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published in

*From Computational Biophysics to Systems Biology (CBSB07),
Proceedings of the NIC Workshop 2007,*
Ulrich H. E. Hansmann, Jan Meinke, Sandipan Mohanty,
Olav Zimmermann (Editors),
John von Neumann Institute for Computing, Jülich,
NIC Series, Vol. 36, ISBN 978-3-9810843-2-0, pp. 275-278, 2007.

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Aggregation of Fragments of the Islet Amyloid Polypeptide as a Phase Transition: A Cluster Analysis

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Aqueous solutions of amyloidogenic polypeptides undergo a phase separation into a water-rich and peptide-rich (fibrillar) phase already at very small peptide concentrations. Studies of the fibrillar phase (stable phase or its metastable intermediates) can be performed by simulations of solution with peptide concentrations deeply inside the two-phase region. In such states, clustering (aggregation) of molecules is extremely sensitive to the system size. We performed MD simulations of 12 amyloidogenic fragments of IAPP (residues 15-19) in liquid water, starting from different random configurations. Analysis of peptide clustering and aggregation evidences features typical for a phase separation. In some simulation runs, the formation of a stable aggregate is hampered by the small system size. We propose to use a clustering analysis to select the configurations relevant for macroscopic systems.

Formation of amyloid fibrils may be the cause of various diseases. Understanding of the driving forces and molecular mechanisms of the fibril formation should elucidate the possibilities to inhibit formation of toxic amyloid fibrils. Aggregation of polypeptides is a cooperative process, which occurs when their concentration in water exceeds some critical value.¹ The critical concentration depends on the characteristics of the considered polypeptide, temperature, ionic strength, pH, etc. The kinetics of aggregation speeds up with increasing peptide concentration and can be facilitated by adding seeds of the peptide-rich phase. All these features are typical for a first-order phase transition in binary mixtures. In the two-phase region the system is separated into a water-rich phase with the critical peptide concentration mentioned above and a peptide-rich phase, which appears as well-ordered solid-like fibrillar structure.

Currently, simulation studies of the fibrillar phase (as stable phase or its metastable intermediates) can be performed by simulations of an aqueous solution with constant peptide concentrations deeply inside the two-phase region. In these states, clustering of molecules may be drastically distorted by the finite size of the simulation system,² however, and may even hamper formation of stable peptide aggregates. To explore the importance of this effect in simulation studies of peptide aggregation, we have simulated an aqueous solution of amyloidogenic fragments of IAPP (residues 15-19) at $T = 330$ K and $P = 1$ atm pressure. The concentration of peptides in the system was about 120 mg/ml (12 peptides and about 3070 to 3087 water molecules), i.e., deeply inside the expected immiscibility region. Simulations were carried out with the GROMACS-3.2.1 software package and TIP3P water model. Several starting configurations were generated by random insertion of the peptides in a cubic box of 125 nm^3 such that each peptide is at least 7 \AA away from its nearest neighbor. The duration of production runs taken were from 50 to 150 ns.

Two peptides were considered as belonging to the same cluster, when the number of the contacts between the atoms of the side chain hydrophobic residues (hydrophobic contacts) exceeds 20. Such contact exists, when a distance between two heavy atoms involved

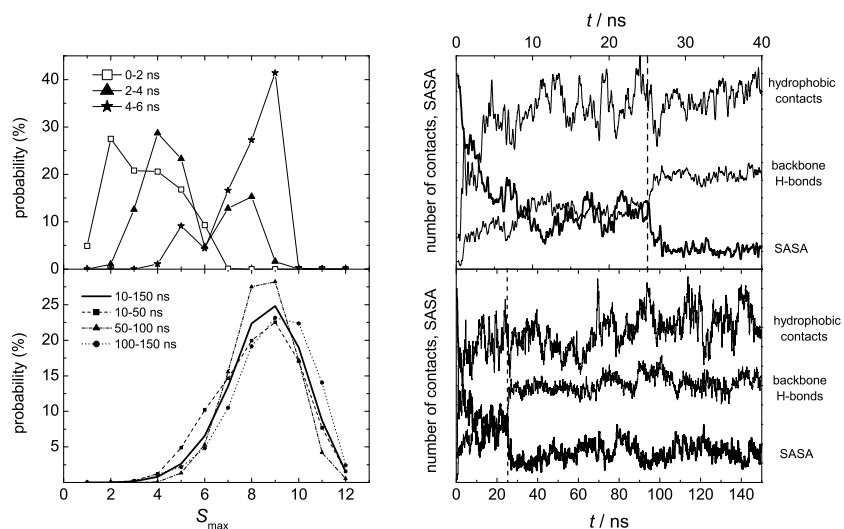


Figure 1. Left panel: probability distributions of the size S_{max} of the largest peptide cluster, averaged over various time intervals of the simulation run. Right panel: Time dependencies of the number of hydrophobic contacts, number of backbone H-bonds and SASA. The equilibration period (up to about 25 ns, indicated by the vertical line) is shown in an enlarged time scale in the upper right panel.

does not exceed the sum of their VdW radii plus 2.8 Å. Additionally, we have analyzed H-bonded peptide clusters, with at least 2 inter-peptide backbone H-bonds being a connectivity criterion. Clustering of the peptides was characterized by calculation of the distribution of sizes S of the clusters, measured as a number of peptides in a cluster. This distribution gives, in particular, the average cluster size and allows analysis of the largest peptide cluster. The secondary structure to each residue is assigned using SEGNO.³ Each peptide is assigned to a particular secondary structure using the criteria that at least three consecutive residues should have the same secondary structure, and no other consecutive secondary structure is present. Two peptides are considered to be forming β -sheets if both peptides are assigned as β -strands and if they are connected by at least two backbone H-bonds.

Upon equilibration, the number of inter-peptide hydrophobic contacts achieve saturation already at $t < 10$ ns (see right panel in Fig. 1). During this time interval, the distribution of the size S_{max} of the largest cluster evolves, but in the time interval 10 ns $< t < 150$ ns, it remains almost intact (see left panel in Fig. 1). The number of inter-peptide H-bonds and, accordingly, the solvent accessible surface area (SASA) require a longer simulation time to achieve their equilibrium values (about 25 ns). In the simulation run considered, the peptides form a rather stable aggregate, which contains on average 8.6, i.e. the majority, of the totally 12 peptides. This aggregate should be considered as an analogue to the stable peptide-rich fibrillar phase, which appears due to the phase separation in the macroscopic aqueous solution of peptides. Therefore, simulation studies of such peptide aggregates may give useful information concerning the formation of ordered fibrils in experimental situations.

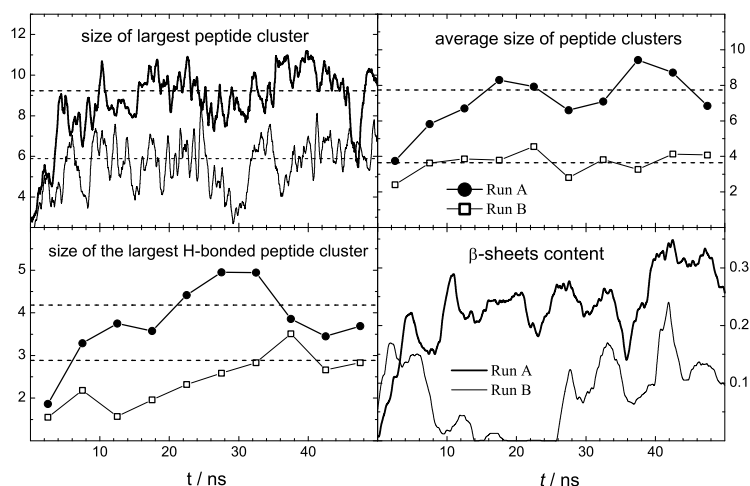


Figure 2. Time dependencies of the size of the largest cluster, of the size of the largest H-bonded cluster, of the average cluster size and of the β -sheet content in two simulation runs: A and B.

However, the large peptide aggregates do not necessarily appear in all simulation runs performed for the same system starting from different initial configurations. Moreover, in some simulation runs, a large peptide aggregate may dissolve after a long simulation time. In Fig. 2, we show two simulation runs, which yield quite different clustering of peptides after an initial equilibration period of about 15 ns. In Run A, there is a large peptide cluster which contains on an average the majority of all peptides. In Run B, the largest cluster contains on an average, less than half of all peptides, and they should be considered as dissolved in water. Such behavior is typical for simulation studies of a small system, whose density (concentration) is kept deeply inside the two-phase region.² Namely, the peptide-rich phase may be in a condensed state or in a dissolved state, but both these states are *stable* in simulations and should substitute each other in the course of a very long simulation run. Obviously, the structural characteristics of these two states are quite different. In particular, β -sheet content is essentially higher in the condensed state (see Fig. 2). Clearly, only the condensed state is relevant to the ordered peptide-rich phase seen in experiments, whereas the dissolved state of peptides is relevant for extremely small systems only. Our results show a strong effect of a small size of the simulation system on the aggregation behavior of peptides. We propose to use a full clustering analysis to select the proper condensed state of peptides, which is relevant for large (macroscopic) systems and, therefore, for comparison with experiments.

Acknowledgments

Financial support from the International Max-Planck Research School in Chemical Biology and from the Zentrum für Angewandte Chemische Genomik is gratefully acknowledged.

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