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Structural character of α-syn12 peptide in solution at high pH

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ABSTRACT

The dynamics and structural character of α-syn12 peptide in aqueous solution at high pH has been investigated through temperature replica exchange molecular dynamics simulations by using GROMOS 43A1 force field. The isolated α-syn12 peptide adopts in water an α-helix structure at high pH. These results are distinct from other amyloid disease protein at neutral pH.

KEYWORDS

Peptide simulation;
Replica exchange;
Force field.

INTRODUCTION

Experimentally, three dimensional protein structures can be analyzed by x-ray diffraction of protein crystals or nuclear magnetic resonance spectroscopy. Due to the absence of a high resolution three dimensional structure of disorder protein in solution, the determination of a unique high-resolution structure and/or the ensemble of conformations sampled experimentally remain a considerable challenge. Currently, the microscopic distributions of protein conformations in time and space are not accessible by experiments. In addition, experiments can not provide detailed dynamics of proteins about how they carry out their functions. With a rapid increase in available computational resources, one of the most prominent theoretical approaches is molecular dynamics simulation.

Molecular dynamics (MD)[1-4] simulations have greatly improved our understanding of how small peptides and protein fold into unique structures in solution. There are two well-known difficulties in molecular simulations, one is the limited accuracy of the potential energy functions of molecular force field and another is limited sampling[5] in the higher dimensional conformational space of protein and peptide, many efforts have been made to solve these questions.

One method to solve the sampling problem is the development of the replica-exchange molecular dynamics (REMD) method[6-8]. Compared to regular MD or MC which sample a conventional canonical ensemble, REMD and REMC sample a generalized ensemble. The equilibrated trajectories sample a generalized ensemble described by a generalized partition function in which temperature is an additional degree of freedom. For each trajectory, the higher temperature phases facilitate the overcoming of energy barriers and the lower temperature phases allow for sufficient equilibrium sampling of local conformational states. REMD simulations are often employed to construct free energy surfaces in reduced dimensions.

Because of the complexity of the systems, biomolecular force fields[9-13] are necessarily empirical.

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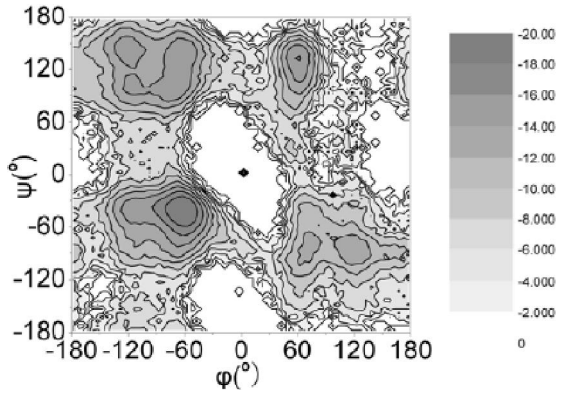

Figure 1: Potential of mean forces obtained from (ϕ, Ψ) distributions of residues 2-11. The gray regions correspond to the lowest energy areas. Neighboring contour lines are separated by 2 kJ.mol-1.

Parameterization plays an important role in achieving the required accuracy after a model capturing the key aspects of the underlying physical interactions has been formulated. Comparisons with quantum mechanical models provided important clues for the improvement of molecular mechanics force fields. The results indicated that there existed systematic biases in the description of protein local conformations by widely-used molecular mechanics force fields[14]. Efforts have been made to refine the treatments of protein backbone conformations in empirical force fields including CHARMM[15], AMBER[16], GROMOS[17], and OPLS-AA[12].

Residues 1-12 (α-syn12 peptide) of the human α-synuclein protein are considered to be important for binding to the coiled-coil domain of synphilin-1[18]. Human α-synuclein protein, is the major component of Lewy bodies deposited in the brains of patients with Parkinson's disease. Typical of Parkinson's disease is the presence of α-synuclein aggregates in a β-structure that can be soluble or insoluble[19]. The structure (PDB ID: 1×q8) of micelle-bound human α-synuclein has been discussed by Ulmer et al.[20] with solution NMR spectroscopy. Val3-Val37 and Lys45-Thr92 form curved α-helices, with a break in the 38-44 region. Synphilin-1 protein is a novel α-synuclein interacting protein also present in lewy bodies.

In the present study, we are analyzing the structural character of α-syn12 peptide in aqueous solution at high pH via temperature replica exchange molecular dynamics simulations. The isolated α-syn12 peptide adopts in water an α-helix structure.

EXPERIMENTAL

We considered α-syn12 peptide (MDVFMK-GLSKAK) known to fold into α-helix structure in membrane-mimicking environments. Molecular dynamics simulation in the NPT ensemble was performed with the GROMACS software package[21] and with the GROMOS 43A1[11] force field. The peptide is solvated in a rectangular box of SPC[22] water model with the minimum solute-box boundary distance set to 1.4nm. Protonation states of ionizable groups were chosen for pH 10.0.

In the explicit-solvent simulations, the non-bonded interactions have been treated using a twin-range cutoff method[23] with generalized reaction field corrections, short-range interactions within 0.8nm evaluated every time step, medium-range interactions between 0.8 and 1.4nm updated every 10 steps and electrostatic interactions beyond 1.4nm approximated by reaction fields generated by a dielectric continuum with a dielectric constant of 54 for water. The temperature and pressure of the system was kept constant by weak coupling to external heat baths with a relaxation time of 0.1 ps[24]. The time step for the MD integrator was set to 2 fs and SHAKE[25] was applied to constrain all bond lengths with a relative tolerance of 10^{-4}.

32 replicas have been simulated at temperatures (in K) of 273, 276, 279, 281, 284, 287, 290, 293, 296, 299, 302, 305, 309, 312, 315, 318, 321, 324, 328, 331, 334, 338, 341, 345, 348, 351, 355, 358, 362, 365, 369 and 373[26]. Each replica had been equilibrated at its respective temperature for 100 ps. Then 90 ns T-REMD simulations were performed, replica exchanges attempted every 2 ps based on the Metropolis criterion. Coordinates and energies have been recorded every 2 ps. The trajectories of 300K have been analyzed.

RESULTS AND DISCUSSION

T-REMD simulations

Effective T-REMD requires sufficient exchange between the different temperatures (the ratio of exchange greater than 0.1). The ratios of successful exchange attempts were between 20% and 40% in these simula-

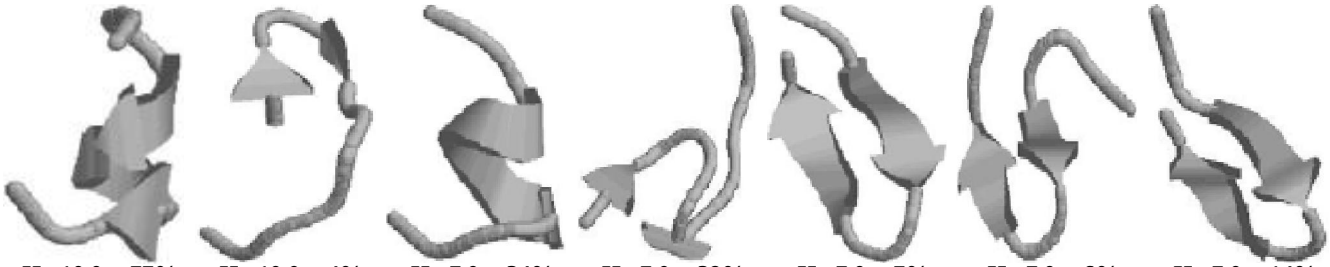

Figure 2 : The central structure of α-syn12 peptide in different conformation clusters. The probability and pH of this cluster is labeled below the images only large clusters with more than 300 conformations have been shown

tions, so the number of replicas was sufficient.

Distributions of backbone dihedral angles in the peptide simulations

We analyze the distributions of the backbone (ϕ, Ψ) angles for each residues (residues 2-11). Data for these residues have been pooled together. The distributions of the Ramachandran (ϕ, Ψ) angles for each residues contained in the native secondary structure excluding glycine were collected from simulations and potentials of mean force were computed (Figure 1). For GROMOS 43A1 force field, residues in the native secondary structures mostly populate the corresponding minima for α region, while the other regions are also populated, but only sparsely.

Different regions are defined as in reference[27], a region: -180°<ϕ<0° and -120° <Ψ<30°; Bridge region: -180°<ϕ<0° and 30° <Ψ<90°; β region: -180°<ϕ<0°, and 90° <Ψ<180° or -180° <Ψ<-120°. For simulation using GROMOS 43A1 force field, the probabilities for (ϕ, Ψ) angles to fall within the α, bridge and β regions are 41%, 3%, 21% respectively. The simulation produced more sampling in the α region.

Figure 1 shows that the simulation produced more sampling in the α region. The probabilities of the conformations show any helical content are calculated with the program STRIDE[28,29] and found that 38%.

Formation of β-turn

The isolated α-syn12 peptide adopts in water a β-sheet structure at neutral pH (in press). Hydrogen bond and β-turn are two factors involved in the folding for β-hairpin structure. The numbers of conformation which has been formed β-turn in the simulations have been computed. $Turn_{i-j}$ is named as residues j-i form a β-turn. In the program STRIDE, the estimation of β-turn was based on the combined use of hydrogen bond energy and backbone dihedral angle information. From the simulation using GROMOS 43A1 force field, the probabilities of conformation have $Turn_{8-5}$ is 32%, $Turn_{9-6}$ is 54%.

Conformation clusters

To understand which is the favorite conformation for GROMOS 43A1 force fields, we partition sampled conformations into different clusters based on their mutual root-mean-square deviations of Cα positions ($RMSD_{C\alpha}$).

We consider conformations sampled by the replica at 300 K. A total of 9000 conformations from the 90 ns trajectory (1 conformation every 10ps) were clustered based on their pair-wise $RMSD_{C\alpha}$. The criteria for clustering have been that for any conformation in a cluster, there is at least one other conformation in the same cluster with an $RMSD_{C\alpha}$ less than 0.1nm from the conformation, and there should be no conformation in any other cluster with an $RMSD_{C\alpha}$ less than 0.1nm. In addition, all conformations in the same cluster should be connected by the $RMSD_{C\alpha}$ criterion. By the clustering criteria, conformations sampled in the simulations fall into clusters of varying sizes (Figure 3). For GROMOS 43A1 force field, 129 clusters have been obtained. Among them, 2 contain at least 300 conformations. For simulation at neutral pH, 387 clusters have been obtained. Among them, 5 contain at least 300 conformations.

CONCLUSIONS

α-syn12 peptide is considered to be important for binding to the coiled-coil domain of synphilin-1. Human α-synuclein protein, is the major component of

Lewy bodies deposited in the brains of patients with Parkinson's disease. The structure (PDB ID: 2jn5) of α-syn12 peptide bound with synphilin-1 has been discussed by Hu et al with solution NMR spectroscopy. However, the isolated α-syn12 peptide in solution at high pH did not analyze by experiment or molecular simulation. In GROMOS 43A1 force field, the isolated α-syn12 peptide adopts in water an α-helix structure. These results are distinct from other amyloid disease protein in solution at neutral pH.

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