Biophysical Chemistry 135 (2008) 95–101

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

A new interpretation of eutectic behavior for distearoylphosphatidylcholine–cholesterol binary bilayer membrane

Nobutake Tamai, Maiko Uemura, Tetsuya Takeichi, Masaki Goto, Hitoshi Matsuki *, Shoji Kaneshina

Department of Life System, Institute of Technology and Science, The University of Tokushima, 2-1 Minamijosanjima-cho, Tokushima 770-8506, Japan
Department of Biological Science and Technology, Faculty of Engineering, The University of Tokushima, 2-1 Minamijosanjima-cho, Tokushima 770-8506, Japan

ARTICLE INFO

Article history:
Received 25 January 2008
Received in revised form 27 March 2008
Accepted 27 March 2008
Available online 4 April 2008

Keywords:
Cholesterol
Distearoylphosphatidylcholine
Eutectic behavior
Hexagonal lattice
Phase diagram

ABSTRACT

We investigated the thermotropic phase behavior of the distearoylphosphatidylcholine (DSPC)–cholesterol binary bilayer membrane as a function of the cholesterol composition (X_{ch}) by fluorescence spectroscopy using 6-propionyl-2-(dimethylamino)naphthalene (Prodan) and differential scanning calorimetry (DSC). The fluorescence spectra, each of which has a single maximum, showed that the wavelength at the maximum intensity (λ_{max}) changed depending on the bilayer state: ca. 440 nm for the lamellar gel (L'_β or L_β) and the liquid ordered (L_o) phases, ca. 470 nm for the ripple gel (P'_β) phase and ca. 490 nm for the liquid crystalline (L_α) phase, respectively. The transition temperatures were determined from the temperature dependences of the λ_{max} and endothermic peaks of the DSC thermograms. Both measurements showed that the pretransition disappears around $X_{ch}=0.035$. The constructed temperature–X_{ch} phase diagram indicated that the phase behavior of the binary bilayer membrane at $X_{ch}\leq 0.15$ is similar to that of general liquid–solid equilibrium for a binary system where both components are completely miscible in the liquid phase and completely immiscible in the solid phase. It was also revealed that the diagram has two characteristic points: a congruent melting point at $X_{ch}=0.08$ and a peritectic-like point at $X_{ch}=0.15$. The hexagonal lattice model was used for the interpretation of the phase behavior of the binary bilayer membrane. These characteristic compositions well correspond to the bilayer states in each of which cholesterol molecules are regularly distributed in the hexagonal lattice in a different way. That is, each composition of 0.035, 0.08 and 0.15 is nearly equal to that for the binary bilayer membrane which is entirely occupied with units, each composed of a cholesterol and 30 surrounding DSPC molecules within the next-next-next nearest neighbor sites (Unit (1:30): $L_\beta(1{:}30)$), with units, each of a cholesterol and 12 surrounding DSPC molecules within the next nearest sites (Unit (1:12): $L_\beta(1{:}12)$) or with units, each of a cholesterol and 6 surrounding DSPC molecules at the nearest neighbor sites (Unit (1:6): $L_\beta(1{:}6)$), respectively. Therefore, the eutectic behavior observed in the phase diagram was fully explainable in terms of a kind of phase separation between two different types of regions with different types of regular distributions of cholesterol. Further, the L_o phase was found in the higher X_{ch}-region ($X_{ch}>0.15$). No endothermic peak over the temperature range from 10 to 80 °C at $X_{ch}=0.50$ suggested that the single L_o phase can exist at $X_{ch}>0.50$.

1. Introduction

Cholesterol is a major and essential constituent of plasma membranes. In mammalian plasma membranes, cholesterol is found to be about 30% of the total lipid mass [1]. Cholesterol also plays an important role in a recent model for membrane structure, the so-called raft model [2]. This model represents the structural feature of heterogeneity in lipid membranes, and the origin of the heterogeneity is closely related to the presence of cholesterol. Therefore, the clarification of the role of cholesterol as a membrane component is indispensable for better understanding the functions, properties and structures of cell membranes. Considerable researches have been made for more than three decades and large amount of information on the role of the cholesterol is now available to us. Cholesterol and the relating matters in cell membranes have been described in some recent review articles [3–8] and a textbook edited by Yeagle [9].

Since the thermotropic and barotropic phase behavior of phospholipid bilayer membranes is intrinsically related to thermodynamic properties of the bilayer membrane [10–14], drawing phase diagrams for cholesterol–phospholipid binary bilayer membranes is useful to elucidate the effect of cholesterol on phospholipid bilayer membranes. There have been many studies dealing with the determination of phase boundaries for cholesterol–phospholipid binary systems using various experimental techniques such as neutron or X-ray scattering [15,16], differential scanning calorimetry (DSC) [17,18], fluorescence spectroscopy [19–21], dilatometry [22,23], EPR (ESR) or NMR [24–28]

* Corresponding author. Tel.: +81 88 656 7513; fax: +81 88 655 3162.
E-mail address: matsuki@bio.tokushima-u.ac.jp (H. Matsuki).

0301-4622/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2008.03.008

and others [29,30]. Most studies commonly showed that the incorporation of a good amount of cholesterol remarkably enhances the stability of the overall bilayer membrane. This stabilization causes the severe uncertainty of the bilayer phase transition at the same time, so the reported phase diagrams are more or less different from one another. This inconsistency indicates that different experimental methods can monitor different aspects of the phase behavior of the binary bilayer system. Lentz et al. [19] have early pointed out the importance of extensive investigation by the combination of several techniques, although the results might render an abstruse phase diagram in terms of thermodynamics.

The phase diagram that is most extensively accepted is probably that of the cholesterol/chain perduterated dipalmitoylphosphatidylcholine (d_{62}-DPPC) system given by Vist and Davis [25]. This is because the phase diagram is consistent with the calculation based on a thermodynamic and a simple microscopic model by Ipsen et al. [31]. These phase diagrams indicate some interesting features. One is that a two-phase region appears at relatively high compositions of cholesterol: a cholesterol-rich phase and a cholesterol-poor phase. The former phase is called liquid ordered (L_o) phase, which is characterized by high conformational order in the hydrocarbon chains like the gel state and relatively fast lateral diffusion. This phase separation is considered to be relevant to the formation of the heterogeneous structure of cell membranes. Another feature is that the cholesterol and the phospholipid molecules are as miscible even in the gel phase (i.e., a solid-like state) as in the liquid crystalline phase in the low cholesterol-composition region. Such miscibility is interesting, because even the binary mixtures of phosphatidylcholine analogues tend to exhibit immiscibility in the gel phase as their difference in the molecular structure, such as the hydrocarbon-chain length and the presence of chain unsaturation, becomes greater [32]. In general, physicochemical behavior in the low composition region, namely dilute region, is of greater importance because properties of an individual solute molecule in the binary mixture can be reflected more definitely. Probably, this view can be applied to also the cholesterol–phospholipid binary membrane, and at least it is reasonable to consider that the miscibility between cholesterol and phospholipid molecules essentially changes depending on the molecular structure of the phospholipid. Nevertheless, less attention has been paid to the phase behavior in the low composition region.

In the present study, we report the thermotropic phase behavior of distearoylphosphatidylcholine (DSPC)–cholesterol binary bilayer membrane investigated by means of DSC and fluorescence spectroscopy using 6-propionyl-2-(dimethylamino)naphthalene (Prodan) as a fluorescent probe. The DSC is highly sensitive to the conformational change of hydrocarbon chains (i.e., chain melting) in the bilayer. On the other hand, the Prodan fluorescence method is sensitive to the microscopic change near the hydrophilic surface in the bilayer [33]. Thus, the combination of the two disparate techniques allows us to observe the phase behavior of the system from different aspects. Much effort has been directed toward elucidating the phase behavior especially at low cholesterol compositions. In addition, we propose a novel interpretation of the phase diagram, including the L_o phase formed at high cholesterol compositions, which can explain the lateral distribution of cholesterol molecules in the bilayer membrane on the basis of a modified superlattice model [3].

2. Materials and methods

Synthetic DSPC (1,2-distearoyl-*sn*-glycero-3-phosphocholine) and cholesterol (5-cholesten-3β-ol) were purchased from Sigma-Aldrich Co. (St. Louis, MO) and used without further purification. The fluorescent probe of Prodan (6-propionyl-2-(dimethylamino) naphthalene) was obtained from Molecular Probes Inc. (Eugene, OR). Water used in preparing sample solutions was distilled twice from dilute alkaline permanganate solution. The multilamellar vesicle (MLV) suspensions of DSPC and mixture of DSPC and cholesterol were prepared by referring to the Bangham's method [34] as follows. The stock solutions of DSPC/chloroform, cholesterol/chloroform and Prodan/ethanol were mixed and the mixed solution was dried in vacuum to remove all residual solvents. The resulting dry film was dispersed by the addition of a given amount of distilled water. The suspension obtained was stirred using a vortex mixer and sonicated for ca. 5 min at a temperature several degrees higher than the main-transition temperature of the DSPC bilayer membrane to form a homogeneous MLV suspension, which was translucent. The concentrations of DSPC and Prodan were fixed at 1 mmol kg^{-1} and 2 μmol kg^{-1} (i.e., molar ratio of DSPC to Prodan is 500:1), respectively. In our previous study [35], it has been verified from the DSC thermogram of the Prodan–DSPC bilayer membrane that such a slight amount of Prodan has no significant influence on the thermotropic phase behavior of the DSPC bilayer membrane. The cholesterol composition (X_{ch}) in the bilayer membrane was varied from 0 to 0.50 in mole fraction. Here, X_{ch} is defined as $m_{ch}/(m_{DSPC}+m_{ch})$, where m_{ch} and m_{DSPC} represent the molalities of cholesterol and DSPC in the sample solution, respectively.

The fluorescence measurements were performed using a fluorescence spectrophotometer (Hitachi Model F-3010) at constant temperatures ranging from 30 to 80 °C. Temperature was controlled within an accuracy of 0.1 °C by circulating thermostated water from a temperature-regulated water bath. The excitation wavelength was 361 nm and the emission spectra were recorded in the range of 400–600 nm.

Differential scanning calorimetry (DSC) measurements were carried out by means of high-sensitivity differential scanning calorimeter (MicroCal MCS). The heating rate was 0.33 °C min^{-1} and the temperature range was from 10 to 80 °C. The temperatures and enthalpy changes associated with the phase transitions were determined from endothermic peaks in the DSC thermograms by use of a data-analyzing software ORIGIN supplied by MicroCal.

3. Results and discussion

3.1. Fluorescence measurements for DSPC bilayer membrane

Fig. 1 shows the emission spectra of Prodan in the DSPC bilayer membrane at every 2 °C from 48 to 60 °C. Every spectrum has a single maximum, and the wavelength at the maximum fluorescence intensity (λ_{max}) shifts from ca. 440 to ca. 490 nm with increasing temperature. It is known that the λ_{max} is sensitive to the polarity of microscopic environment around the fluorescent probe molecule. We also have previously confirmed that the λ_{max} values of Prodan in various solvents vary depending on the dielectric constant of the solvent and that there is a good correlation between the dielectric constant and the λ_{max} value [33]. Therefore, the shift of the λ_{max} of

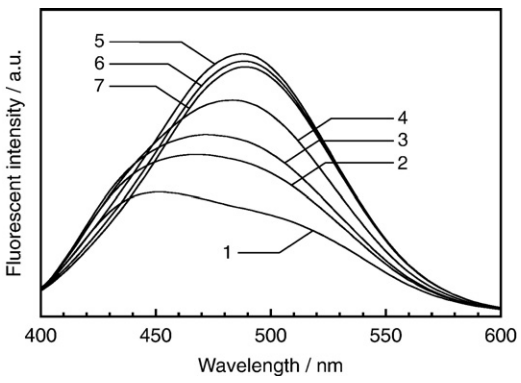

Fig. 1. Emission spectra of Prodan in DSPC bilayer membrane at every 2 °C from 48 to 60 °C: spectrum 1, 48 °C; 2, 50 °C; 3, 52 °C; 4, 54 °C; 5, 56 °C; 6, 58 °C; 7, 60 °C.