

UCLA

UCLA Previously Published Works

Title

Folding events in the 21-30 region of amyloid β -protein ($A\beta$) studied in silico

Permalink

<https://escholarship.org/uc/item/34q1v3vc>

Journal

Proceedings of the National Academy of Sciences of the United States of America, 102(17)

ISSN

0027-8424

Authors

Borreguero, Jose M
Urbanc, Brigita
Lazo, Noel D
et al.

Publication Date

2005-04-26

DOI

10.1073/pnas.0502006102

Peer reviewed

Folding events in the 21–30 region of amyloid β -protein ($A\beta$) studied *in silico*

Jose M. Borreguero*[†], Brigita Urbanc*, Noel D. Lazo[‡], Sergey V. Buldyrev[§], David B. Teplow[‡], and H. Eugene Stanley*

*Center for Polymer Studies and Department of Physics, Boston University, Boston, MA 02215; [‡]Department of Neurology, David Geffen School of Medicine, University of California, Los Angeles, CA 90095; and [§]Department of Physics, Yeshiva University, New York, NY 10033

Contributed by H. Eugene Stanley, March 10, 2005

Oligomeric assemblies of the amyloid β -protein ($A\beta$) have been implicated in the pathogenesis of Alzheimer's disease as a primary source of neurotoxicity. Recent *in vitro* studies have suggested that a 10-residue segment, Ala-21–Ala-30, forms a turn-like structure that nucleates the folding of the full-length $A\beta$. To gain a mechanistic insight, we simulated $A\beta(21-30)$ folding by using a discrete molecular dynamics algorithm and a united-atom model incorporating implicit solvent and a variable electrostatic interaction strength (EIS). We found that $A\beta(21-30)$ folds into a loop-like conformation driven by an effective hydrophobic attraction between Val-24 and the butyl portion of the Lys-28 side chain. At medium EIS [1.5 kcal/mol (1 cal = 4.18 J)], unfolded conformations almost disappear, in agreement with experimental observations. Under optimal conditions for folding, Glu-22 and Asp-23 form transient electrostatic interactions (EI) with Lys-28 that stabilize the loop conformations. Glu-22–Lys-28 is the most favored interaction. High EIS, as it occurs in the interior of proteins and aggregates, destabilizes the packing of Val-24 and Lys-28. Analysis of the unpacked structures reveals strong EI with predominance of the Asp-23–Lys-28 interaction, in agreement with studies of molecular modeling of full-length $A\beta$ fibrils. The binary nature of the EI involving Lys-28 provides a mechanistic explanation for the linkage of amino acid substitutions at Glu-22 with Alzheimer's disease and cerebral amyloid angiopathy. Substitutions may alter the frequency of Glu-22 or Asp-23 involvement in contact formation and affect the stability of the folding nucleus formed in the $A\beta(21-30)$ region.

molecular dynamics | Alzheimer's disease | nucleation | protein folding

Alzheimer's disease (AD) is a neurodegenerative disorder characterized pathologically by the extracellular deposition of amyloid fibrils and the intracellular formation of neurofibrillary tangles. Amyloid fibrils are composed of the amyloid β -protein ($A\beta$), which exists predominantly *in vivo* as a 40- or 42-residue protein produced by cleavage from the $A\beta$ precursor. Amyloid fibrils were shown to be neurotoxic (1), but subsequent biophysical, biological, and clinical data indicated that smaller assemblies also were neurotoxic (2–4). More recent studies addressed the toxicity of soluble oligomeric $A\beta$ assemblies (5–9). Taken together, these data suggest that small oligomers, not fibrils, may be the proximate toxin that causes neurodegeneration. An attractive strategy for drug development suggests itself: Prevent the formation of toxic oligomers. To do so, knowledge of the structure and dynamics of $A\beta$ monomer under conditions that favor oligomerization is necessary.

Recent limited proteolysis experiments on $A\beta(1-40)$ and $A\beta(1-42)$ identified protease-resistant segments under conditions favoring oligomerization (10). A 10-residue segment, Ala-21–Ala-30, was highly resistant to proteolytic attack, indicating the presence of a folded structure. Lazo *et al.* (10) postulated that this structure nucleates the intramolecular folding of $A\beta$ monomer. Notably, the homologous decapeptide $A\beta(21-30)$ showed similar protease resistance when studied in monomeric solution, lending support to the hypothesis. Determination of the solution structure of $A\beta(21-30)$ by NMR yielded two families of struc-

tures containing a turn-like motif centered at residues Gly-25–Ser-26. Putative stabilization factors are (i) intrinsic turn propensities of Gly-25, Ser-25, and Asn-27; (ii) hydrophobic interaction between Val-24 and the butyl portion of the Lys-28 side chain; and (iii) electrostatic interactions (EI) between Glu-22 and Lys-28 or between Asp-23 and Lys-28. These latter interactions defined the two families and led to the question, Which of the two families of structures is preferred? Lazo *et al.* also postulated that partial unfolding of the Ala-21–Ala-30 region may be necessary for the subsequent fibrillization of $A\beta$. How might this unfolding occur?

To address these questions, we used an *in silico* approach to visualize the folding/unfolding of $A\beta(21-30)$. Computer simulations, particularly molecular dynamics (MD) simulations, can provide information that cannot be obtained by experimental methods, including conformational transitions that occur at every step along the folding/unfolding pathway. MD simulations also can contribute to a better understanding of the forces (e.g., electrostatic versus hydrophobic) controlling protein folding. Discrete MD (DMD) combined with a coarse-grain protein model has been used to study protein folding (11–13) and aggregation (14–17, 22). We present here results of studies of the conformational dynamics of $A\beta(21-30)$ folding obtained by using DMD simulations in conjunction with a united-atom model and implicit solvent. The results agree with NMR-determined structures for monomeric $A\beta(21-30)$ in aqueous solution, provide a theoretical basis for understanding the pathologic effects of mutations at position 22, and suggest that our model will be useful for *in silico* testing of mechanistic hypotheses about $A\beta$ folding and its therapeutic control.

Methods

Protein Model. Our united-atom model, which is an extension of a recently used DMD model (18), represents all protein atoms except hydrogen. Of relevance to our study are the atomic interactions implemented, namely (i) backbone hydrogen bonds, (ii) effective interactions mimicking EI between charged atoms of the side chains, and (iii) implicit solvent effects mediated by hydrophobic interactions between side chain atoms.

MD. We performed DMD simulations and monitored the time evolution of $A\beta(21-30)$ trajectories. We simulated the conformational changes of $A\beta(21-30)$ for 10^6 time units (≈ 50 ns with the selected energy scale). This time span is much smaller than typical folding times of isolated hairpins in solution (19). However, we can effectively sample the conformational space of $A\beta(21-30)$ because of our implicit treatment of solvent and hydrogen atoms, thus increasing the conformational freedom of the peptide and reducing the complexity of the energy landscape (20). We simulated $A\beta(21-30)$ folding at six different EI

Abbreviations: $A\beta$, amyloid β -protein; AD, Alzheimer's disease; MD, molecular dynamics; DMD, discrete MD; EI, electrostatic interaction(s); EIS, EI strength; SASA, solvent-accessible surface area.

[†]To whom correspondence should be addressed. E-mail: jmborr@bu.edu.

© 2005 by The National Academy of Sciences of the USA

Table 1. 1-loop and 2-loop radii for amino acids of the distal hairpin of the c-Crk Src homology 3 (SH3) domain

	c-Crk SH3(173–178)	
	1-loop, r_1	2-loop, r_2
$i - 1$	5.9	30.0
i	6.4	13.0
$i + 1$	3.9	5.0
$i + 2$	3.9	4.4
$i + 3$	4.0	7.5
$i + 4$	5.1	17.0

strengths (EIS) in the range of 0.00–2.50 kcal/mol (1 cal = 4.18 J) and recorded $N = 2,000$ conformations for each trajectory.

Structural Determinants. We characterized the structures that $A\beta(21-30)$ adopts in the course of the simulation by computing (i) the propensity of each amino acid to be at the center of a loop (see below); (ii) the combined solvent-accessible surface area (SASA) (21) of the Val-24 and Lys-28 side chains, which assumes a much smaller area in the $A\beta(21-30)$ loop conformation centered at Ser-26 than in a random coil or in a loop conformation centered at a residue other than Ser-26; (iii) average distances and deviations between all pairs of C^α atoms; and (iv) the α angle, defined as the angle between the vector \vec{v} joining C^α and N^ϵ atoms of Lys-28 and the plane generated by the C^α atoms of Val-24, Ser-26, and Lys-28. α is positive if the projection of \vec{v} onto the normal vector to the loop-plane, \vec{n} , is positive (Fig. 2*b Inset*). The Lys-28 side chain points above the plane for positive α and below the plane for negative α .

We defined the k -loop radius for amino acid i as the radius of the circle passing through the C^α atoms of amino acids ($i - k$), i , and ($i + k$). We computed for each amino acid the 1-loop ($k = 1$) and the 2-loop ($k = 2$) radii during the simulation span and obtained the histograms of 1- and 2-loop radii. The 1-loop radius depends only on the local (Φ and Ψ) angles. Small 1-loop values indicate a preference for a local sharp bend or a part of a turn, and large values indicate a preference for locally extended conformations. Small 2-loop values indicate a preference of the amino acid to be at the center of a loop comprising five amino acids, which could be classified either as a turn or as an unstructured loop.

We computed 1-loop and 2-loop radii for the distal hairpin of c-Crk Src homology 3 domain protein (residues 173–178), which we take as a model hairpin (Table 1). Residues in the turn are labeled as i , ($i + 1$), ($i + 2$), and ($i + 3$). Residues ($i + 1$) and ($i + 2$) show the lowest 1-loop and 2-loop radii and serve as a reference for our results with the $A\beta(21-30)$ simulations (Table 2).

Results

In preliminary studies of $A\beta(21-30)$, we used a simplified, four-bead model with amino acid-specific hydrophobic interactions (17) and effective EI. $A\beta(21-30)$ folding studied with this model indicated a bend in the Gly-25–Asn-27 region only under strong EI ($\cong 4$ kcal/mol) (data not shown). However, it is not clear if and how such a strong EI can occur when charged amino acids are exposed to the solvent. A more sophisticated model was needed to test the nature of $A\beta(21-30)$ folding at the atomic level; therefore, we adopted the united-atom model with implicit solvent.

Relaxation Time. Fluctuations of the potential energy at equilibrium conditions take $\approx 16 \times 10^3$ computer time steps to relax. During this time span, we recorded ≈ 32 conformations. Thus, the number of statistically independent measurements in our

Table 2. 1-loop and 2-loop radii for amino acids of the distal hairpin of the $A\beta(21-30)$

	$A\beta(21-30)$	
	1-loop, $r_1 \pm \sigma(r_1)$	2-loop, $r_1 \pm (r_2)$
Glu-22	6.1 ± 0.9	—
Asp-23	5.1 ± 1.0	8.6 ± 4.3
Val-24	4.7 ± 0.9	10.2 ± 8.7
Gly-25	6.0 ± 1.6	13.4 ± 14.4
Ser-26	6.3 ± 0.9	8.3 ± 2.5
Asn-27	5.1 ± 1.1	8.3 ± 4.4
Lys-28	5.2 ± 1.2	10.9 ± 5.2
Gly-29	5.6 ± 1.6	—

Data are shown as mean \pm SD. Standard error of the average equals the SD divided by $\sqrt{N_{\text{idp}}} = 7.9$.

simulation, N_{idp} , is the total number of measurements, N ($N = 2,000$), divided by the number of consecutive correlated measurements, $N_{\text{idp}} = N/32 \cong 62$ (23).

1-Loop and 2-Loop Radii. We first simulated $A\beta(21-30)$ dynamics without the hydrophobic and electrostatic components of the force field. Under these conditions, Val-24 and Ser-26 have the smallest and biggest average 1-loop radii, respectively, and both values are bigger than the typical 1-loop radii of a β -turn (see *Methods* and Table 2). The peptide tends to bend in the immediate vicinity of Val-24 and tends to adopt more locally extended conformation around Ser-26. Gly-25 and Gly-29 have the biggest standard deviations of 1-loop radii, reflecting the wider range of allowed Φ and Ψ values for Gly. Regarding the average 2-loop radii (Table 2), Ser-26 and Asn-27 display the two smallest values. The emerging picture for the preferred peptide conformation under no hydrophobicity and electrostatics interactions is a broad loop with the center at Ser-26 and Asn-27. The loop is broad because of the large 1-loop radius for Ser-26.

SASA. Under conditions of no hydrophobicity and irrespective of the EIS, the SASA distribution shows a single peak corresponding to the sum of the separate SASA values for solvent-exposed Val-24 and Lys-28 (≈ 350 Å²) (Fig. 1*a*, peak A). “Switching on” hydrophobic interactions results in an additional peak with a smaller SASA (≈ 245 Å², a 30% decrease) (Fig. 1*b*, peak B). We denote the set of conformations with SASA values within peak B as conformations of class *B*. For these conformations, $A\beta(21-30)$ adopts a loop conformation centered at Ser-26 and the Val-24 propyl side chain packs against the butyl portion of the Lys-28 side chain because of an effective hydrophobic attraction. EI modulate the population of the peaks but does not shift the average SASA values (Table 3). Thus, EI affect the probability that the loop will form but do not alter the way in which Val-24 and Lys-28 pack against each other once the loop has formed. The Val-24–Lys-28 packing probability increases when we increase the EIS from 0 to 1.5 kcal/mol and then decreases upon a further EIS increase. Analysis of the unpacked conformations at a high EIS reveals the formation of contacts Glu-22–Lys-28 (23% of the cases), Asp-23–Lys-28 (48% of the cases), or both (29% of the cases). These interactions inhibit the ability of Val-24 and Lys-28 to pack against each other.

C^α – C^α Distances and σ Values. The C^α – C^α distance between Val-24 and Lys-28 when in a loop conformation (5.3 ± 0.5 Å) is 32% smaller than the distance in the absence of hydrophobicity and EI (7.8 ± 1.3 Å) (Fig. 1*c*). Once the loop forms, the EIS has no apparent effect on the C^α – C^α distance, indicating again that electrostatics does not alter the way in which Val-24 and Lys-28 pack against each other. Contacts involving either Val-24 or

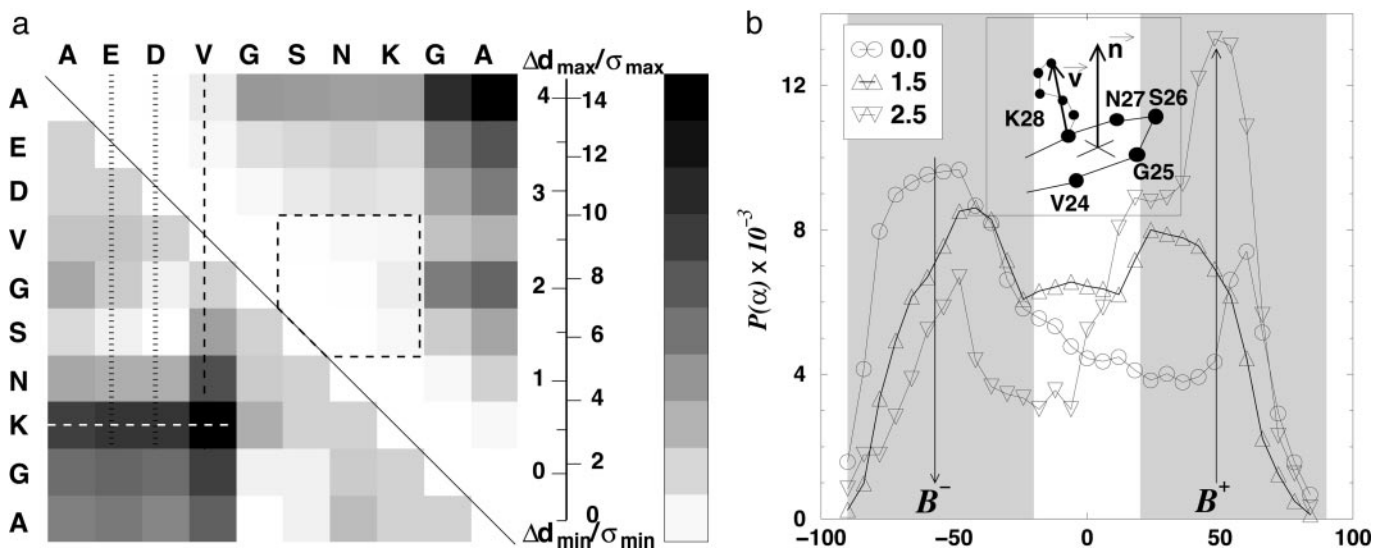


Fig. 2. Geometric characterizations of folded $A\beta$ (21–30) conformations. (a) The upper-right triangle shows the standard deviation, σ , of the C^α - C^α distance ($\sigma_{\min} = 0.0 \text{ \AA} < \sigma < \sigma_{\max} = 13.8 \text{ \AA}$) for loop conformations. Contacts within the square have minimal σ values and correspond to loop contacts. The lower-right triangle shows the average distance reduction in the C^α - C^α distance when $A\beta$ (21–30) adopts a loop conformation, ($\Delta d_{\min} = 3.8 \text{ \AA} < \Delta d < \Delta d_{\max} = 14.9 \text{ \AA}$). (b) α angle distributions for different EIS are all bimodal. The heights of the peaks follow the arrows with increasing EIS. (Inset) Vectors \vec{v} and \vec{n} for a loop conformation.

are highly flexible. Similar conclusions were made by Hou *et al.* (31) based on NMR experiments of $A\beta$ (1–40) and $A\beta$ (1–42). The finding that Lys-28 flips its orientation during the simulation is a direct consequence of the flexibility of the termini. In a hairpin configuration, a flip in the orientation of Lys-28 would require breaking three backbone hydrogen bonds, an energetically unfavorable process.

Under optimal conditions for loop stability (EI ≈ 1.5 kcal/mol), Lys-28 shows no preference to point above or below the loop plane. Lys-28 preferentially points above the loop plane at a high EIS because the bias of the Glu-22–Lys-28 interaction with Lys-28 orientation becomes prominent under these conditions. The bias is in agreement with structure calculations by Lazo *et al.* (10), who observed a Coulombic interaction between Glu-22 and Lys-28 for the model structure in which Lys-28 is pointing above the loop plane. In the structural model of $A\beta$ (1–40) fibrils proposed by Petkova *et al.* (25), Lys-28 points above the loop plane and forms a salt bridge with Asp-23. Our results suggest that Glu-22 would inhibit formation of this salt bridge because Lys-28 preferentially interacts with Glu-22 when pointing above the plane.

EI affect loop stability differently than do hydrophobic interactions. Whereas hydrophobic effects are “bulky” in nature (for instance, the whole side chain of Val-24 interacting with the butyl portion of Lys-28), EI are selective because they involve only two charged atoms. Thus, we expected that EI might modulate, but not determine, the conformational dynamics of $A\beta$ (21–30). EI stabilize the loop conformations in the range of EIS typical of interacting charged residues at the surface of proteins (32, 33)

Table 4. Percent probability of forming Glu-22–Lys-28 and Asp-23–Lys-28 contacts for conformations of class B , B^- , and B^+

EI, kcal/mol	Glu-22–Lys-28			Asp-23–Lys-28		
	B	B^-	B^+	B	B^-	B^+
0.0	7	1	5	6	1	1
1.5	28	6	16	22	7	9
2.5	48	5	21	40	11	13

(0.0–1.5 kcal/mol). Thus, we argue that EI and hydrophobic interactions cooperate to maximize the stability of loop conformations when $A\beta$ (21–30) is solvent-exposed. Glu-22 is more likely to interact with Lys-28 than is Asp-23 (Table 4) and, thus, may be more prominent in stabilizing the $A\beta$ (21–30) monomer fold.

In contrast, EI destabilize the loop conformations in the range of the EIS of interacting charged residues in the interior of proteins and aggregates (EI > 1.5 kcal/mol) (34). Asp-23 is more likely to interact with Lys-28 than is Glu-22, inhibiting the ability of Lys-28 to pack against Val-24 (Fig. 3a). Thus, we hypothesize that before fibril formation, the region $A\beta$ (21–30) partially unfolds, disrupting the Val-24–Lys-28 contact. The observed prevalence of the Asp-23–Lys-28 interaction is in agreement with molecular models of fibrils formed by full-length $A\beta$ (25) and $A\beta$ (16–35) (27), which show stabilization through Asp-23–Lys-28 interactions and no Glu-22–Lys-28 interaction or Val-24–Lys-28 packing. In recent kinetics experiments, Sciarretta *et al.* (35) observed an increase of three orders of magnitude of fibrillogenesis upon stabilization of the Asp-23–Lys-28 interaction by means of an engineered lactam bridge. This observation led the authors to suggest that stabilization of the salt bridge Asp-23–Lys-28 in the core of the fibril may be the rate-limiting step in the process of fibril formation. A hypothesis that may explain this observation is a desolvation barrier upon burial of Asp-23 inside the core of the protofibril. This hypothesis rationalizes the observed increase in the rate of fibril formation (36) associated to the familial AD mutation Asp-23–Asn (Iowa mutation). The desolvation barrier for Asn may be lower than that for Asp, and Asn-23 still can form a stable hydrogen bond with Lys-28 in the core of the fibril.

Increases in the rate of fibril growth also are observed in peptides with amino acid substitutions linked to other familial AD mutations, including Glu-22–Gly (Arctic), Glu-22–Gln (Dutch), and Glu-22–Lys (Italian). However, the desolvation hypothesis barrier does not hold when applied to Glu-22, because Glu-22 is solvated in the model fibril (25). Our results suggest a different mechanism for the “protective” role of Glu-22. If fibril formation requires a rearrangement of the $A\beta$ (21–30) region involving denaturation of the Val-24–Lys-28

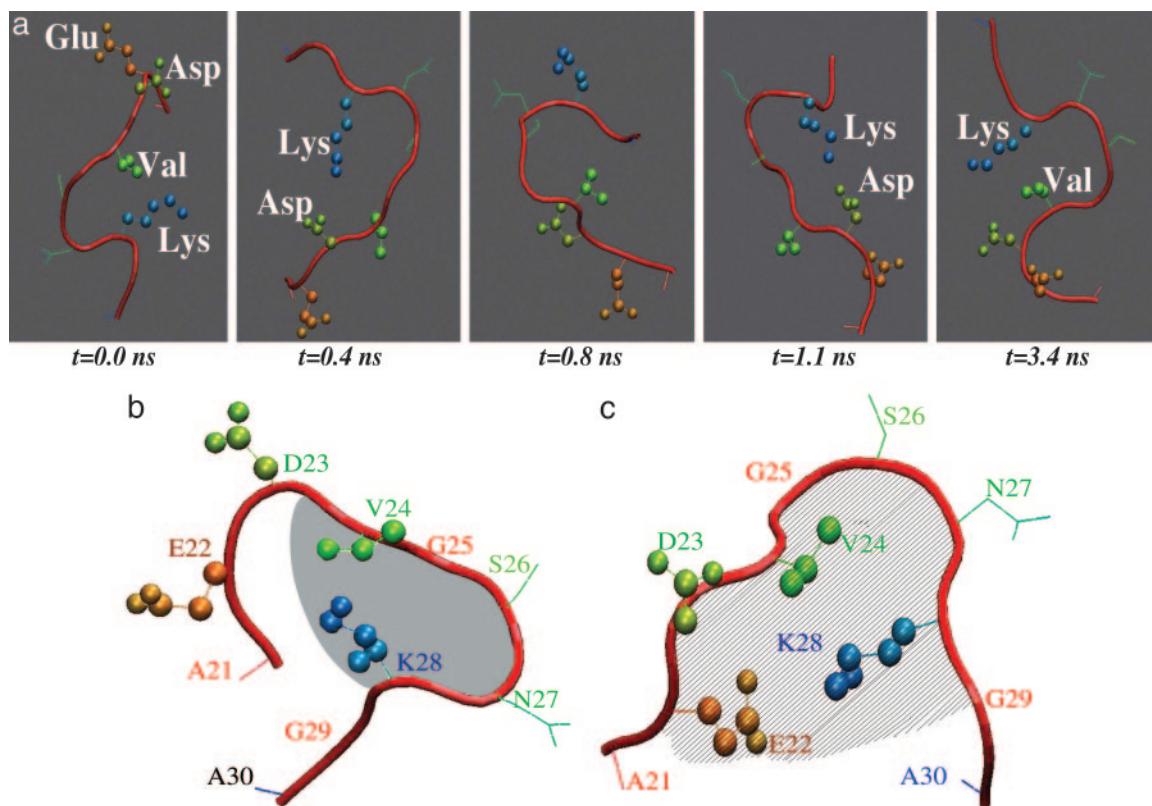


Fig. 3. Representative A β (21–30) conformations. (a) Series of alternate Val-24–Lys-28 unpacking/packing events due to formation of transient Asp-23–Lys-28 contact. (b) Representative loop conformation of class B⁻. Lys-28 side chain points opposite to the normal vector of the loop plane. (c) Representative conformation for class B⁺. Glu-22 is close to Lys-28 in this conformation. Lys-28 points along the normal vector to the loop plane.

fold, perturbations in loop stability could enhance or block fibril formation. A substitution of Glu-22 by a nonnegatively charged amino acid could enhance the model fibril formation through two different mechanisms: (i) decrease of loop stability and subsequent increase in the population of aggregation-prone unpacked conformations and (ii) increase in the rate of Asp-23–Lys-28 contact formation because Glu-22 no longer competes with Asp-23 for a stable interaction with Lys-28.

Summary

We employed discrete MD and a united-atom model to visualize the conformational dynamics of A β (21–30), a region of A β hypothesized to be the nucleation center of A β monomer folding. Simulations at equilibrium conditions reveal a stable loop structure in the central region (Val-24–Lys-28), which is stabilized by hydrophobic interactions, and a high degree of

flexibility in other areas. Correlation of perturbations of loop stability with changes in the electrostatic component of the force field provide an energy-based interpretation for the effects of familial AD mutations causing amino acid substitution at Glu-22. Our simulations are consistent with experimental studies of A β (21–30) and provide mechanistic insight into how conformational changes in the structure of the A β monomer may affect peptide self-assembly and aggregation.

We thank F. Ding and N. V. Dokholyan (University of North Carolina, Chapel Hill) for providing the DMD source codes and for helpful discussions and Gal Bitan and Samir Maji for continued intellectual support. This work was supported by a fellowship from the Bechtel Foundation (to J.M.B.); National Institutes of Health Grants R21-AG-D23661 (to H.E.S.), NS38328 (to D.B.T.), NS44147 (to D.B.T.), and AG18921 (to D.B.T.); and a grant from the Foundation for Neurologic Diseases (to D.B.T.).

- Pike, C. J., Walencewicz, A. J., Glabe, C. G. & Cotman, C. W. (1991) *Brain Res.* **563**, 311–314.
- Walsh, D. M., Lomakin, A., Benedek, G. B., Condron, M. M. & Teplow, D. B. (1997) *J. Biol. Chem.* **272**, 22364–22372.
- Harper, J. D., Wong, S. S., Lieber, C. M. & Lansbury, P. T. (1997) *Chem. Biol.* **4**, 119–125.
- Lambert, M. P., Barlow, A. K., Chromy, B. A., Edwards, C., Freed, R., Liosatos, M., Morgan, T. E., Rozovsky, I., Trommer, B., Viola, K. L., et al. (1998) *Proc. Natl. Acad. Sci. USA* **95**, 6448–6453.
- Yong, W., Lomakin, A., Kirkitadze, M. D., Teplow, D. B., Chen, S. H. & Benedek, G. B. (2002) *Proc. Natl. Acad. Sci. USA* **99**, 150–154.
- Walsh, D. M., Klyubin, I., Fadeeva, J. V., Cullen, W. K., Anwyl, R., Wolfe, M. S., Rowan, M. J. & Selkoe, D. J. (2002) *Nature* **416**, 535–539.
- Kayed, R., Head, E., Thompson, J. L., McIntire, T. M., Milton, S. C., Cotman, C. W. & Glabe, C. G. (2003) *Science* **300**, 486–489.
- Nilsberth, C., Westlind-Danielsson, A., Eckman, C. B., Condron, M. M., Axelman, K., Forsell, C., Stenh, C., Luthman, J., Teplow, D. B., Younkin, S. G., et al. (2001) *Nat. Neurosci.* **4**, 887–893.
- Klein, W. L., Stine, W. B. & Teplow, D. B. (2004) *Neurobiol. Aging* **25**, 569–580.
- Lazo, N. D., Grant, M. A., Condron, M. C., Rigby, A. C. & Teplow, D. B. (2005) *Protein Sci.*, in press.
- Zhou, Y. Q. & Karplus, M. (1997) *Proc. Natl. Acad. Sci. USA* **94**, 14429–14432.
- Borreguero, J. M., Dokholyan, N. V., Buldyrev, S. V., Shakhnovich, E. I. & Stanley, H. E. (2002) *J. Mol. Biol.* **318**, 863–876.
- Borreguero, J. M., Ding, F., Buldyrev, S. V., Stanley, H. E. & Dokholyan, N. V. (2004) *Biophys. J.* **87**, 521–533.
- Ding, F., Dokholyan, N. V., Buldyrev, S. V., Stanley, H. E. & Shakhnovich, E. I. (2002) *Biophys. J.* **83**, 3525–3532.
- Smith, A. V. & Hall, C. K. (2001) *J. Mol. Biol.* **312**, 187–202.
- Peng, S., Ding, F., Urbanc, B., Buldyrev, S. V., Cruz, L., Stanley, H. E. & Dokholyan, N. V. (2004) *Phys. Rev. E Stat. Phys. Plasmas Fluids Relat. Interdiscip. Top.* **69**, 041908.

17. Urbanc, B., Cruz, L., Yun, S., Ding, F., Buldyrev, S. V., Bitan, G., Teplow, D. B. & Stanley, H. E. (2004) *Proc. Natl. Acad. Sci. USA* **101**, 17345–17350.
18. Ding, F., Buldyrev, S. V. & Dokholyan, N. V. (2004) *Biophys. J.* **88**, 147–155.
19. Eaton, W. A., Muñoz, V., Hagen, J. S. J., Jas, G. S., Lapidus, L. J., Henry, E. R. & Hofrichter, J. (2000) *Annu. Rev. Biophys. Biomol. Struct.* **29**, 327–359.
20. Onuchic, J. N., Nymeyer, H., García, A. E., Chahine, J. & Socci, J. N. (2000) *Adv. Protein Chem.* **53**, 87–152.
21. Sanner, M. F., Olson, A. J. & Spehner, J. (1996) *Biopolymers* **38**, 305–320.
22. Urbanc, B., Cruz, L., Ding, F., Sammond, D., Khare, S., Buldyrev, S. V., Stanley, H. E. & Dokholyan, N. V. (2004) *Biophys. J.* **87**, 2310–2321.
23. Pathria, R. K. (1996) *Statistical Mechanics* (Butterworth–Heinemann, Boston), 2nd Ed.
24. D’Ursi, A. M., Armenante, M. R., Guerrini, R., Salvadori, S., Sorrentino, G. & Picone, D. (2002) *J. Med. Chem.* **47**, 4231–4238.
25. Petkova, A. T., Ishii, Y., Balbach, J. J., Antzutkin, O. N., Leapman, R. D., Delaglio, F. & Tycko, R. (2002) *Proc. Natl. Acad. Sci. USA* **99**, 16742–16747.
26. Li, L., Darden, T. A., Bartolotti, L., Kominos, D. & Pedersen, L. G. (1999) *Biophys. J.* **76**, 2871–2878.
27. Ma, B. & Nussinov, R. (2002) *Proc. Natl. Acad. Sci. USA* **99**, 14126–14131.
28. Hutchinson, E. G. & Thornton, J. M. (1994) *Protein Sci.* **3**, 2207–2216.
29. Ramírez-Alvarado, M., Kortemme, T., Blanco, F. J. & Serrano, L. (1999) *Bioorg. Med. Chem.* **7**, 93–103.
30. Espinosa, J. F., Muñoz, V. & Gellman, S. H. (2001) *J. Mol. Biol.* **306**, 397–402.
31. Hou, L., Shao, H., Zhang, Y., Li, H., Menon, N. K., Neuhaus, E. B., Brewer, J. M., Byeon, I. L., Ray, D. G., Vitek, M. P., *et al.* (2004) *J. Am. Chem. Soc.* **126**, 1992–2005.
32. Horovitz, A. & Fersht, A. R. (1992) *J. Mol. Biol.* **224**, 733–740.
33. Searle, M. S., Griffiths-Jones, S. R. & Skinner-Smith, H. (1999) *J. Am. Chem. Soc.* **121**, 11615–11620.
34. Kumar, S. & Nussinov, R. (1999) *J. Mol. Biol.* **293**, 1241–1255.
35. Sciarretta, K. L., Gordon, D. J., Petkova, A. T., Tycko, R. & Meredith, S. C. (2005) *Biochemistry*, in press.
36. Nostrand, W. E. V., Melchor, J. P., Cho, H. S., Greenberg, S. M. & Rebeck, G. W. (2001) *J. Biol. Chem.* **276**, 32860–32866.