Colloids and Surfaces B: Biointerfaces 72 (2009) 135–140

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Interdigitation and vesicle-to-micelle transformation induced by a local anesthetic tetracaine in phospholipids bilayers

Koichi Takeda[a,1], Hiroko Okuno[a], Takashi Hata[b], Makoto Nishimoto[a], Hitoshi Matsuki[a,c,*], Shoji Kaneshina[a,c]

[a] Department of Biological Science and Technology, Faculty of Engineering, The University of Tokushima, 2-1 Minamijosanjima-cho, Tokushima 770-8506, Japan
[b] Department of Material Science and Technology, Kochi National College of Technology, 200-1 Monobe-otsu, Nankoku, Kochi 783-8508, Japan
[c] Department of Life System, Institute of Technology and Science, The University of Tokushima, 2-1 Minamijosanjima-cho, Tokushima 770-8506, Japan

ARTICLE INFO

Article history:
Received 28 January 2009
Received in revised form 26 March 2009
Accepted 26 March 2009
Available online 5 April 2009

Keywords:
Bilayer interdigitation
Lipid membrane
Local anesthetic
Phospholipids
Vesicle-to-micelle transformation

ABSTRACT

The phase transitions of distearoyl- (DSPC), dipalmitoyl- (DPPC) and dimyristoyl-phosphatidylcholine (DMPC) bilayer membranes were observed by means of differential scanning calorimetry as a function of the concentration of a local anesthetic tetracaine hydrochloride (TC·HCl). The depression of both temperatures of the main- and pre-transition, which is accompanied by a decrease in enthalpy changes for both transitions, was observed initially by the addition of TC·HCl. Bilayer interdigitation, which is accompanied by an increase in enthalpy change for the main transition from the interdigitated gel phase to the liquid crystalline phase, was followed by disappearance of the pretransition. The TC·HCl concentration necessary for the bilayer interdigitation was found to be 10, 21 and 6 mmol kg^{-1} for DSPC, DPPC and DMPC bilayers, respectively, which was not consistent with the order of acyl-chain length of lipids. Biphasic interactions for the interdigitation, that is, repulsive interaction between polar head groups and van der Waals attractive interaction between hydrophobic chains of lipids, were discussed. On the other hand, vesicle-to-micelle transformation, which is accompanied by a cooperative decrease in enthalpy change for the main transition, was observed at higher concentration of TC·HCl and was confirmed by the vesicle size determined by the dynamic light scattering. The longer the acyl-chain length of lipids, the higher the TC·HCl concentration necessary for the vesicle-to-micelle transformation.

1. Introduction

In order to elucidate the molecular interactions between biological membranes and anesthetic molecules including short-chain alcohols, so far many physico-chemical approaches have been reported using model biomembranes such as phospholipid bilayers, although current studies on molecular mechanism of anesthesia concentrate on the interaction between proteins and anesthetic molecules. The most representative phospholipid, dipalmitoylphosphatidylcholine (DPPC) has been widely used in the study of model biomembranes. It is well known that the DPPC bilayer membrane without thermal annealing undergoes two phase transitions with increasing temperature: the pretransition from the lamellar gel (L'_β) phase to the ripple gel (P'_β) phase and the main transition from the P'_β phase to the liquid crystalline (L_α) phase, in turn [1,2]. The main-transition temperature of DPPC bilayer membrane was depressed by the addition of anesthetics in a dose-dependent manner [3–8]. This depression of the main-transition temperature has been often analyzed as a colligative property of bilayer membranes and has led to the partition coefficients of anesthetics into the membrane [5,6]. Rowe [9,10] has shown that short-chain alcohols induce a biphasic chain-melting behavior in phosphatidylcholine (PC) bilayers with different acyl-chain lengths; that is, the main-transition temperature of PCs is reduced at low concentrations of alcohol but increased at high concentrations. It was shown that the longer the acyl-chain length of the lipid, the lower the alcohol concentration at which the inflection in the main-transition temperature occurs [9]. It was subsequently shown by Simon and McIntosh [11] that the alcohol-induced biphasic behavior observed in PCs is a consequence of acyl-chain interdigitation. The existence of the interdigitated gel ($L_\beta I$) phase has been confirmed in PC membrane systems including other surface active molecules such as benzyl alcohol, chloropromazine, tetracaine, ethylene glycol, etc. [12,13]. The $L_\beta I$ phase has also been observed in the absence of any inducer in an ether-linked phospholipid dihexadecylphosphatidylcholine

* Corresponding author at: Department of Life System, Institute of Technology and Science, The University of Tokushima, 2-1 Minamijosanjima-cho, Tokushima 770-8506, Japan. Tel.: +81 88 656 7513; fax: +81 88 655 3162.
E-mail address: matsuki@bio.tokushima-u.ac.jp (H. Matsuki).
[1] Present address: Pharmaceutical Technology Institute, Otsuka Pharmaceutical Factory, Inc., Muya-cho, Naruto, Tokushima 772-8601, Japan.

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doi:10.1016/j.colsurfb.2009.03.026

bilayer [14,15], and in saturated diacyl PC bilayers under high pressure [16–19].

On the other hand, some local anesthetics such as tetracaine (TC·HCl) and dibucaine hydrochlorides are known to form micelles in the aqueous solution [20–23]. Therefore, the vesicle–micelle transformation has been reported in the systems of phospholipids and micelle-forming surfactants such as TC·HCl, bile salts and so on [24–31]. In our previous study, the phase transitions of DPPC bilayer membrane were observed by means of differential scanning calorimetry (DSC) as a function of the concentration of local anesthetics including TC·HCl [32].

In the present study, interaction of three kinds of PCs, distearoyl-PC (DSPC), DPPC and dimyristoyl-PC (DMPC), with a local anesthetic TC·HCl has been investigated by the DSC and the dynamic light scattering (DLS) techniques. The study of molecular interactions between lipid bilayer membranes and a local anesthetic is fundamentally useful for the molecular mechanism of anesthesia and is also interesting from the viewpoint of colloidal and interfacial aspects.

2. Materials and methods

2.1. Materials

Three kinds of phospholipids, 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), were purchased from Sigma–Aldrich Corp. (St. Louis, MO) and used without further purification. A local anesthetic tetracaine hydrochloride (TC·HCl), 2-(dimethylamino)ethyl-4-(butylamino) benzoate, was obtained from Sigma–Aldrich Corp. in the crystalline form and recrystallized several times from ethanol. Water was distilled twice after a deionization, where the second step was done from dilute alkaline permanganate solution.

Powder lipid was first suspended in anesthetic solution with various concentrations to give a lipid concentration of 2.0 mmol kg^{-1}. The suspensions adjusted at various concentrations of TC·HCl up to 160 mmol kg^{-1} were sonicated for a few minutes by using a Branson model 185 sonifier and a cup horn at a temperature several degrees above the main-transition temperature of lipid, in order to prepare the phospholipid multilamellar vesicles suitable for the phase transition measurements [18,19].

2.2. Differential scanning calorimetry

The phase transitions of DSPC, DPPC and DMPC bilayer membranes in the presence of a local anesthetic TC·HCl were observed by a Microcal MCS high-sensitivity differential scanning calorimeter (Northampton, MA). Before the measurements, the suspension was degassed for 3 min. The heating rate was 0.75 K min^{-1}.

2.3. Dynamic light scattering

The average size of phospholipids–TC·HCl aggregates was measured by a dynamic light scattering spectrophotometer DLS-7000 (Otsuka Electrics, Osaka, Japan) with a He–Ne laser operating at 633 nm. All measurements were made at temperatures higher than the main-transition temperature for respective phospholipid bilayers: at 55 °C for DSPC–TC·HCl, 45 °C for DPPC–TC·HCl and 25 °C for DMPC–TC·HCl bilayer systems, respectively. The intensity auto correlation function was analyzed by the method of histograms. Sample solutions were filtered through 0.45 μm Millipore filters prior to each measurement.

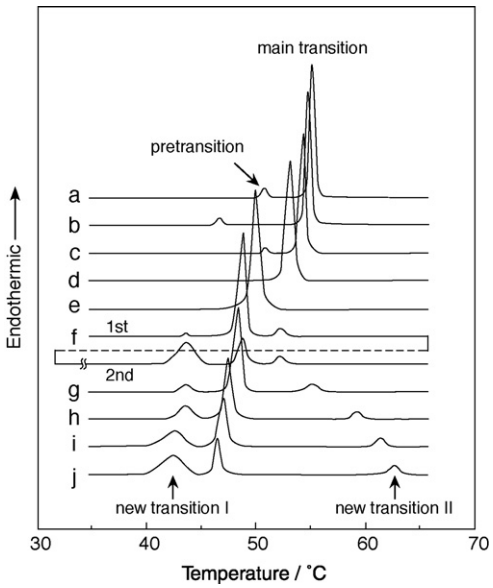

Fig. 1. DSC thermograms of DSPC bilayer membrane including tetracaine of various concentrations: (a) without tetracaine, (b) 5, (c) 13, (d) 14, (e) 111, (f) 119, (g) 126, (h) 132, (i) 137 and (j) 142 mmol kg^{-1}. Thermogram (f) contains second scan. DSPC concentration is 2.0 mmol kg^{-1}.

3. Results and discussion

3.1. Effect of tetracaine on the thermotropic behavior of lipid bilayers

Fig. 1 shows typical DSC thermograms of DSPC bilayer membranes at several concentrations of TC·HCl. The temperatures of main transition (T_m) and pretransition (T_p) in the absence of TC·HCl (curve a in Fig. 1) were observed at 55.0 and 50.8 °C, respectively, which are in good agreement with previous data [2,19]. Both of T_m and T_p were depressed by the addition of TC·HCl (curve b). The endothermic peak of the pretransition from the L'_β phase to the P'_β phase became smaller by the addition of TC·HCl and has almost disappeared at the concentration of 10 mmol kg^{-1} TC·HCl. Sequentially the elevation of the pretransition temperature was observed in the narrow range of TC·HCl concentrations from 10 to 14 mmol kg^{-1} (shown in curve c). This small endothermic peak was incorporated with the main transition peak above 14 mmol kg^{-1} TC·HCl. Similar phase behavior of DSPC and DPPC bilayer membranes in the presence of ethanol has been observed by Ohki et al. [33]; the appearance of the $L_\beta I$ phase instead of the L'_β phase and subsequently the disappearance of the P'_β phase. Present behavior of pretransition could be elucidated with the aid of the phase diagram, which is shown in Fig. 2. The pretransition observed in the narrow range of TC·HCl concentration from 10 to 14 mmol kg^{-1} can be assigned as the transition from the $L_\beta I$ phase to the P'_β phase because of the appearance of the $L_\beta I$ phase instead of the L'_β phase at the TC·HCl concentration above 10 mmol kg^{-1}. In the range of TC·HCl concentration from 14 to 111 mmol kg^{-1} (curves d and e), only a main transition from the $L_\beta I$ phase to the L_α phase was observed. In the presence of TC·HCl of higher concentration than 112 mmol kg^{-1}, we found two types of new transitions except for the main transition (curves f∼j). One is the transition observed at a lower temperature than the temperature of the main transition, which is called the new transition I. The temperature of new transition I was depressed gradually as the concentration of TC·HCl increases. The other is the transition at a higher temperature than the temperature of the main transition, which is called the new