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STRUCTURE OF MACROMOLECULAR COMPOUNDS

Modeling of the Interaction of Cytochrome *c* with Cardiolipin

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Abstract—Interactions of horse heart cytochrome *c* (Cyt *c*) with cardiolipin molecules were modeled by molecular dynamics simulations. It was shown that the Cyt *c* molecule forms a complex with cardiolipin molecules. The steps of the formation of the Cyt *c*–cardiolipin complex, the interactions of the Cyt *c* molecule with cardiolipin molecules, and conformational changes of the Cyt *c* molecule upon formation of the Cyt *c*–cardiolipin complex are described. These data provide better insight into the mechanism of action of Cyt *c*.

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INTRODUCTION

Cytochrome *c* (Cyt *c*) is a small heme-*c* containing protein [1, 2] having two functions. Thus, Cyt *c* is involved in the respiratory chain [3] and acts as one of activators of apoptosis, a form of programmed cell death. It is known that the interaction of Cyt *c* with cardiolipin clusters involved in the inner mitochondrial membrane is one of key steps of apoptosis [4–7]. It was shown that cardiolipin molecules form a complex with Cyt *c* [8, 9], and the structure of this complex was proposed [10, 11]. However, this complex was not described at the molecular level. This complex is hydrophobic and can catalyze the formation of free radicals both in an aqueous medium and in the hydrophobic environment. This process leads to lipid peroxidation in mitochondrial membranes, thereby triggering a cascade of apoptosis and cell death reactions [12, 13]. It was shown that the effect of the Cyt *c*–cardiolipin complex on cancer cell lines causes the activation of apoptosis and death of cells, in particular, those resistant to conventional anticancer drugs [14]. Due to the development of computer science and technologies and greater accessibility of supercomputers, molecular dynamics (MD) simulations can currently be used to study rather large systems, such as protein–protein complexes and complexes of proteins with bilipid membranes at the molecular level [15–17]. The molecular modeling of ion flows through ion channels, which are transmembrane proteins or protein complexes, was accomplished [18]. In order to determine the structure of the Cyt *c*–cardiolipin complex at the molecular level, in this work we studied the molecular dynamics of Cyt *c* placed in an aqueous environment with the addition of cardiolipin molecules. The dynamics of the formation and the properties of the Cyt *c*–cardiolipin complex are described.

MATERIALS AND METHODS

Construction of the Initial Model of the System

The initial model of the system was built up using the PackMol package [19] and the AmberTools19 software package [20]. The topology of the heme was built using the parameters determined in [21].

Molecular Dynamics Simulations

Molecular dynamics simulations were performed using the Amber18 software suite [20] in the ff14SB force field [22]. Water molecules were described by the TIP3P model, which is most suitable for MD simulations using the ff14SB force field. Potassium chloride (0.15 M KCl) and several ions were added to the system to neutralize the total charge of the system. To relax the structure and avoid steric clashes, the system was subjected to potential-energy minimization. The pressure and the temperature in the system were equilibrated in six 25–50-ps steps to 1 atm and 310 K, respectively, by performing simulations in NVT and NPT ensembles with restraints on atoms. Then the restraints on atom motion were removed, and the system was equilibrated for 2 ns. The temperature and the

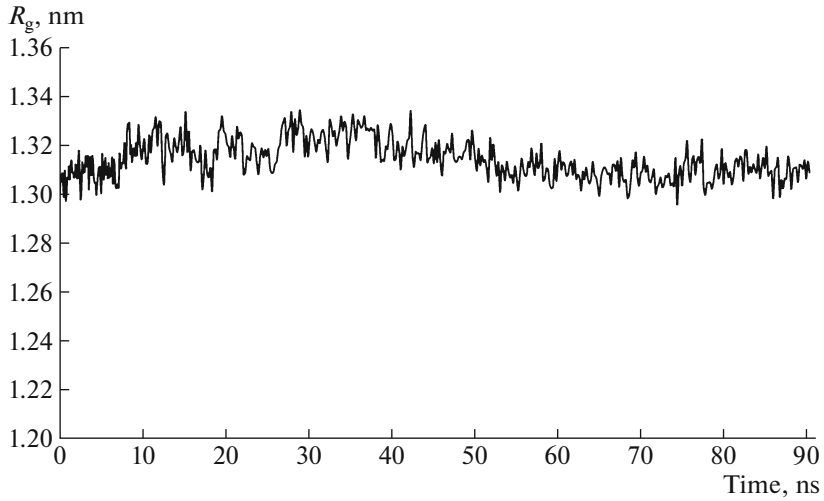

Fig. 1. Time dependence of the radius of gyration of the Cyt *c* molecule.

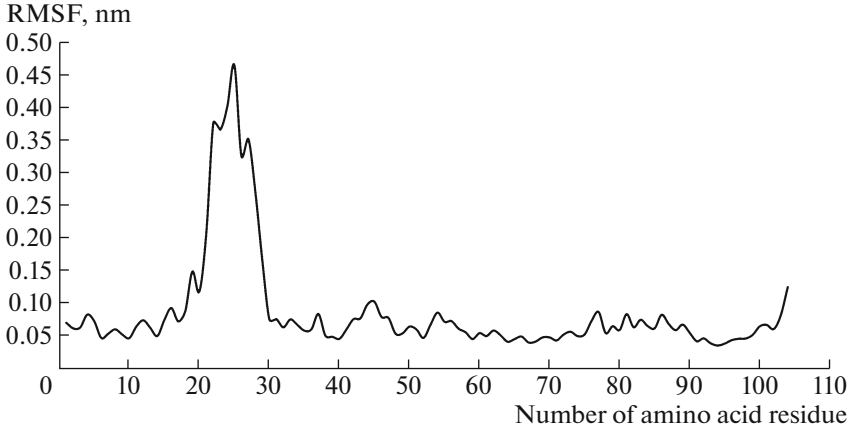

Fig. 2. Root-mean-square fluctuations (RMSF) of Cα atoms of the Cyt *c* molecule.

pressure in the system were controlled using the Berendsen thermostat [23] and the Parrinello–Rahman barostat [24], respectively. Productive 90-ns MD simulations were performed for each system in the isothermal-isobaric ensemble with a step of 2 fs. The vander-Waals and Coulomb interactions were truncated at a cut-off radius of 1.4 nm, which is an optimal value for the force field used [22].

RESULTS AND DISCUSSION

The initial trajectory analysis involved calculations of the dependences of the root-mean-square deviations of the radius of gyration of the Cyt *c* molecule (Fig. 1) and the root-mean-square fluctuations (RMSF) of Cα atoms of Cyt *c* (Fig. 2). As is seen in Fig. 1, there are no significant changes in the radius of gyration during simulations, which indicates that the compactness of the Cyt *c* molecule remains unchanged along the MD trajectory. Figure 2 shows that the Cyt *c* molecule is very flexible in the amino-acid region 20–30 and is stable in other regions. The region 20–30 is a disordered loop, which does not include, as opposed to other disordered loops of the Cyt *c* molecule, residues bound to the heme iron through coordination bonds. Apparently, this fact is responsible for the flexibility of this loop.

In [25] it was shown that under certain conditions, Cyt *c* can undergo conformational changes, being transformed into the molten globule state. Besides, the presence of cardiolipin in the solution can induce the transformation of the cytochrome molecule into the molten globule state [26]. The transformation of Cyt *c* into the molten globule state is related to the axial residues coordinated to the heme iron [27]. In horse heart Cyt *c*, the heme is coordinated by Met-80. Figure 3 represents the time dependence of the distance between the sulfur atom of Met-80 and the heme iron. It is interesting that, although the coordination bond is

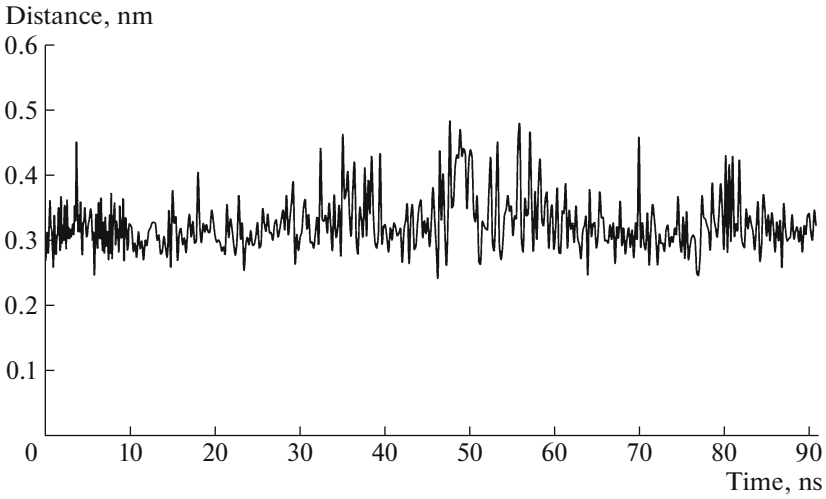

Fig. 3. Time dependence of the distance between the heme iron and the sulfur atom of methionine 80 in the Cyt *c* molecule.

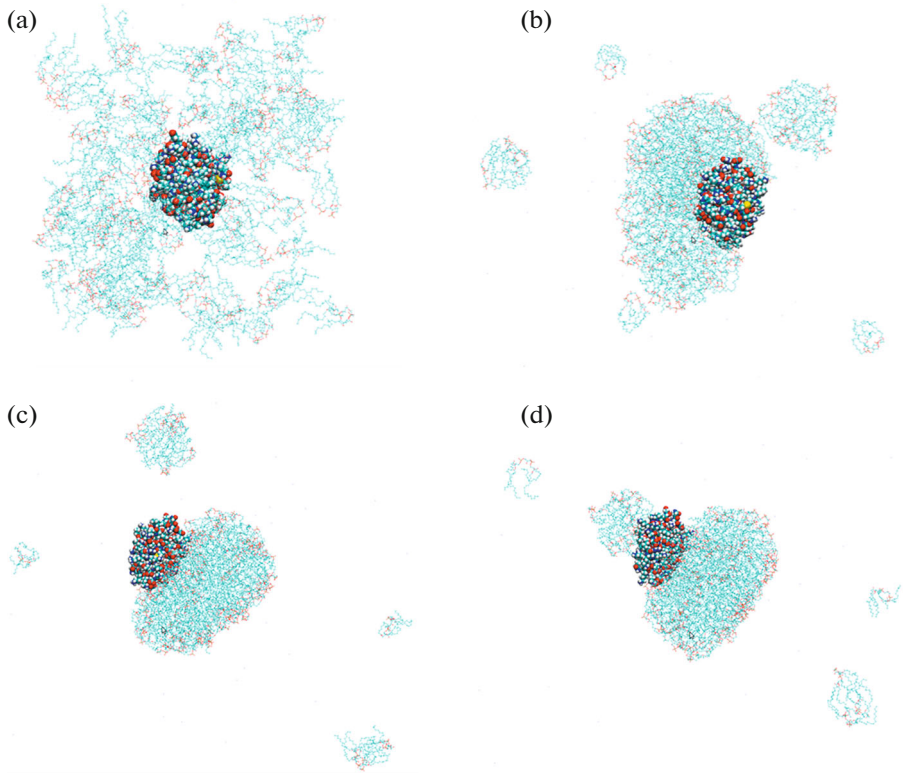

Fig. 4. Simulated system (a) in the initial state and at (b) 3.6, (c) 40, and (d) 54 ns of simulation time. The Cyt *c* molecules are represented as spheres; cardiolipin molecules are indicated by lines.

not fully broken, the distance between the sulfur atom of Met-80 and the heme iron varies in a wide range reaching 4.8 A at 47 ns of simulation time. This may be indicative of the onset of the transformation of the Cyt *c* molecule into the molten globule state.

The dynamics of the formation of the Cyt *c*–cardiolipin complex can be arbitrarily divided into four steps. Initially (Fig. 4a), the cytochrome molecule is randomly surrounded by lipids; however, structures formed by lipid molecules appear within 3.6 ns (Fig. 4b). It is seen that most lipid molecules are located near the cytochrome molecule; however, the resulting structure is unstable and nonhomogeneous. There are also five small lipid structures consisting of from one to eight cardiolipin molecules. The largest structure con-

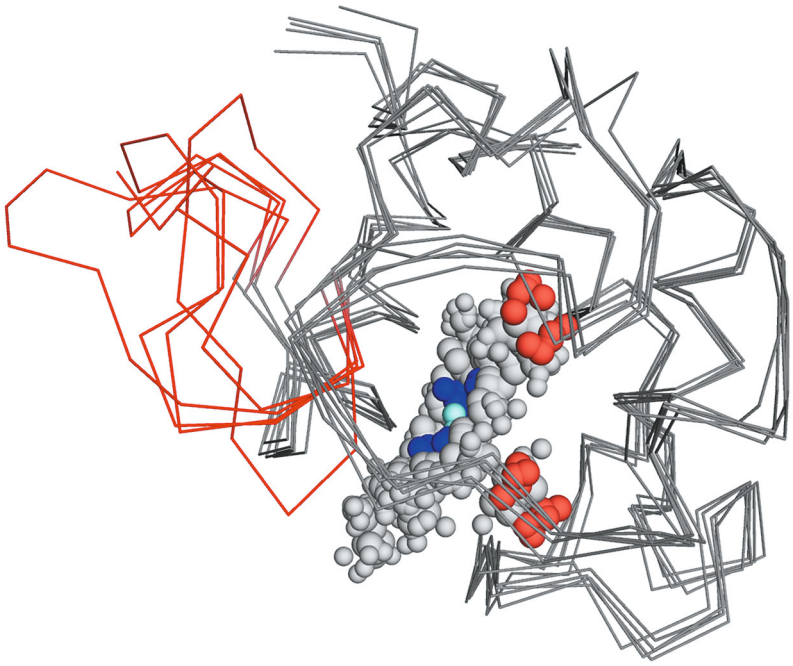

Fig. 5. Comparison of the conformations of the Cyt *c* molecule in the initial state and at 10, 30, 60, and 90 ns of simulation time. The flexible loop 20–30 is shown in red. The hemes are represented as spheres.

tains eight lipid molecules; one structure, three lipid molecules; and the other three structures, one lipid molecule each. At this moment, lipid molecules located near cytochrome do not yet form energetically favorable structures, as evidenced by the fact that the polar heads of lipids are sometimes incorporated into lipid micelles. Starting from 40 ns (Fig. 4c), the basic lipid structure slightly changes and it becomes more planar compared to the structure considered previously. Besides, all polar heads of lipids are located outside micelles from now on. The number of small lipid structures decreases from five to four, the largest of which still consists of eight lipids. Starting from 54 ns (Fig. 4d), the major lipid micelle is stabilized in a complex with a cytochrome molecule. Besides, the structure consisting of eight lipids is attached to the cytochrome on the side opposite to the major lipid micelle. It is worth noting that the Cyt *c* molecule does not undergo significant conformational changes (Fig. 5).

In [28, 29] it was suggested that cardiolipin molecules interact with the horse heart Cyt *c* molecule predominantly through lysine clusters on the surface of the cytochrome molecule. In this work we showed that cardiolipin molecules interact with the following positively charged residues on the surface of the cytochrome: Lys7, Lys8, Lys22, Lys25, Lys27, Arg38, Lys53, Lys55, Lys72, Lys73, Lys79, Lys86, Lys87, and Arg91. This corresponds to the previous suggestions and also demonstrates an important role of arginines in the interaction of the Cyt *c* molecule with cardiolipin.

The results of this study are not only important for the elucidation of the mechanism of the process essential for the cellular metabolism and the body as a whole but are also of practical importance for medicine. The action of available anticancer drugs is based mainly on the induction of apoptosis or ferroptosis in cancer cells. In [14] it was demonstrated that the catalytically reactive Cyt *c*–cardiolipin complex can induce apoptosis and cause cancer cell death in the culture. It was shown that the Cyt *c*–cardiolipin complex produces lipoperoxide radicals in the following two reactions: through the decomposition of lipid hydroperoxides and lipid peroxidation with hydrogen peroxide. Antioxidants inhibit the formation of lipid radicals. It should be noted that the Cyt *c*–cardiolipin complex rather than Cyt *c* by itself sharply increases the level of apoptosis and causes cell death in the following two cell lines: the drug-sensitive cell line A2780 and the doxorubicin-resistant cell line A2780-Adr [14]. Since enzyme complexes with lipids are component of mammal cells, the effect of these complexes or their analogs on cancer (or bacterial) cells can serve as a tool for designing drugs, against which foreign cells or microorganisms do not develop defense mechanisms.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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