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Modeling the molecular dynamics of cytochrome C in aqueous and water–methanol environment

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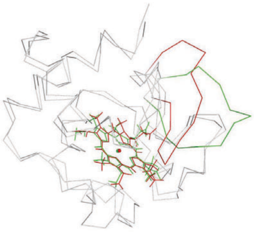
One-microsecond molecular dynamics of horse heart cytochrome C was modeled in aqueous and water–methanol environment. It was shown that the coordination bond between Met-80 sulfur and heme iron is broken in water–methanol solution.



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Cytochrome C is a small protein containing heme C.[1] The main function of cytochrome C is participation in the respiratory electron transfer chain.[2] In addition, cytochrome C is involved in apoptosis initiation, alongside with a few other functions.[3] It was previously shown that under certain conditions the conformation of cytochrome C can change in such a manner, that cytochrome C becomes a so-called 'molten globule'.[4] This conformational change of the cytochrome C molecule is associated with the appearance of its peroxidase activity,[5] which is connected with the process of apoptosis.[6–9] Thus, the information about the conformational changes in cytochrome C, related to its peroxidase activity, is important for apoptosis research. It was shown that the presence of methanol and/or cardiolipin in solution can serve as a promoting factor in the transition of the cytochrome C molecule into the molten globule state.[10–12] This transition is associated with axial amino acid residues coordinating heme iron.[13] To demonstrate this process at the molecular level, we simulated 1000 ns of molecular dynamics of cytochrome C in aqueous and water–methanol environment.

Initial system models were built using PackMol[14] and AmberTools19,[15] and then converted to GROMACS[16] format using the ACPYPE program.[17] To build the topology of heme, we used the parameters obtained by Giamonna.[18] The ff14SB force field was used for the simulation.[19] Modeling was carried out using the GROMACS 2020.3 package.[16] The TIP3P model was chosen as the water model, being the most suitable for use with the ff14SB force field. At the first stage, the energy was minimized with 1 fs time step to a maximum force of 1000.0 kJ $\mathrm{mol^{-1}\ nm^{-1}}$. Then the system was equilibrated at a temperature of 293 K and a pressure of 1 atm by simulating in NVT and NTP ensembles (100 ps each), respectively. The temperature and pressure in the systems were controlled using a modified Berendsen thermostat[20] and the Parinello–Rahman barostat,[21] respectively. A productive simulation of 1 μs of molecular dynamics for each system was carried out in an isothermal-isobaric ensemble with 2 fs time step. The LINCS[22] algorithm was used to restrict bonds involving hydrogen atoms. Long-range electrostatic interactions were calculated using the Ewald summation scheme.[23] Van der Waals and Coulomb interactions have been truncated to 1.4 nm, which is optimal for the ff14SB force field.[19] Before trajectory analysis, artifacts arising from quasi-infinite periodic boundary conditions were eliminated using the GROMACS 2020.3[16] package, returning the cytochrome C molecule back to the simulation cell.

To analyze the simulation results, RMSD (Root Mean Square Standard Deviations), RMSF (Root Mean Square Fluctuations), radius of gyration R_g as well as the distance between Met-80 sulfur and heme iron were calculated (Figure 1). The RMSD plots for two systems are shown in Figure 1(*a*). Given the size of the systems, these plots indicate the stability of the protein molecule during the simulation. Figure 1(*b*) shows the change in R_g of the cytochrome C molecule during the simulation. The cytochrome C molecule in water–methanol solution becomes less compact than in an aqueous one. Figure 1(*c*) shows RMSFs for Cα-atoms of the cytochrome C molecule in aqueous and water–methanol environments. The region from 20th to 30th amino acid residues is the most mobile in both cases. This region is a disordered loop, which, unlike other disordered loops in the cytochrome C molecule, does not contain amino acid residues coordinated to heme iron. This is the probable reason for the mobility of this loop. The additional mobile region from 76th to 86th amino acid residues arises in water–methanol environment. This region contains Met-80, which coordinates the heme iron atom by the sulfur atom in the crystal structure. The difference in the mobility of this region of the cytochrome C molecule in aqueous and water–methanol solutions is explained by the cleavage of the coordination bond between heme iron and Met-80 sulfur atom in water–methanol medium [see Figure 1(*d*)]. This coordination bond is retained in aqueous solution throughout the

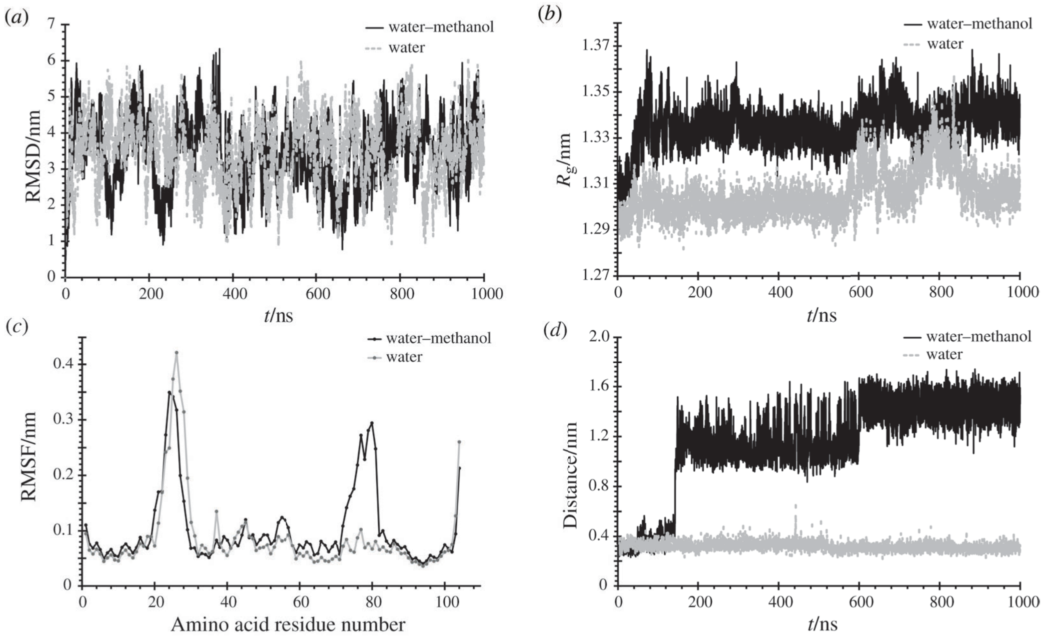

Figure 1 Analysis of structural changes of cytochrome C in water and water–methanol solution for a time interval of 1000 ns: (*a*) change in RMSD for cytochrome C Cα-atoms; (*b*) change in the radius of gyration R_g of the cytochrome C molecule; (*c*) RMSF plots for cytochrome C Cα-atoms; (*d*) change in the iron–sulfur bond length.

entire simulation period, while in water–methanol environment it irreversibly breaks at the timepoint around 200 ns.

The breaking of this coordination bond leads to significant conformational changes in the cytochrome C molecule. These conformational changes are shown in Figure 2 and represent the change in the position of the mobile loop from 76th to 86th amino acid residues. Methionin-80 ends up on the surface of the molecule.

Thus, the 'molten globule' state of the cytochrome C molecule was modeled at the molecular level for the first time. The native and the 'molten globule' states of cytochrome C molecule were compared, and the observed structural differences were analyzed.

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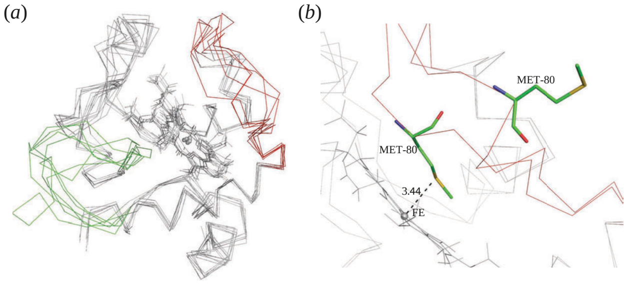

Figure 2 (*a*) Superposition of the cytochrome C molecule at the start of the simulation and at 200, 400, 600, 800 and 1000 ns. The red color shows the mobile loop from 76th to 86th amino acid residues, the green color shows the mobile loop from 20th to 30th amino acid residues. Heme is depicted by gray lines, iron is indicated by gray spheres. (*b*) Comparison of the mobile loop from 76th to 86th amino acid residues at the start of the simulation and at 1000 ns. The coordination bond between Met-80 sulfur and heme iron is broken and Met-80 appears on the surface of the cytochrome C molecule.

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