Molecular dynamics simulation of mitochondrial ADP/ATP carrier in absence and in presence of its natural inhibitor carboxyatractyloside

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Motivation

The transport of various metabolites across the mitochondrial membranes is essential for eukaryotic metabolism. Specific transport through the inner mitochondrial membrane is achieved by nuclear encoded carriers which form a large transport family, the mitochondrial carrier family. The structure of the ADP/ATP carrier in complex with its inhibitor carboxyatractyloside (CATR) has been recently solved by X-ray crystallography providing for the first time an insight into one conformation of the protein. In order to shed light on the possible conformation sampled by the protein and on the effect of CATR on constraining a definite configuration we have carried out two 10 ns molecular dynamics simulation of the protein embedded in a lipid bilayer of palmitoyl-oleoyl-phosphatidyl-choline (POPC) with and without its co-crystallized inhibitor CATR.

Methods

The starting coordinates of the ADP/ATP carrier were obtained from X-ray diffraction (PDB entry 1OKC). The POPC membrane bilayer, composed by 255 lipids, was generated by using the VMD v1.8.2 program. The insertions of protein in the membrane and molecular dynamics simulations were carried out by using the GROMACS 3.2.1 program. Two simulation systems were build up, one keeping and one removing the CATR inhibitor (66906 and 67054 atoms, respectively). Each system was thermalized and after simulated in water for 10.0 ns at constant temperature and pressure. Periodic boundary conditions have been used and the electrostatic interactions were calculated with the Particle Mesh Ewald method. The atomic positions were saved every 0.5 ps for the analysis.

Results

The RMSF calculated on the trajectories and averaged over each residue well reproduces the crystallographic B-factors but reveals a different behaviour of selected protein loops that exhibit larger or lower fluctuations in the presence or in the absence of CATR, respectively. The trans-membrane helices in the simulations are characterized by RMSF values lower than the corresponding crystallographic B-factors, likely because of a stabilizing effect induced by the POPC bilayer that is absent in the crystal. A different stable network of salt bridges has been found in the two trajectories suggesting that in the absence of CATR the carrier undergoes a conformational switch, characterized by a conformation that is more opened on the matricial side. The volume present in the internal protein channel is constant in the inhibited protein while

in the CATR-free carrier the main cavity initially present tends to decrease and a new cavity is appearing, localized in the matricial side of the carrier according to this switching hypothesis. The interactions of the ADP/ATP carrier with POPC lipids and water is different in the two trajectories indicating that they are dependent on the different conformational state sampled by the protein in the two simulations.

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