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Molecular dynamics simulations of pore formation in stretched phospholipid/cholesterol bilayers

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ABSTRACT

Molecular dynamics (MD) simulations of pore formation in stretched dipalmitoylphosphatidylcholine (DPPC) bilayers containing different concentrations of cholesterol (0, 20, 40, and 60 mol%) are presented. The stretched bilayers were simulated by constant $NP_ZA_{||}T$ MD simulations with various constant areas. The effects of the cholesterol concentration on pore formation are examined in terms of the critical areal strain where the pore is formed, the processes of pore formation, and the change in molecular orientation of the DPPC molecules by analyzing the order parameters and radial distribution functions of the DPPC molecules. With increasing cholesterol concentration, the critical areal strain initially increases, peaks at 40 mol%, and then decreases, which agrees well with the available experimental data. For the bilayers containing cholesterol, DPPC molecules become disordered at low areal strains, whereas the order slightly increases when the areal strain exceeds a certain value depending on the cholesterol concentration. For 40 mol% cholesterol, the two monolayers in the bilayer interpenetrate under high areal strains, inducing an increase of the order parameters and the peak positions of the radial distribution function compared with their states at low areal strains, indicating the formation of an interdigitated gel-phase-like structure. The transient increasing of the order of the molecular orientations may inhibit water penetration into the bilayer, resulting in increased critical areal strain in the phospholipid/cholesterol bilayers.

1. Introduction

Cholesterol is an important component of biological cell membranes and occurs at a wide range of concentrations depending on the cell type (Alberts et al., 1994). The roles of cholesterol in normal cell functions have long been recognized (Vance and Van den Bosch, 2000; Yeagle, 1985) and, to understand the underlying mechanisms, the interactions between cholesterol and various types of lipids, which are the primary components of cell membranes, have been comprehensively studied (Róg et al., 2009; Demel, 1972; Almeida et al., 1992; Pike, 2009; Needham and Nunn, 1990; Evans et al., 2003). One of the important findings is that cholesterol increases the ordering of coexisting lipids, the so-called ordering effect (Róg et al., 2009), which is considered to cause the reduction in the passive permeability of the lipid membrane (Demel, 1972), the modulation of lateral diffusion of lipids (Almeida et al., 1992), the instigation of the formation of a rigid micro-domains, called a lipid raft (Pike, 2009), and the increase of mechanical stiffness of the lipid membranes (Needham and Nunn, 1990).

The lipid bilayer is the fundamental structure of biological cell membranes and acts as a barrier between the inside and outside of a cell, and, when stressed, the tension exerted on the bilayer will cause unstable pore formation and failure of the bilayer (Evans et al., 2003). The effects of cholesterol on pore formation and the failure have been investigated experimentally. Needham and Nunn (1990) performed a micropipette aspiration experiment for giant bilayer vesicles consisting of various lipids and cholesterol, and showed that the critical areal strain, where the failure of the vesicle occurs, non-linearly changes with the cholesterol rate. Koronkiewicz and Kalinowski (2004) performed an electroporation experiment using constant-current measurements for a planar bilayer lipid membrane including cholesterol and showed that the presence of cholesterol in the bilayer causes an increase in the value of the breakdown potential. Both experiments suggest that cholesterol increases the resistance of the bilayer to mechanical and electoral stresses, but little is known about the molecular details because pore formation and failure are usually extremely fast events and thereby elusive in experiments. Molecular dynamics (MD) simulation of the bilayer is a promising method to reveal

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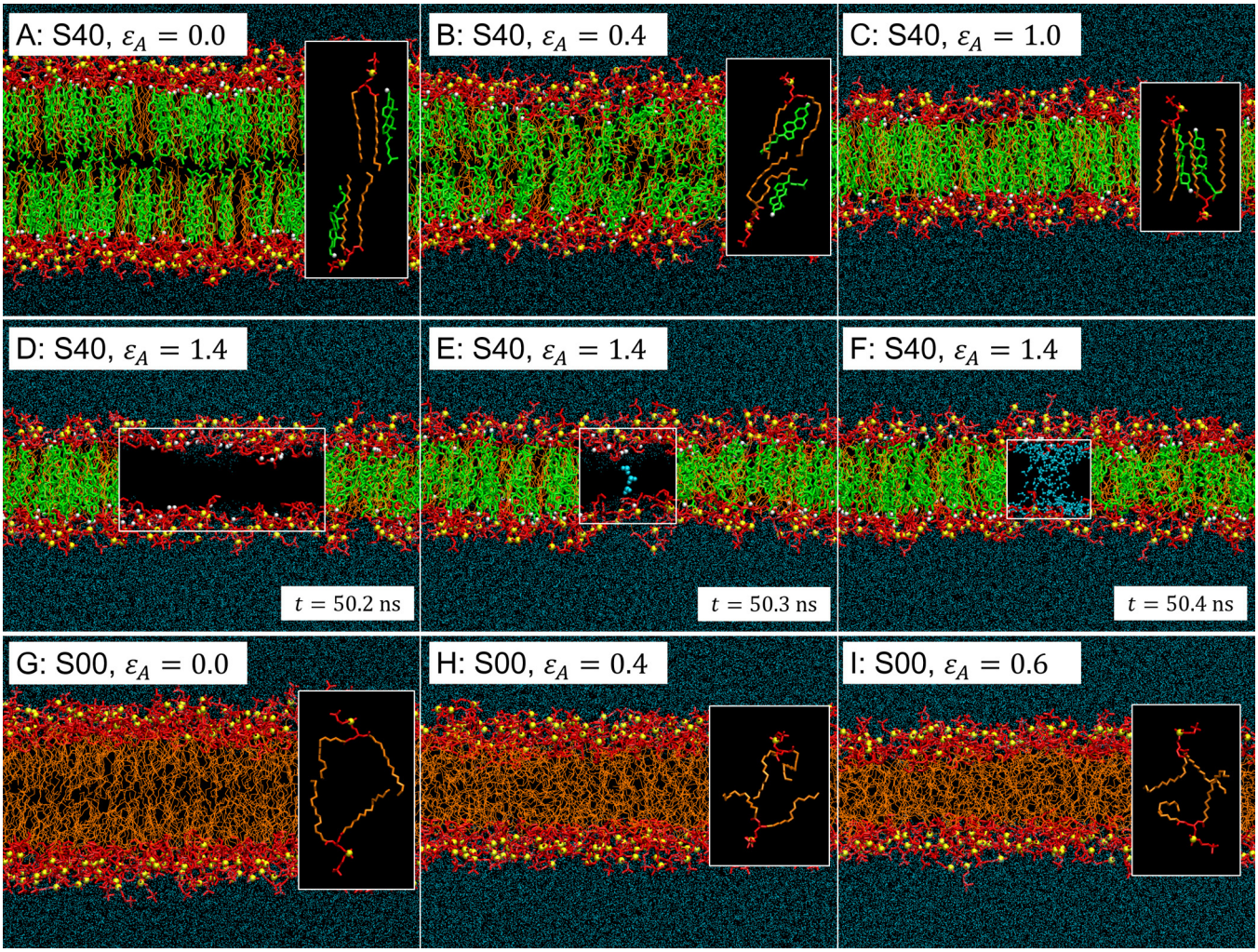

Fig. 1. Representative snapshots of the stretched bilayers for S40 (A–F) and S00 (G–I). The applied areal strains are 0.0 (A and G), 0.4 (B and H), 0.6 (I), 1.0 (C), and 1.4 (D–F). The DPPC headgroups are shown in red, the DPPC tails in orange, the cholesterol molecules in green, phosphorus atoms of the DPPC as yellow spheres, hydrogen atoms of the cholesterol molecules as white spheres, and the water molecules in blue. In the sections shown in the white frames in panels D–F, the DPPC tails and cholesterol molecules are not shown, and the water molecules in the hydrophobic part of the membrane are emphasized for clarity. The insets in panels A–C and G–I are representative positions and orientations of the DPPC and cholesterol molecules.

such molecular level phenomena in the bilayer (Tieleman et al., 2003). In fact, MD simulation studies of pore formation in stretched pure lipid bilayers (Leontiadou et al., 2004; Tolpekina et al., 2004; Koshiyama and Wada, 2011) and various cholesterol effects in the unstressed bilayer (Róg et al., 2009; Berkowitz, 2009; Niemelä et al., 2009) have been reported. Nevertheless, to our knowledge, MD simulations have not been applied to clarify the molecular details of the effect of cholesterol on pore formation and failure under stretching. Furthermore, understanding pore formation in realistic cell membranes is very important, especially for the development of various medical and experimental techniques that require delicate control of the permeability or failure of the membrane, e.g., electroporation (Gehl, 2003), sonoporation (Delalande et al., 2013), ventricular assisted devices (Rother et al., 2005), and synthetic red blood cells (Doshi et al., 2009). The lipid bilayer containing cholesterol is a more realistic model of biological cell membranes than pure lipid bilayers, and, hence, a detailed understanding of the effect of cholesterol at the molecular level is essential.

In this paper, we perform a series of molecular dynamics simulations of stretched dipalmitoylphosphatidylcholine (DPPC) bilayers containing different concentrations of cholesterol (0, 20, 40, and 60 mol%) and analyze how the processes of pore formation, the critical area where the pore is formed, and the molecular orientations in the stretched bilayers are related to the concentration of cholesterol. We discuss the relationships between the resistance of the bilayer to pore formation and the change of the membrane structure induced by stretching. These analyses will reveal the difference of the molecular mechanism of the pore formation process in the phospholipid/cholesterol bilayers with different compositions of lipid and cholesterol molecules, and provide insight into the toughness of red blood cell membranes.

2. Methods

2.1. Bilayer systems

Planer phospholipid/cholesterol bilayer systems with cholesterol concentrations of 0, 20, 40, and 60 mol% were used. Each system comprised 200 lipid molecules of dipalmitoylphosphatidylcholine (DPPC) and cholesterol molecules in rectangular simulation boxes with periodic boundary conditions. To avoid the effects of the periodic images in the bilayer normal direction during stretching, a large number of water molecules (at least 11,918) were added to the systems. The pure DPPC bilayer and DPPC/cholesterol bilayers with 20, 40, and 60 mol% cholesterol were labeled as S00, S20, S40, and S60, respectively. DPPC and cholesterol molecules were