoming peptide shows the largest decrease in formation probability. Specifically, $\langle C^{(23,11)} \rangle$ is decreased 10-fold, from 0.65 (WT) to 0.05 (MT). Importantly, in the WT the contact Asp23-Glu11 is the most stable among all peptide-fibril side chain contacts.²⁸ Hence, the Asp23Tyr mutation affects essential peptide-fibril interaction, which in the WT contributes to off-registry binding. In other words, the changes in peptide-fibril aggregation interface and stabilization

of the locked state should be attributed to the elimination of Asp23 side chain interactions. (As a byproduct of Asp23Tyr mutation significant destabilization of the contact between the fibril Ala21 and Glu11 from incoming peptide is also observed.) Analysis of implicit solvent REMD trajectories indicates that Asp23-Glu11 interactions are due to the formation of stable HB between one of the oxygen acceptor atoms (OD1 or OD2) in Asp23 side chain and the backbone amino group of Glu11 (Fig. 1b).

Therefore, because the substitution of Asp23 with Tyr increases the free energy gap between the locked and docked states and stabilizes parallel in-registry β -sheets, it is expected to promote fibril growth. Our data also suggest that strong off-registry side chain interactions (such as Asp23-Glu11) disfavor locked state and their elimination should enhance the formation of parallel peptide-fibril β -sheets. Finally, the strong impact of Asp23Tyr mutation is consistent with the prediction that the A β aggregation interface mainly involves the sequence region 10–23. This prediction has been made by us^{46,57} and other investigators.^{29,64}

Testing implicit solvent simulations with explicit solvent model

It is important to test the stability of Asp23-Glu11 interactions using explicit solvent model. To this end, we produced two 20 ns MD trajectories, one for the WT and the other for Asp23Tyr mutant. We used NAMD molecular dynamics program⁶⁵ and CHARMM22 force field. The charge states of amino acids correspond to pH 7 conditions. The hexamer systems were solvated in the periodic boundary box with the dimensions 79Åx 69Åx 47Å. The simulations were performed at 360K and started with the implicit solvent structure, in which the HB between the residue 23 (fibril peptide) and Glu11 (incoming peptide) is formed (Fig. 1b). Fig. 7 shows as a function of time t the energy $E_{nb}(t)$ of non-bonded interactions between Asp23 (Tyr23 in the MT) and the backbone of the N-terminal of the incoming peptide. After few ns attractive interactions between the WT Asp23 and the N-terminal backbone are established ($E_{nb} \sim -30\text{kcal/mol}$), which last for about 10 ns. Within this time interval Asp23 forms HBs with the backbone of the N-terminal of incoming peptide (mostly, with amino group of Val12). Once these HBs are disrupted at $t > 12\text{ns}$, E_{nb} approaches zero. Interestingly, during 20 ns MT trajectory Tyr23 does not form HBs with the N-terminal of incoming peptide and, consequently, its E_{nb} is relatively high. If the interactions between Asp23 (Tyr23) and the N-terminal of incoming peptide include also the side chains, then the non-bonded interaction energy averaged over $2 < t < 11\text{ns}$ is -10.7kcal/mol (WT) and -6.9kcal/mol (MT). Thus, as in implicit water simulations Asp23 in explicit water model forms, at least temporarily, stable interactions with the N-terminal of incoming peptide, which supercede the repulsion between Asp and Glu. (In contrast to implicit solvent, Asp23 establishes HB with Val12 rather than with Glu11.) The stability of the HB between Asp23 and the N-terminal is significant, because it occurs in dehydrated environment. It follows from the WT explicit solvent trajectory that Asp23 is about 80% buried when this HB is formed. Therefore, explicit water simulations are consistent with our conclusion that Asp23Tyr mutation compromises attractive off-registry peptide-fibril side chain interactions.

Side chain interactions can impede fibril growth: Evidence from simulations and experiments

In our previous study of fibril growth we performed perturbations of binding free energy landscape by scanning partial deletions of side chain interactions at various A β_{10-40} sequence positions.²⁸ By comparing the free energy gaps between the locked and docked states for the WT and “mutants” we proposed that side chain contacts may impede fibril growth. More specifically, if a perturbation partially deletes stable side chain contact, which favors out-of-registry (random) peptide-fibril binding, a formation of parallel β -sheets or locking should be promoted. It appears that the simulations of Asp23Tyr mutant are in accord with this proposal. As shown above the mutation Asp23Tyr eliminates strong peptide-fibril off-registry

interactions and considerably stabilizes the locked in-registry state. Therefore, it seems plausible that targeted mutagenesis may accelerate fibril elongation by suppressing selected side chain interactions.

It is important to note that we cannot rule out that the stabilization of the locked state is, in part, due to enhanced aromatic interactions and higher hydrophobicity introduced by Asp23Tyr mutation. These factors may structurally stabilize the turn sequence region, which, as suggested by previous MD simulations,⁶⁶ nucleates the A β folding.

We now compare the results of REMD simulations with experimental data. Using A β -green fluorescent protein fusion system Kim and Hechts have mapped the mutations, which increase A β aggregation propensity.³² They found that one of the most aggressive mutations is Asp23Tyr (WM5, in their designation), which forces A β ₁₋₄₀ to aggregate even more readily than A β ₁₋₄₂. This observation is striking, because A β ₁₋₄₂ is highly aggregation-prone and forms fibrils without detectable time lag.⁶⁷ The study of Kim and Hechts also showed that Asp23Tyr mutation drastically reduces A β ₁₋₄₀ solubility.

There are other indications that deleting aspartic acid at the position 23 enhances amyloid assembly. Shirasawa and coworkers have studied the aggregation behavior of A β ₁₋₄₀ variants isomerized at Asp23 to produce unusual β -linkage, which incorporates Asp side chain into peptide backbone.³⁹ They showed that Asp23 isomer aggregates more aggressively than the WT and becomes as aggregation-prone as a well known wild-type Dutch A β mutant Glu22Asn. Despite backbone modification Asp23 isomers formed the amyloid fibrils morphologically indistinguishable from the WT. In contrast, isomerization at the position Asp7 had no discernible effect on amyloid assembly. Finally, Wetzel and coworkers performed proline mutagenesis scan of A β ₁₋₄₀ sequence and measured the free energy gap between the fibrillized and soluble states.⁶⁸ Asp23Pro was one of the four mutants out of 30 tested, which decreased the free energy of the fibril. This result is unusual, because as a rule Pro substitutions destabilize fibril structures as they are incompatible with β -sheets.

In the solid-state NMR fibril structure of A β ₁₋₄₀ the side chain of Asp23 forms intermolecular salt bridge with Lys28.¹² Therefore, the mutation of Asp23 should, in principle, destabilize the fibril structure. The fact that the opposite is experimentally observed could be the consequence of disruption of side chain interactions formed by Asp23, which destabilize locked states. According to our REMD simulations these interactions (e.g., Asp23-Glu11) are incompatible with the parallel fibril-like β -sheets formed by incoming peptides on the fibril edge. Therefore, our simulations appear to provide microscopic explanation for the changes in amyloidogenesis caused by Asp23 modification.

Conclusions

Using REMD simulations we probed the effect of Asp23Tyr mutation on the mechanism of A β ₁₀₋₄₀ fibril growth. The consequences of the mutation were evaluated by computing binding free energy landscapes, distributions of peptide-fibril interactions, and through the comparison with the wild-type A β ₁₀₋₄₀ peptide. We showed that Asp23Tyr mutation has limited impact on the docking of A β peptides to the fibril, which as for the WT remains barrierless. In contrast, the locking stage is strongly affected by the mutation due to profound stabilization of the parallel in-registry β -sheets formed by the peptides on the fibril edge. The enhanced stability of parallel β -sheets results from the deletion of strong side chain interactions formed by Asp23, which are incompatible with the locked state. Based on our data we expect Asp23Tyr mutation to promote fibril growth. The analysis of Asp23Tyr mutation therefore suggests that strong off-registry side chain interactions may slow down fibril assembly as it occurs for the wild-

type A β peptide. This observation can be useful in predicting the effects of mutations on fibril growth. The available experimental data appear to support our *in silico* conclusions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Dobson CM. Nature 2003;426:884–890. [PubMed: 14685248]
2. Selkoe DJ. Nature 2003;426:900–904. [PubMed: 14685251]
3. 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. Nature Medicine 2008;14:837–842.
4. Kaye R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, Glabe CG. Science 2003;300:486–489. [PubMed: 12702875]
5. Haass C, Selkoe DJ. Nature Rev. Mol. Cell. Biol 2007;8:101–112. [PubMed: 17245412]
6. Pastor MT, Kummer N, Schubert V, Esteras-Chopo A, Dotti CG, de la Paz ML, Serrano L. J. Mol. Biol 2008;375:695–707. [PubMed: 18036611]
7. Murphy RM, Pallitto MM. J. Struct. Biol 2000;130:109–122. [PubMed: 10940219]
8. Carulla N, Caddy GL, Hall DR, Zurdo J, Gair M, Feliz M, Giralt E, Robinson CV, Dobson CM. Nature 2005;436:554–558. [PubMed: 16049488]
9. Martins IC, Kuperstein I, Wilkinson H, Maes E, Vanbrabant M, Jonckheere W, van Gelder P, Hartmann D, Hooze RD, de Strooper B, Schymkowitz J, Rousseau F. EMBO J 2008;27:224–233. [PubMed: 18059472]
10. Serpell LC. Biochim Biophys. Acta 2000;1502:16–30.
11. Burkoth TS, Benzinger T, Urban V, Morgan DM, Gregory DM, Thiyagarajan P, Botto RE, Meredith SC, Lynn DG. J. Am. Chem. Soc 2000;122:7883–7889.
12. Petkova AT, Yau W-M, Tycko R. Biochemistry 2006;45:498–512. [PubMed: 16401079]
13. Luhrs T, Ritter C, Adrian M, Loher B, Bohrmann DR, Dobeli H, Schubert D, Riek R. Proc. Natl. Acad. Sci. USA 2005;102:17342–17347. [PubMed: 16293696]
14. Nelson R, Sawaya MR, Balbirnie M, Madsen AO, Riek C, Grothe R, Eisenberg D. Nature 2005;435:773–778. [PubMed: 15944695]
15. Meersman F, Dobson CM. Biochim. Biophys. Acta 2006;1764:452–460. [PubMed: 16337233]
16. Kirkitadze MD, Bitan G, Teplow DB. J. Neurosci. Res 2002;69:567–577. [PubMed: 12210822]
17. Murthy RM. Ann. Rev. Biomed. Eng 2002;4:155–174. [PubMed: 12117755]
18. Esler WP, Stimson ER, Jennings JM, Vinters HV, Ghilardi JR, Lee JP, Man-tyh PW, Maggio JE. Biochemistry 2000;39:6288–6295. [PubMed: 10828941]
19. Ma B, Nussinov R. Curr. Opin. Struct. Biol 2006;10:445–452.
20. Wu C, Lei H, Duan Y. J. Amer. Chem. Soc 2005;127:13530–13537. [PubMed: 16190716]
21. Nguyen PH, Li MS, Stock G, Straub JE, Thirumalai D. Proc. Natl. Acad. Sci. USA 2007;104:111–116. [PubMed: 17190811]
22. Krone MG, Hua L, Soto P, Zhou R, Berne BJ, Shea J-E. J. Amer. Chem. Soc. 2008
23. Takeda T, Klimov DK. Biophys. J 2008;95:1758–1772. [PubMed: 18502791]
24. Takeda T, Klimov DK. Biophys. J 2009;96:442–452. [PubMed: 19167295]
25. Buchete N-V, Tycko R, Hummer GJ. Mol. Biol 2005;353:804–821.

26. Zheng J, Jang H, Ma B, Tsai C-J, Nussinov R. *Biophys. J* 2008;93:3046–3057. [PubMed: 17675353]
27. Buchete N-V, Hummer G. *Biophys. J* 2007;92:3032–3039. [PubMed: 17293399]
28. Takeda T, Klimov DK. *Biophys. J* 2009;96:4428–4437. [PubMed: 19486667]
29. Cecchini M, Curcio R, Pappalardo M, Melki R, Caflisch A. *J. Mol. Biol* 2006;357:1306–1321. [PubMed: 16483608]
30. Cannon MJ, Williams AD, Wetzel R, Myszkowski DG. *Anal. Biochem* 2004;328:67–75. [PubMed: 15081909]
31. O’Nuallain B, Shivaprasad S, Kheterpal I, Wetzel R. *Biochemistry* 2005;44:12709–12718. [PubMed: 16171385]
32. Kim W, Hechts MH. *J. Mol. Biol* 2008;377:565–574. [PubMed: 18258258]
33. Peim A, Hortschansky P, Christopeid T, Schroeckh V, Richter W, Fandrich M. *Prot. Sci* 2006;15:1801–1805.
34. Pawar AP, Dubay KF, Zurdo J, Chiti F, Vendruscolo M, Dobson CM. *J. Mol. Biol* 2005;350:379–392. [PubMed: 15925383]
35. Tartaglia G, Cavalli A, Pellarin P, Caflisch A. *Protein Sci* 2005;14:2723–2734. [PubMed: 16195556]
36. Luheshi LM, Tartaglia GG, Brorsson A-C, Pawar AP, Watson IE, Chiti F, Vendruscolo M, Lomas DA, Dobson CM, Crowther DC. *PLoS Biology* 2007;5:e290. [PubMed: 17973577]
37. Esler WP, Stimson ER, Ghilardi JR, Lu Y-A, Felix AM, Vinters HV, Man-tyh PW, Lee JP, Maggio JE. *Biochemistry* 1996;35:13914–13921. [PubMed: 8909288]
38. Meinhardt J, Tartaglia GG, Pawar A, Christopeid T, Hortschansky P, Schroeckh V, Dobson CM, Vendruscolo M, Fandrich M. *Prot. Sci* 2007;16:1214–1222.
39. Shimizu T, Fukuda H, Murayama S, Izumiyama N, Shirasawa T. *J. Neurosci. Res* 2002;70:451–461. [PubMed: 12391606]
40. Brooks BR, Brucoler RE, Olafson BD, States DJ, Swaminathan S, Karplus M. *J. Comp. Chem* 1982;4:187–217.
41. Ferrara P, Apostolakis J, Caflisch A. *Proteins Struct. Funct. Bioinform* 2002;46:24–33.
42. Hasel W, Hendrickson TF, Still WC. *Tetrah. Comp. Methodol* 1988;1:103–116.
43. Ferrara P, Caflisch A. *Proc. Natl. Acad. Sci. USA* 2000;97:10780–10785. [PubMed: 10984515]
44. Hiltbold A, Ferrara P, Gsponer J, Caflisch A. *J. Phys. Chem. B* 2000;104:10080–10086.
45. Cecchini M, Rao F, Seeber M, Caflisch A. *J. Chem. Phys* 2004;121:10748–10756. [PubMed: 15549960]
46. Takeda T, Klimov DK. *J. Phys. Chem. B* 2009;113:6692–6702. [PubMed: 19419218]
47. Paravastu AK, Petkova AT, Tycko R. *Biophys. J* 2006;90:4618–4629. [PubMed: 16565054]
48. Nostr WE, Melchor JP, Cho HS, Greenberg SM, Rebeck GW. *J. Biol. Chem* 2001;276:32860–32866. [PubMed: 11441013]
49. Zhou Y, Vitkup D, Karplus M. *J. Mol. Biol* 1999;285:1371–1375. [PubMed: 9917381]
50. Sugita Y, Okamoto Y. *Chem. Phys. Lett* 1999;114:141–151.
51. Garcia AE, Onuchic JN. *Proc. Natl. Acad. Sci. USA* 2003;100:13898–13893. [PubMed: 14623983]
52. Tsai H-H, Reches M, Tsai C-J, Gunasekaran K, Gazit E, Nussinov R. *Proc. Natl. Acad. Sci. USA* 2005;102:8174–8179. [PubMed: 15923262]
53. Baumketner A, Shea J-E. *J. Mol. Biol* 2006;362:567–579. [PubMed: 16930617]
54. Jang S, Shin S. *J. Phys. Chem. B* 2008;112:3479–3484. [PubMed: 18303879]
55. Klimov DK, Thirumalai D. *Structure* 2003;11:295–307. [PubMed: 12623017]
56. Kabsch W, Sander C. *Biopolymers* 1983;22:2577–2637. [PubMed: 6667333]
57. Takeda T, Klimov DK. *Proteins Struct. Funct. Bioinf.* 2009
58. Ferrenberg AM, Swendsen RH. *Phys. Rev. Lett* 1989;63:1195–1198. [PubMed: 10040500]
59. 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. *J. Amer. Chem. Soc* 2004;126:1992–2005. [PubMed: 14971932]
60. Landau, LD.; Lifshitz, EM. *Statistical Physics (Course of Theoretical Physics, Volume 5)*. Oxford: Butterworth-Heinemann; 1984.

61. Grosberg, AY.; Khokhlov, AR. Statistical Physics of macromolecules. Wood-bury: AIP Press; 1994.
62. Fawzi NL, Okabe Y, Yap E-H, Head-Gordon T. J. Mol. Biol 2007;365:535–550. [PubMed: 17070840]
63. Kellermayer MSZ, Karsai A, Benke M, Soos K, Penke B. Proc. Natl. Acad. Sci. USA 2008;105:141–144. [PubMed: 18162558]
64. Melquiond A, Dong X, Mousseau N, Derreumaux P. Curr. Alzh. Res 2008;5:244–250.
65. Kale L, Skeel R, Bhandarkar M, Brunner R, Gursoy A, Krawetz N, Phillips J, Shinozaki A, Varadarajan K, Schulten K. J. Comp. Phys 1999;151:283–312.
66. Krone MG, Baumketner A, Bernstein SL, Wyttenbach T, Lazo ND, Teplow DB, Bowers MT, Shea J-E. J. Mol. Biol 2008;381:221–228. [PubMed: 18597778]
67. Jarrett JT, Berger EP, Lansbury PT. Biochemistry 1993;32:4693–4697. [PubMed: 8490014]
68. Williams AD, Portelius E, Kheterpal I, Guo J, Cook K, Xu Y, Wetzel R. J. Mol. Biol 2004;335:833–842. [PubMed: 14687578]
69. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. J. Comp. Chem 2004;25:1605–1612. [PubMed: 15264254]

Fig. 1.

(a) Cartoon representation of the MT A β_{10-40} hexamer. Four A β peptides in grey form fibril fragment. Two incoming peptides in color with side chains shown are bound to the fibril edge. Fibril protofilament consists of four in-registry parallel β -sheets formed by the $\beta 1$ and $\beta 2$ strands (panel (c)). Asp23Tyr mutation in A β promotes similar in-registry parallel β -sheets formed by incoming peptides on the fibril edge. Spatial allocation of $\beta 1$ and $\beta 2$ in the fibril results in the appearance of two distinct fibril edges - concave (CV) and convex (CX). (b) Representative snapshot of binding the WT A β_{10-40} peptides to the WT fibril. A HB between the side chain of the fibril residue Asp23 and the backbone of the residue Glu11 from incoming peptide is shown by dashed line. This peptide-fibril interaction favors off-registry binding of

WT incoming peptides, which is incompatible with parallel in-register β -sheets. The WT fibril structure is derived from NMR measurements¹² and visualized using Chimera.⁶⁹ (c) The sequence of the MT A β_{10-40} monomer and the allocation of the β 1 and β 2 fibril β -strands and the turn.

Fig. 2.

Temperature dependence of binding of A β_{10-40} peptides to amyloid fibril is probed by the thermal averages of the number of hydrophobic contacts $\langle C_{hh}(T) \rangle$ (a), the number of HBs $\langle N_{hb}(T) \rangle$ (b), and the number of parallel HBs $\langle N_{phb}(T) \rangle$ (c). The data in black and grey correspond to the MT and WT, respectively. These plots show that Asp23Tyr mutation enhances binding to the fibril.

Fig. 3.

Docking of A β_{10-40} peptides to the fibril. (a) Free energy of incoming peptide $\Delta F(N_{hb})$ as a function of the number of peptide-fibril HBs N_{hb} the MT (data in black), the WT (in grey). The free energy of the state with $N_{hb} = 0$ is set to zero. Plot is obtained at $T_d = 370 K$. The inset shows the temperature dependence of the free energy of MT hexamer system, $\Delta F(T)$ (circles). The parabolic fit $\Delta F(T) = -\alpha(T - T_d)^2$, where $\alpha = 0.0082 \text{ kcal}/(\text{mol K}^2)$ and $T_d = 370 K$ is a docking temperature, is shown by continuous line. (b) The thickness $D(T)$ of the layer formed by the MT peptides bound to the fibril edge as a function of temperature (open circles). Continuous line represents the fit with the inverse temperature function $D_0(T_u - T)^{-1}$, where $D_0 = 580 \text{\AA}$ and $T_u = 581 K$. The data at $T < T_d$ are in grey. Barrierless free energy profile and

the temperature dependences $\Delta F(T)$ and $D(T)$ suggest that docking is a continuous transition. The inset shows the MT probability distribution $P(z)$ of the position of incoming peptide's center of mass along the axis z at $360K$. The shaded area marks the fibril fragment; CV and CX denote concave and convex fibril edges (Fig. 1). The distribution $P(z)$ demonstrates that the MT binds exclusively to the fibril edges.

Fig. 4.

The probabilities of concave (CV, full circles) and convex (CX, open circles) edge binding as a function of temperature, $P_{CV}(T)$ and $P_{CX}(T)$: the MT (data in black), the WT (in grey). To compute P_{CV} we assumed that a peptide is bound to the CV edge, if the z -component of its center of mass is > 0 (Fig. 3b). The probability of CX binding is $P_{CX} = 1 - P_{CV}$. Because peptides do not bind to the fibril sides, these definitions capture the binding to its edges. For the MT and WT the binding to the CV edge is strongly preferred.

Fig. 5.

Free energy surface $\Delta F(N_{phb}, N_{ahb})$ as a function of the numbers of parallel N_{phb} and antiparallel N_{ahb} peptide-fibril HBs is computed for the MT at $T = 360K$. Four basins are observed - the docked (D, $N_{phb} = 0, N_{ahb} = 0$), the locked (L, $N_{phb} > 3, N_{ahb} = 0$), the antiparallel (AP, $N_{phb} = 0, N_{ahb} > 1$), and the mixed (M, $N_{phb} > 1, N_{ahb} > 1$). The free energy of the D state is set to zero. Compared to the WT the locked state of the MT is stabilized.

Fig. 6.

(a) The MT thermal contact map $\langle C(i,j) \rangle$ displays the probabilities of forming side chain contacts between the fibril residues i and the residues j from incoming peptide. (b) The difference contact map $\langle \Delta C(i,j) \rangle$ visualizes the changes in peptide-fibril contact probabilities occurring in the MT relative to the WT. (c) The MT thermal map $\langle N_{hb}(i,j) \rangle$ of HBs formed between the fibril residues i and the residues j from incoming peptide. $\langle C(i,j) \rangle$, $\langle \Delta C(i,j) \rangle$, and $\langle N_{hb}(i,j) \rangle$ are color coded according to the scales. The panels (a–c) suggest that the mutation Asp23Tyr promotes nearly in-registry binding of incoming peptides to the fibril edge. (d) The fraction of pHBs $n_{phb}(j)$ is plotted for residues j in incoming peptide: the MT (in black),

the WT (in grey). The plot demonstrates that the mutation Asp23Tyr increases the fraction of pHBs. The residues from $\beta 1$ and $\beta 2$ strands are boxed (Fig. 1c).

Fig. 7.

The energy $E_{nb}(t)$ of non-bonded interactions between the WT residue Asp23 (or Tyr23 in the MT) and the N-terminal backbone of incoming peptide as a function of time t : thick black line (WT), grey line (MT). The thin black line represents the number of HBs formed between Asp23 and the N-terminal backbone of incoming peptide. The N-terminal includes five residues Tyr10-His14 (Fig. 1c). The figure is obtained using explicit solvent simulations. It suggests that Asp23Tyr mutation deletes attractive peptide-fibril interactions, which are incompatible with parallel β -sheets formed by incoming peptides on the fibril edge.

Table 1
Peptide-fibril aggregation interface for Asp23Tyr mutant

(a) Average numbers of side chain contacts between incoming peptide and the fibril $\langle C(s1,s2) \rangle^{\ddagger}$		
	$\beta 1$	$\beta 2$
$\beta 1$	12.2 (+2.7)	6.1 (-5.3)
$\beta 2$	5.9 (-2.9)	8.7 (+5.4)
(b) Average registry offsets $\langle R(s1,s2) \rangle$ for the MT peptides bound to the fibril edge ‡		
	$\beta 1$	$\beta 2$
$\beta 1$	1.2	15.5
$\beta 2$	15.8	1.4

‡ the row $s1$ and column $s2$ indices are the β -strands $\beta 1$ or $\beta 2$ in fibril and incoming peptides, respectively.

‡ numbers in parenthesis indicate the change $\Delta \langle C(s1,s2) \rangle = \langle C(s1,s2) \rangle - \langle C(s1,s2) \rangle_{WT}$ in the number of peptide-fibril contacts observed for the MT relative to the WT.