Peptide folding in non-aqueous environments investigated with molecular dynamics simulations
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5 Is it possible to simulate peptide aggregation? Case study: Spontaneous aggregation and fibril stability of SIV gp- 32 peptide.

Based on: Patricia Soto, Josep Cladera, Alan E. Mark, Xavier Daura. Stability of SIV gp32 fusion peptide single layer protofibrils as monitored by molecular dynamics simulations

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Abstract

Molecular Dynamics (MD) simulations have been performed on a set of model protofibrils of different length built from chains of the twelve residue SIV N-terminal gp32 peptide. The suprastructures spontaneously twist as the whole geometry stabilizes. This result offers an atomistic picture of amyloid fibrils, polymeric aggregates associated with several diseases like Alzheimer's disease, Bovine Spongiform Encephalopathy (BSE) in cattle and Creutzfeld-Jakob disease (CJD) in humans. The simulation results provide insight into the structural features of the overall geometry that depend both on the backbone and side chain of the peptide and show the ability of the technique to study fibrils at levels that experiment cannot access. In addition, the spontaneous aggregation of the same peptide has been simulated in different environments: water, hexane and DMSO. These simulations show how that the general process of oligomerization of the SIVwt peptide into ordered β-sheets depends on the chemical environment.
5.1 Introduction

Amyloid fibrils are filamentous structures formed by peptides and proteins with widely varying sequences and lengths.\textsuperscript{136,137} Such non-covalent assemblies are at the heart of a wide variety of diseases\textsuperscript{3} such as Alzheimer's disease, Bovine Spongiform Encephalopathy (BSE) in cattle and Creutzfeld-Jakob disease (CJD) in humans. These so called protein conformational diseases are particularly intriguing because evidence is accumulating that, given the appropriate conditions, the formation of highly organized amyloid aggregates is a generic property of polypeptides whatever the source protein.\textsuperscript{3} The biophysical factors that determine whether specific peptides or proteins can form a stable amyloid suprastructure are largely unknown. In addition, high-resolution experimental data has proved difficult to obtain because of the non-crystalline and insoluble nature of the fibrils,\textsuperscript{136} although there have been some recent advances. Models have been proposed for amyloid protofilaments that consist of multilayered helically twisted $\beta$-sheets based on cryoelectron microscopy and image processing\textsuperscript{144} and on solid state NMR.\textsuperscript{52} The major features of amyloid-fibre diffraction patterns\textsuperscript{138} are consistent with a cross-$\beta$ structure.\textsuperscript{138} The similar structural features of the fibrillar forms of many different proteins suggest that they form via a common mechanism. Insight into the process of assembly of any specific system could therefore be relevant to understanding processes occurring in a range of amyloid diseases.\textsuperscript{3,136,137} Here, we report results from a series of molecular dynamics simulations in which we study the supramolecular organization of a single layer protofibril in atomic detail using realistic models for the peptide and explicit solvent.

The synthetic peptide SIVwt, corresponding to the 12-residue N-terminal region of the simian immunodeficiency virus (SIV) transmembrane glycoprotein gp32 (GVFVLGFLGFLA) is involved in triggering the fusion between the viral and the target cell membranes, a process which facilitates the entry of the virus into the cell. This 12 residue long fusion sequence is very hydrophobic, a property shared with the C-terminal segment (residues 30-40) of the A$\beta$(1-40) amyloid peptide (AIIGMLVGGVIA), which is part of the so called functional domain of the amyloid peptide, required for cytotoxicity in Alzheimer's disease. This part of the A$\beta$(1-40) peptide has been shown to adopt $\beta$-strand conformations and to form parallel $\beta$-sheets.\textsuperscript{139} Interestingly, the 12-residue SIV fusion peptide has been claimed to form aggregated $\beta$-structures based primarily on evidence from infrared spectroscopy performed under different experimental conditions.\textsuperscript{140,141} When the peptide is studied by Attenuated Total Reflectance Spectroscopy (ATR), the presence of $\beta$-aggregates can be inferred. The spectroscopic characteristics of such aggregates cannot be distinguished from those of an amyloid fibril. In both cases a typical infrared band for $\beta$-aggregates forming strong hydrogen bonds is observed. It also should be noted that the preparation of fusion peptide for the ATR studies described in Martin et al\textsuperscript{141} involves drying a solution of the peptide in dimethyl sulfoxide.
(DMSO) on the ATR crystal. This means that the infrared spectrum used to infer the presence of aggregates corresponds to a fusion peptide sample in which the concentration of the peptide is very high and the concentration of DMSO very low.

The fact that the gp32 peptide forms β-aggregates in DMSO provides a unique opportunity to study the process of fibril formation in atomic detail using molecular dynamics simulation techniques. Due both to its size and mass DMSO is a much less expensive solvent to simulate explicitly than water. This means that it is possible to use the N-terminal SIV gp32 peptide in DMSO to study the collective properties of a large scale aggregate and thus shed light on the nature of superstructure formation of amyloid fibrils.

5.2 SIVwt protofibril stability

5.2.1 Methods

In total four systems were simulated. Three were of β-sheet constructs containing 10, 20 and 30 parallel SIVwt chains, respectively. The simulation times were 100ns for the 10-chain construct (pb₁₀), and 50ns for the 20- (pb₂₀) and 30-chain (pb₃₀) constructs. The initial arrangement of the β-sheets was planar, with an average distance between facing Cα atoms of 0.4nm. The long axis of the β-sheet in this planar configuration measured ~4 nm for pb₁₀, ~8 nm for pb₂₀, and ~12 nm for pb₃₀. Each β-sheet was introduced into a periodic rectangular box of 8x(D+4)x7 nm, with D the length of the β-sheet's long axis, and solvated with DMSO. In addition to these three systems, a fourth system was simulated that consisted of 10 peptide chains in an antiparallel arrangement (ab₁₀). The purpose of this simulation was to test the stability of the protofibril as a function of the relative orientation of the peptide chains. All simulations were performed using the GROMACS software and the GROMOS 43A1 force field. The temperature and the pressure were coupled to external baths at 300 K and 1 bar with relaxation times of 0.1 and 0.5 ps, respectively. Bond lengths were constrained with the LINCS algorithm. The equations of motion were integrated with the leap-frog algorithm using a time step of 2 fs. Non-bonded interactions were evaluated with a twin-range cut-off of 0.8/1.4 nm, using a charge-group pair list that was updated every 10 time steps. A similar setup has been reported for other peptide simulations that reproduce available experimental data.

5.2.2 Results and discussion

The β-sheet with 10 parallel SIVwt chains spontaneously adopts a helical suprastructure in
the simulation (figure 5.1). After an initial period of about 20 ns in which the β-sheet twists away from the initial planar configuration, the helical twist per chain oscillates between a maximum angle of ~15° and a minimum angle of ~5°. Thus, pb₁₀ is in dynamic equilibrium involving partial unwinding and rewinding of the helical suprastructure, with an average twist angle of ~10° and an average helical pitch of ~15 nm (calculated over the interval 20-100 ns). These measurements include from the second to the seventh chain of pb₁₀, since the first and three last chains have less than five hydrogen bonds to their respective adjacent chains after 30 ns of simulation. For this fragment of six chains, the average number of interstrand hydrogen bonds is ~50, i.e., approximately 8 hydrogen bonds per strand. The β-sheets with 20 and 30 parallel SIVwt chains show a similar behavior (figure 5.1). The average twist angle is ~10° for pb₂₀ and ~9° for pb₃₀, while the average pitches are ~16 nm and ~20 nm, respectively (calculated over the time period 20-50 ns). The final structure of pb₃₀ is shown in figure 5.2. For both systems, only the chains with an average of 5 hydrogen bonds or more were included in the calculation (7-19 for pb₂₀ and 5-20 for pb₃₀). In all simulations, the extremes of the filament tend to adopt a higher twist angle than the central section (figure 5.2). Models of short fragments would thus overestimate the degree of twist. Indeed, models pb₁₀ and pb₂₀ show a slightly larger twist than pb₃₀. In some cases, short fragments at the extremes are observed to orient in a different direction relative to the main axis of the fragment (helix bending) while still maintaining a helical twist. However this does not appear to destabilize the helical suprastructure.

Figure 5.1
Twist angle per chain in simulations of pb₁₀, pb₂₀, and pb₃₀. The twist angle is calculated as \( \theta = \arccos(\mathbf{u}_m \cdot \mathbf{u}_n)/(n-m+1) \), where \( \mathbf{u} \) is the unit vector from the Cα atom of residue 1 to the Cα atom of residue 12, and m and n are the first and last chains considered (chains with a minimum of 5 interstrand hydrogen bonds): 2-7 for pb₁₀, 7-19 for pb₂₀ and 5-20 for pb₃₀.
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The supramolecular structure that develops spontaneously in the simulations of the three parallel β-sheet arrangements of SIVwt chains has been previously described as a left-handed twisted ribbon with saddle-like curvature, a structure thermodynamically more stable than helical ribbons due to the chirality of the constituent chains. In the simulations we see that there is a cooperative twist of the β-sheet until a maximum twist angle is reached. At the point of maximum stress in the geometry, there is a subsequent relaxation toward a smaller angle, followed by alternating events of rewinding and partial unwinding around an equilibrium twist angle. The twisted ribbon structure sampled in the simulations is highly dynamic.

The cross-β structure of amyloid fibrils (i.e., β-strands of the precursor polypeptide arranged perpendicular to, and ribbon like β-sheets parallel to, the fibril axis), inferred from diffraction data, may accommodate different forms of packing of the polypeptide chains. The packing in a parallel or antiparallel arrangement, for example, may to some extent depend on the sequence of the polypeptide owing to the nature of the interactions and steric constraints between side chains of neighboring chains. It has been recently suggested that increasing the amphiphilicity of the Aβ(16-22) peptide changes the β-sheet structure in the fibrils from antiparallel to parallel. To check whether the stability and twisting of the SIVwt protofibril was dependent on a parallel or antiparallel orientation of the peptide chains, a simulation of 10 antiparallel chains in DMSO (ab10) was performed. In the resulting trajectory, a rapid loss of interchain hydrogen bonds was observed. After 50 ns, only two adjacent chains have more than five interstrand hydrogen bonds (results not shown). Therefore, the results suggest that the parallel β-sheet arrangement is in this case dictated by specific (non-polar) side-chain/side-chain interactions.

This is the first example of an MD simulation of the spontaneous twisting of a protofibril into an equilibrium twisted-ribbon suprastructure. In a recent study, Fishwick et al observed the spontaneous twisting of two 20-membered β-sheets formed by glutamine-rich 11-peptides, in 100ps MD simulations using an implicit solvent model. The extremely short time of the simulations puts serious doubts on the reliability of the results. In previous computational studies of amyloid-protofibrils the ribbon twist was imposed on the initial structure and the short simulations performed served only to relax the system from its initial configuration. Simulations aimed at the spontaneous aggregation of this and other peptides into β-sheet structures are described below. It is hoped that by combining this two types of studies, it will be possible to explore the initial stages of protofibril formation at the atomic level.
Figure 5.2

Configuration of the protofibril pb30 at time 50 ns. The last chain has dissociated. The average twist angle per chain is 9° and the average pitch is 20 nm (20-50 ns interval). The pitch $P$ is calculated as $P = N_c \times D_c$, where $N_c$ is the number of chains per 360° turn and $D_c$ is the translation per chain along the β-sheet's long axis. $N_c$ and $D_c$ are calculated for chains 2-7 of pb10, 7-19 of pb20 and 5-20 of pb30 (all three fragments have an average of 8 interstrand hydrogen bonds per chain).

5.3 Spontaneous aggregation of SIVwt

5.3.1 Methods

In total seven simulations were performed. Four were of SIVwt chains solvated in hexane (one trajectory of 90ns, trajectory A, and the others of 30 ns each, trajectories B, C, and D), two in DMSO (one trajectory of 50 ns and the other of 20 ns, trajectories E and F) and one in water (50 ns). Initially the SIVwt chains were randomly distributed inside the simulation box (figure 5.3). Each arrangement was introduced into a periodic dodecahedron box with an initial minimum distance between peptide atoms and box walls of 0.8 nm, and solvated in hexane, in DMSO,142 and in water. All simulations were performed using the GROMACS software85,86,131 and the GROMOS 43A1 force field.65,66 The temperature and the pressure
were coupled to external baths at 300 K and 1 bar with relaxation times of 0.1 and 0.5 ps, respectively. Bond lengths were constrained with the LINCS algorithm. The equations of motion were integrated with the leap-frog algorithm using a time step of 2 fs. Non-bonded interactions were evaluated with a twin-range cut-off of 0.8/1.4 nm, using a charge-group pair list that was updated every 10 time steps. A similar setup has been reported for other peptide simulations that reproduce available experimental data.

Hydrogen bonds: A hydrogen bond is defined to exist if the donor - acceptor distance was less than 0.275 nm and the hydrogen - donor - acceptor angle less than 60°.

Side chain contacts: A contact between two atoms was defined to exist if the distance between them is less than 0.6 nm.

Figure 5.3
Initial random distribution of SIVwt chains in the simulation box.

5.3.2 Results and discussion

Aggregation in hexane occurs rapidly in the simulations (see figure 5.4). Stabilization of the number of hydrogen bonds (after ~20 ns) and side chain contacts (~40 ns) motivated us to monitor shorter trajectories. In the four trajectories the qualitative behavior observed is the same (see figure 5.5): An initial collapse dominated by the formation of backbone hydrogen bonds is followed by a slower stabilization of side chain interactions. Strikingly, in all simulations the SIVwt chains assemble into ordered (parallel) β-sheets (see figure 5.6). The fact that most of the β-strands orient in a parallel arrangement supports our previous hypothesis on protofibril stability according to which for hydrophobic sequences, side chain packing highly influences the relative orientation between adjacent β-strands (see section 5.2.2). No α-helical content was observed in any of the trajectories.

In the four trajectories in hexane, a common overall mechanism is observed for the assembly of peptide the chains. The precise paring varies however on the process observed and is not
dominated by the initial conditions. When extended (random coil) chains interact, they rapidly dimerize and such dimers assemble into a core aggregate. When individual chains fold into $\beta$-hairpins, the folded chain is incorporated more slowly into the aggregate core.

Aggregation in water shows a similar overall behavior (see figure 5.5) in terms of the backbone collapse and further side chain stabilization. However, the distinction between the contributions from the backbone and the side chains is not as clear as in hexane. This is most likely because the dynamics of peptide chains is slower in water. The ordering of the peptide chains into $\beta$-sheets is less than in hexane. It may be possible that chains will further rearrange in time and exhibit higher proportion of $\beta$-sheet. It is interesting to note that the evolution of the backbone and side chain interactions during aggregation does not correlate directly with $\beta$-sheet formation (see figure 5.7). This suggests that other indicators should be investigated to account for $\beta$-sheet population (work currently under development).

**Figure 5.4**

Time series of main chain to main chain hydrogen bonds and inter-peptide side chain contacts (atom-atom distance less than 0.6 nm). Data shown for one trajectory in hexane and in water. The other trajectories in hexane exhibit a similar behavior.

The behavior of the SIVwt chains in each solvent was different although the peptide concentration was the same. Overall, the simulations in hexane and water show
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oligomerization of the peptide chains while the simulations in DMSO do not. These conclusions are based on the number of backbone interchain hydrogen bonds and the extent of side chain interchain contacts calculated along the trajectories (see figure 5.4). The value of these indicators increases as a function of time in the trajectories run in water and in hexane while decreasing in DMSO. In hexane, interchain interactions are stabilized due to the non-polar nature of the solvent. In particular, this facilitates the formation of inter-peptide hydrogen bonds. In water, although there is competition for hydrogen bond formation between the solvent and the peptide chains, oligomers are formed. Nevertheless, these interactions stabilize more slowly than in hexane, at least in the simulations. In contrast, there is no increase in the number of hydrogen bonds nor side chain contacts for the simulations in DMSO. This observation is rationalized, considering that the concentration of peptides is the same, as being a consequence of the aprotic nature of DMSO and of the viscosity higher than water and hexane.

Figure 5.5

Number of inter-peptide side chain contacts versus number of main chain to main chain hydrogen bonds. Note the different trend in the graph of the trajectory in water (see discussion in the text)

To investigate the energetic contributions to oligomerization, the non-bonded terms of the interaction energy function were investigated (see figure 5.8). The values obtained correspond to intermolecular Coulomb and Lennard-Jones interactions and are interpreted qualitatively and not quantitatively. For the simulations in hexane, figure 5.8 indicates that there are favorable electrostatic interactions between chains. This combined with the
repulsion for solvent molecules gives a net enthalpic contribution to favor oligomeric states. In addition, due to the nature of the interactions peptide-solvent, the translational entropy of the chains will easily decrease. Under these conditions, an aggregate is a thermodynamically favorable state.

Figure 5.6
Snapshots of trajectories A and D in hexane and trajectory in water. The behavior of trajectories B and C in hexane is similar to the one depicted in this figure.

The energetic trend observed in water is different. Although hydrophobic effect would induce aggregation, the electrostatic competition between solvent and peptide chains is strong and the net enthalpic contribution leads to an oligomeric state that is likely to be a transient intermediate to a stable state. Nevertheless, from the simulations it is not possible to predict the characteristics of such stable state.

This set of simulations demonstrate the ability of molecular dynamics simulation techniques to model initial states of peptide aggregation and suggest a common mechanism for aggregation independent of the solvent. Details into specific β-sheet formation are, however, highly dependent on the environment and are linked to a step by step ordering of pre-assembled units.
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Figure 5.7
The number of residues in β-sheet secondary structure according to the DSSP algorithm.122 The behavior of all trajectories in hexane is similar. Therefore the results only of trajectory A are shown.

Figure 5.8
Non bonded terms of the interaction function. This corresponds to the sum of Coulomb and Lennard-Jones interactions between peptide chains and between peptide chains and the solvent. The behavior of all trajectories in hexane is similar. Therefore the results only of trajectory A are shown.
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