Journal of Biochemistry and Molecular Biology, Vol. 33, No. 1, January 2000, pp. 54-58

JOURNAL OF
Biochemistry
and
Molecular Biology

Molecular Dynamic Simulations of the Fatty Acid Bilayer Containing Very Long Chain Transmembrane Dicarboxylic Acids

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Received 4 September 1999, Accepted 14 October 1999

Recent research results regarding the very long chain transmembrane α,ω-dicarboxylic components in the membrane of extremophilic eubacteria, such as *Sarcina ventriculi*, *Thermotoga maritima*, and *Thermoanaerobacter ethanolicus* have raised interesting questions concerning the physical and biochemical function on these components in the membrane. In order to understand the dynamic characteristics of these acids which reside in the bilayer membrane, 580 ps molecular dynamic simulations at 300 K were performed for two model systems. These systems were the bilayer with regular chain (C16:0 or C18:1) fatty acid methyl esters and the fatty acid bilayer containing very long chain transmembrane dicarboxylic acid methyl esters (α,ω-15,16-dimethyltriacotane-dioate dimethyl ester; C32:0). Our analyses indicate that very long chain transmembrane dicarboxylic acids have a noticeable influence on the bilayer dynamics at a sub-nanosecond time scale. The center-of-mass mean-squared-displacement (MSD) of regular chain fatty acids adjacent to the very long chain transmembrane dicarboxylic acids decreased, the long-axis order parameter increased, and the reorientational motions of methylene groups were slowed along the hydrocarbon chains. These results indicate that the very long chain transmembrane dicarboxylic acids reduce the molecular order of the whole bilayer membrane.

Keywords: Dicarboxylic acid, Extremophile, Molecular dynamics.

Introduction

Membranes are critical components of the cell surface of living organisms that form a barrier between the cell cytoplasm and its surroundings through which many metabolites must pass. Membranes must possess very sophisticated molecular mechanisms for maintaining sufficient fluidity for molecular events to take place and sufficient rigidity to maintain cellular integrity (Lee *et al.*, 1998). Most microorganisms use homeoviscous adaptability to change the chemical properties of the membrane so as to restore the original viscosity or fluidity after an environmental perturbation which changes membrane viscosity (Sinensky, 1974; Yoon *et al.*, 1997; Nam *et al.*, 1999). In general, microorganisms genetically regulate the ratio of unsaturated to saturated fatty acids of their biological membrane in response to external conditions, such as a change in temperature (Choi *et al.*, 1999; Yeo *et al.*, 1999). Some extremophilic eubacteria, such as *Sarcia ventriculi* (Goodwin and Zeikus, 1987; Jung *et al.*, 1993), *Butyrivibrio sp.*, (Hauser *et al.*, 1979; Hazlewood and Dawson, 1979; Clarke *et al.*, 1980), *Thermatoga maritima* (Klein *et al.*, 1979), and *Thermoanaerobacter ethanolicus* (Jung *et al.*, 1994), can tolerate large changes in environmental conditions, including high temperature, high or low pH, a high salt concentration, and the presence of organic solvents or antibiotics (Ingram and Vreeland, 1980). Unlike general homeoviscous adaptation, this tolerance is linked to the production of very long chain transmembrane dicarboxylic acids. It has been proposed that these bifunctional fatty acids span the cell membrane and are synthesized by the tail-to-tail joining of membrane lipid chains between the bilayer, forming bipolar transmembrane species that stabilize the membrane structure (Jung and Hollingsworth, 1994; Jung and Hollingsworth, 1995). Transmembrane dicarboxylic acids may modulate the mechanical and transport properties of membranes (Lee *et al.*, 1998).

Despite a great deal of recent experimental progress in understanding the effects of transmembrane dicarboxylic acids on biological membranes, the molecular picture of transmembrane dicarboxylic acid is not clear. Computer

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modeling has the potential to provide some of the details of the molecular mechanism. In this study we performed 580 ps constant temperature and volume molecular dynamic (MD) simulations of a regular fatty acid bilayer and a fatty acid bilayer containing transmembrane dicarboxylic acid at 300K. The effects of various transmembrane dicarboxylic acids on fatty acid bilayer dynamics were investigated by comparing our results with a simulation of a regular fatty acid bilayer under the same conditions.

Materials and Methods

General methods Molecular modeling calculations were performed using the InsightII/Discover program (version 97.0, Molecular Simulations Inc., San Diego, U.S.A.) with a consistent valence force field (CVFF) (Dauber-Osguthorpe *et al.*, 1988) on a Silicon Graphics Origin 2000 workstation (Silicon Graphics Inc., Mountain View, U.S.A.). MD calculations were performed using the Velocity Verlet algorithm (Swope *et al.*, 1982) at a constant volume with a time step of 1 fs. Electrostatic and van der Waals interactions were calculated every time step with a 1.8 nm group-based cutoff (Mers and Roux, 1996). The non-bond list was updated whenever any atom moved more than one-half the buffer width of 0.5Å. Initial atomic velocities were assigned from a Gaussian distribution corresponding to a temperature of 300K. Temperature was controlled based on the Berendsen algorithm with a coupling constant of 0.1443 ps (Berendsen *et al.*, 1984). We did not consider the water-screening effect in the bilayer interior and assumed a dielectric constant of $\varepsilon = 1$.

Determination of the fatty acid density in the bilayer A preliminary molecular model of the fatty acid bilayer was constructed with 20 palmitic acid (C16:0) methyl esters and 28 oleic acid (C18:1) methyl esters by randomly arraying these fatty acid esters into a bilayer. These fatty acids are abundant in microorganism membranes (Lehninger *et al.*, 1993). The resulting bilayer was then energy-minimized. In order to determine the correct density of these fatty acids in the bilayer for further calculation, preliminary MD simulations were performed for 100 ps at 300K without any constraints (without periodic boundary conditions, or PBC). The density of this bilayer was monitored until a stable value was reached. Within 30 ps the value of average area per fatty acid reached an equilibrium value of approx. 23.5Å^2 /fatty acid.

MD simulation of the fatty acid bilayer The model of the regular fatty acid bilayer was constructed with 10 palmitic acid (C16:0) methyl esters and 14 oleic acid (C18:1) methyl esters with the density obtained above. The very long chain transmembrane dicarboxylic acid was constructed by linking two palmitic acids at the ω-1 position of each fatty acid, as suggested by Jung *et al.* (1994, 1995). Four molecules of palmitic acid methyl ester were replaced with two transmembrane dicarboxylic acid molecules yielding a bilayer containing transmembrane dicarboxylic acids (2 transmembrane fatty acids and 20 bilayer fatty acids). Simulations were performed using periodic boundary conditions (PBC, $a = b = 18$Å, $c = 60$Å, $\alpha = \beta = 90°$, $\gamma = 60°$). The bilayers were solvated with pre-equilibrated SPC water on the top and bottom of the bilayer (Berendsen *et al.*, 1981). Water molecules were then subjected to MD simulation for 25 ps to allow further relaxation of the solvent molecules. The whole system was energy-minimized followed by a 580 ps fully flexible constant-volume and temperature MD simulation at 300K. Intermediate structures were saved every 1000 fs for analysis.

Results and Discussion

The time series of potential energy during the 580 ps MD simulation of the bilayers is shown in Fig. 1. These numbers drifted from their initial values up to 230 ps elapsed time, at which point we regarded equilibration had occurred. Trajectory files obtained during the last 350 ps were used for analyses. The potential energy values reached equilibration near stable average values of 163.3 kcal/mol for the normal bilayer and 218.3 kcal/mol for the bilayer containing transmembrane dicarboxylic acids, respectively.

A snapshot from the end of the MD simulation of the bilayer containing transmembrane dicarboxylic acids is shown in Fig. 2. The average tilt, defined as the angle between the bilayer normal and the vector representing the transmembrane fatty acid, was 3.8±1.6°, which is smaller than the tilt of fatty acids in the regular bilayer (7.8±6.3°) and in the bilayer containing dicarboxylic acids (6.7±5.4°). The lower average tilt value and the standard deviation indicate that this transmembrane component restricted the overall motion of adjacent fatty acids.

The average hydrocarbon chain conformation was also analyzed in terms of the long-axis order parameter,

$$S = 1/2\langle 3\cos^2\theta - 1\rangle$$

where θ is the angle between a vector along the fatty acid and the bilayer normal. The brackets denote an average over time and chains (Collings, 1990). A higher value of S indicates a higher packing order in the fatty acids. Fig. 3 shows the values

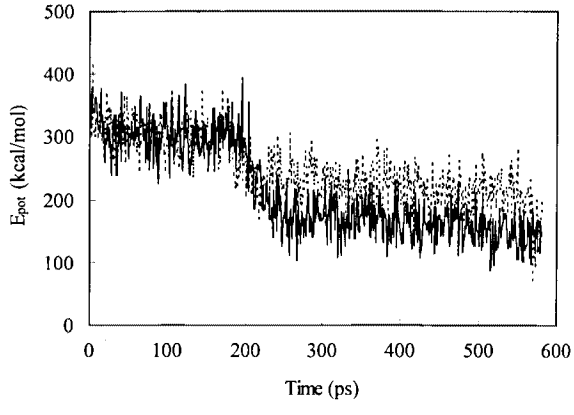

Fig. 1. Time series of potential energy during MD simulation. The energy values converge after 230 ps. (— regular fatty acid bilayer, ------ transmembrane fatty acid containing bilayer)

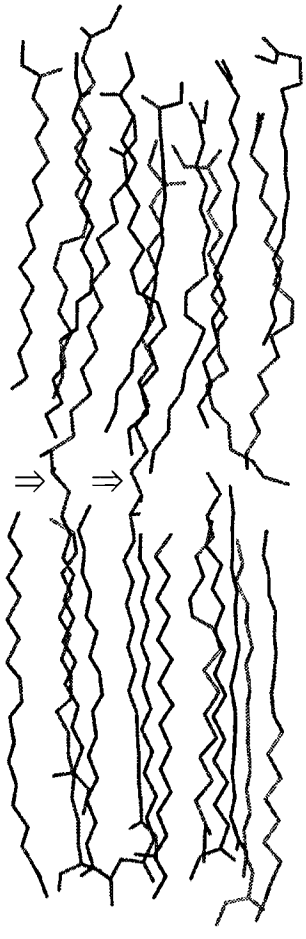

Fig. 2. Snapshot of a configuration from the MD simulation of the bilayer containing transmembrane dicarboxylic acids. Water molecules and lipid hydrogen atoms are not displayed for clarity. Transmembrane dicarboxylic acids are indicated by arrows (⇒).

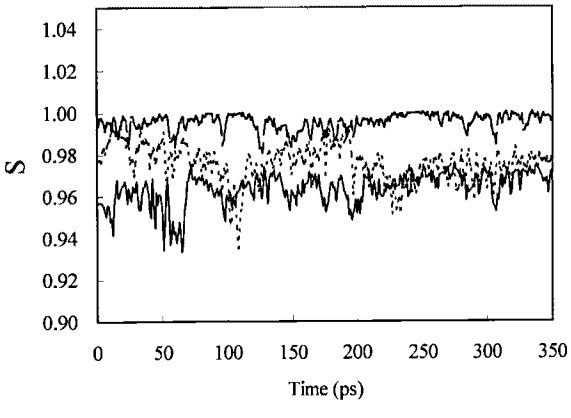

Fig. 3. Comparison of the fatty acid long axis order parameters. (— regular fatty acids, ----- regular fatty acids adjacent to transmembrane dicarboxylic acids, – – transmembrane dicarboxylic acid)

of S calculated from our simulations for each class of fatty acid in the bilayers. Transmembrane dicarboxylic acids raised the molecular order in the bilayer environment, as reflected by the S values.

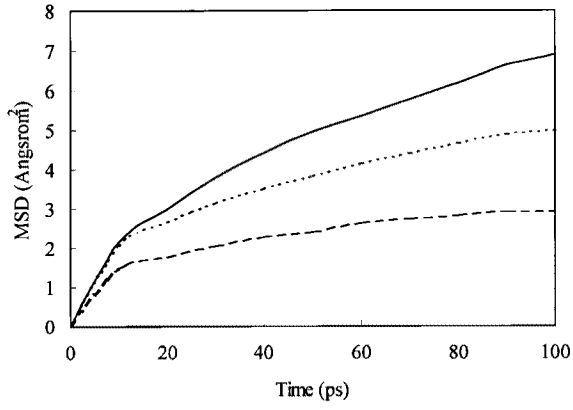

Fig. 4. Center-of-mass mean-squared-displacements (MSDs) of fatty acid molecules. (— regular fatty acids, ----- regular fatty acids adjacent to transmembrane dicarboxylic acids, – – transmembrane dicarboxylic acid)

On pico to nano second time scales the whole molecules in bilayer form exhibited a distinct lateral diffusion (Tu *et al.*, 1998). The effect of transmembrane dicarboxylic acids on the lateral motion at the 100-ps time scale is quantitatively illustrated by the time dependence of the center-of-mass mean-squared-displacements (MSDs) in the bilayer plane plotted in Fig. 4. Although the initial slopes for regular fatty acids only and the slopes for fatty acids adjacent to transmembrane dicarboxylic acids are similar, the overall slope of the latter is roughly 75% of the former. This indicates that the diffusive motion (quantified by the slope of MSD) of regular fatty acids is restricted by the presence of transmembrane dicarboxylic acids. Thus, transmembrane dicarboxylic acids induce a reduction of passive permeability of solute molecules passing through the bilayer.

The internal dynamics of the hydrocarbon chains (fluidity of the bilayer interior) is discussed in terms of the time correlation function which is plotted in Fig. 5:

$$C(t) = 1/2< 3[\mu(t) \cdot \mu(0)]^2 - 1>$$

where μ is a unit vector along a methylene C-H bond (Swope *et al.*, 1982; Mers and Roux, 1996). C8-H and C15-H bonds were used in the calculations. The reorientational correlation function indicates the degree to which the orientation of a vector at a time t is related to its orientation at time 0. A slow decrease in the function indicates lower membrane fluidity. Note here that transmembrane fatty acids increased the reorientational relaxation times along the chains. Thus, transmembrane dicarboxylic acids apparently increased the microscopic viscosity of the bilayer interior.

Our analysis suggests that in the bilayer interior the transmembrane dicarboxylic acids noticeably affect the dynamics of the hydrocarbon chains. The center-of-mass motion of fatty acids is clearly restricted, and the C-H reorientational motion is slowed along the hydrocarbon chains when transmembrane dicarboxylic acids are added. The

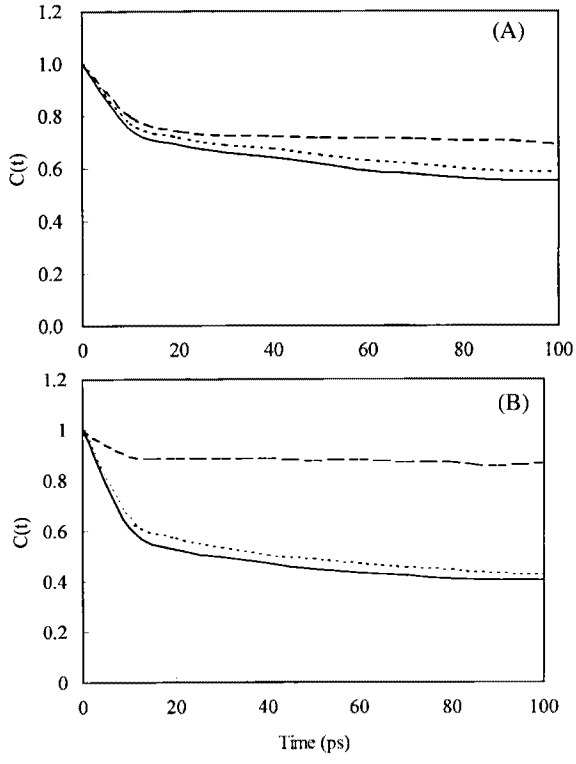

Fig. 5. Reorientational time correlation functions for the selected C-H vectors computed from the simulations with and without transmembrane dicarboxylic acids. A. C8-H vector, B. C15-H vector. (— regular fatty acids, ----- regular fatty acids adjacent to the transmembrane dicarboxylic acid, – – transmembrane dicarboxylic acid)

freezing of the center-of-mass and fatty acid chain motions may be translated into increased friction and rigidity of the bilayer.

Through simulation we have elucidated the molecular function of transmembrane components. The results of this simulation provide a valuable insight concerning the way transmembrane dicarboxylic acids affect bilayer dynamics.

Acknowledgments This research was supported by grant No. KOSEF 981-0513-064-2 from the Korea Science and Engineering Foundation.

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