Communication

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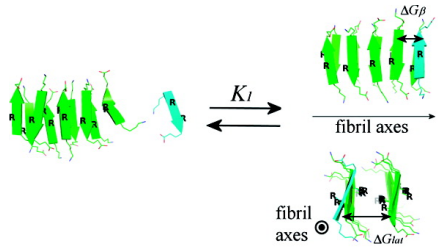

	K_1 (M^{-1})	ΔG_β (RT)	ΔG_{lat} (RT)
KFFE	$2.9 \cdot 10^4$	-5.4	-4.9
KLLE	$2.5 \cdot 10^3$	-3.3	-4.5
KVVE	$1.5 \cdot 10^4$	-4.3	-5.4

K_1 = association constant
ΔG_β = ΔG of β-sheet interaction
ΔG_{lat} = ΔG of lateral interaction

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Quantitative Prediction of Amyloid Fibril Growth of Short Peptides from Simulations: Calculating Association Constants To Dissect Side Chain Importance

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Amyloid fibrils are organized peptide aggregates with high β-sheet character. Their occurrence under pathological conditions, e.g., Alzheimer and Huntington disease, has gained this class of structures widespread attention.[1,2] Functional amyloid fibrils like silk[1] have been known for a long time. Recent interest has been raised in the field of materials science, i.e., for the fabrication of nanowires on amyloid fibril templates.[3,4]

An amyloid fibril is composed of multiple peptides that are linked through intermolecular β-sheet interactions, which extend along the fibril axes to form a cross-β-sheet. Perpendicular to the fibril axes the cross-β-sheets interact laterally by hydrophobic clustering of the side chains. The structural details of an amyloid fibril are sequence specific, and although the fibril structures can be solved experimentally, this still presents a challenging task.[5,6]

It appears that every peptide or protein has the ability to form amyloid fibrils; however, the propensity varies strongly for different peptides.[7] Peptides as small as four residues have been shown to possess the properties necessary to form fibrils at physiological conditions.[8] Analyses of these small peptides by computer simulations[9−12] together with a broad analysis of natural occurring mutations in disease related amyloid fibrils[13,14] show that physico-chemical interactions such as charge, hydrophobicity, and secondary structure preference direct fibril formation. Although these considerations are successfully used to make a qualitative assessment of the propensity of (part of) a peptide to form fibrils,[15−17] a quantitative assessment requires more detail.

At present, the mechanism of amyloid fibril formation is considered to be a nucleation−growth mechanism.[2,18,19] Formation of a stable nucleus precedes rapid growth of the fibril until equilibrium is reached. Although the first step is under kinetic control, fibril growth is under thermodynamic control[20−23] and can be evaluated quantitatively in terms of equilibrium properties such as association constants. Here we examined the propensity of different polypeptides for amyloid fibril growth calculated by the association constant using all-atom MD with explicit solvent.

We examined the fibril growth properties of four tetrapeptides, KFFE, KVVE, KLLE, and KAAE. Previously it was shown that KFFE and KVVE form amyloid fibrils, while KLLE and KAAE do not.[24] A dimerization study (nucleation) of these tetrapeptides showed that the fibril forming propensity of KFFE and KVVE is the result of a hydrophobic collapse. Although KLLE also benefits from this collapse it suffers from a large entropy penalty upon formation of the dimers.[9] While KLLE and KAAE clearly face a higher kinetic barrier to form a nucleus, the question remains whether they can still form full length amyloid fibrils once the nucleus is formed. Therefore we assessed the growth propensity of mature fibrils.

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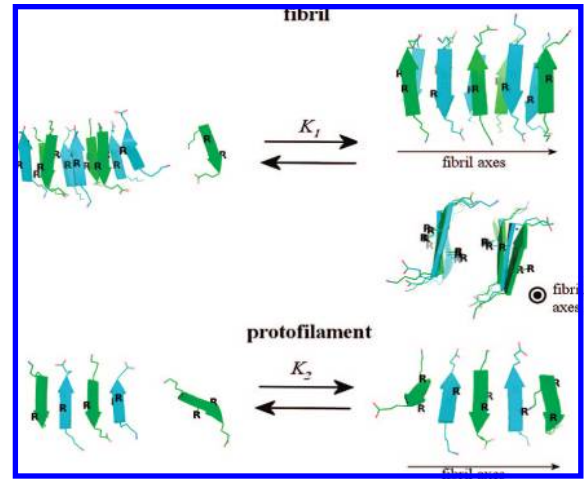

Figure 1. Association−dissociation of one peptide from a hypothetical amyloid fibril and protofilament structure. R is the side chain of F, L, or V for the KFFE, KLLE, or KVVE peptide, respectively. K_1 and K_2 are the association constants.

We started by constructing hypothetical amyloid fibrils from 10 peptides (see Supporting Information) for each tetrapeptide and tested the stability by a 10 ns standard MD simulation. The 10 peptides have been arranged in two cross-β-sheets of five peptides each that interact through a lateral interaction (Figure 1). Within this simulation the KFFE, KLLE, and KVVE fibrils appeared to be stable, while the KAAE fibril quickly converted to a random aggregate.

For the three stable fibrils we sampled the distance between the dissociated peptide and the closest cross-β-sheet bonded peptide in an umbrella sampling simulation[25] combined with replica exchange to accelerate convergence.[26,27] From this simulation we calculated the potential of mean force (PMF) that describes the association−dissociation of one peptide from the fibril (Figure 2) with the weighted histogram analysis method.[28,29] From the PMF we calculated the association constants[30] K_1, listed in Table 1. To obtain quantitative results we used the Gromos force field 56a6, parametrized to calculate accurate solvation free energies.[31]

We repeated this procedure with a hypothetical amyloid protofilament composed of five peptides in a cross-β-sheet conformation (Figure 1) to calculate PMFs (Figure 2). The corresponding association constants K_2 are listed in Table 1.

Table 1. Association Constants and Free Energies Related to Amyloid Fibril Growth

	K_1 (M^{-1})	K_2 (M^{-1})	ΔG_β (RT)	ΔG_{lat} (RT)
KFFE	$2.9 \cdot 10^4$	$2.5 \cdot 10^2$	-5.4	-4.9
KLLE	$2.5 \cdot 10^3$	26	-3.3	-4.5
KVVE	$1.5 \cdot 10^4$	73	-4.3	-5.4

10.1021/ja806606y CCC: $40.75

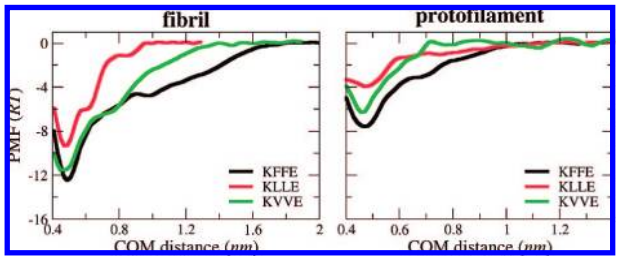

Figure 2. Potential of mean force associated with the association−dissociation of one peptide. The COM distance is between the dissociated peptide and the closest cross-β-sheet bonded peptide.

If we assume the number of fibrils in solution to be constant the critical monomer concentration for fibril formation is $1/K_1$. We found critical monomer concentrations of 34, 400, and 67 μM for KFFE, KLLE, and KVVE, respectively. For a 200 μM monomer concentration, used in the experiments of Tjernberg et al.,[24] we expect KFFE and KVVE to form fibrils as opposed to KLLE, in very good agreement with their findings.

The association constant is related to the free-energy difference through $\Delta G = -RT \ln K$. The free-energy difference associated with the cross-β-sheet interaction ΔG_β can thus be estimated from the protofilament association constant. Similarly the fibril association constant is related to $\Delta G_\beta + \Delta G_{\text{lat}}$, with ΔG_{lat} as the free-energy difference of the lateral interaction between two peptides. ΔG_β and ΔG_{lat} are given in Table 1.

The origin of the overall negative value of ΔG_β and ΔG_{lat} is the hydrophobic collapse as indicated by the hydrophobic solvent accessible surface (SAS). We observed a reduction in the hydrophobic SAS corresponding to ΔG_β of 2.4, 2.1, and 2.0 nm^2 per peptide of KFFE, KLLE, and KVVE, respectively. For ΔG_{lat} we found a reduction of 0.6, 0.8, and 0.3 nm^2 per peptide of KFFE, KLLE, and KVVE, respectively. However, the hydrophobic collapse alone cannot explain the order of ΔG_β and ΔG_{lat}.

The propensity to form cross-β-sheets is KFFE > KVVE > KLLE (ΔG_β in Table 1). To explain this order the side chain orientation was compared between the monomer and the protofilament in addition to an evaluation of the hydrophobic SAS. KFFE then ranks first, as it has the largest burial of the hydrophobic SAS and the orientation of the F side chains does not require any adjustment (Figure 3a,b). Next, the hydrophobic SAS slightly favors KLLE over KVVE while both peptides change side chain orientation (Figure 3d,e + 3g,h), to obtain an optimal packing. For KVVE this change only results in a small entropy loss as one orientation is now favored over another, whereas for KLLE the favorable interaction resulting in the orientation preference of the monomer has to be disrupted in addition to the entropy loss.

The order for ΔG_{lat} is KVVE > KFFE > KLLE. Although both KFFE and KLLE show a larger reduction in hydrophobic SAS than KVVE, they also suffer from excluded volume effects. The excluded volume of the F and L side chains forces them to adopt a less favorable orientation (Figure 3a,c and 3d,f respectively). This is not observed for KVVE (Figure 3g,i).

The quantitative assessments made here are in very good agreement with experimental results. In addition we show that the physicochemical properties used to make a qualitative assessment are a consequence of the hydrophobic collapse (fibril growth promoting), conformational entropy, and excluded volume effects (both decreasing fibril growth propensity).

The hydrophobic collapse, conformational entropy, and excluded volume effect all contribute to both ΔG_β and ΔG_{lat}. As the relative contributions of these three interactions vary strongly for different side chains, so do the relative strengths of the cross-β-sheet and lateral interaction. This explains why the morphology and details of an amyloid fibril are sequence specific.

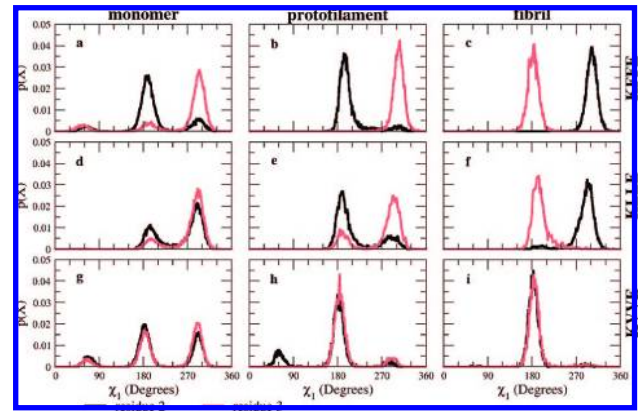

Figure 3. Side chain orientation. Probability distributions of the χ_1 dihedral angle of the KFFE, KLLE, and KVVE peptides in a monomer, protofilament, and fibril environment.

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Supporting Information Available: System, Method, and details of the simulations. This material is available free of charge via the Internet at http://pubs.acs.org.

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