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EFFECT OF SUBSTITUTION OF HYDROGEN OXIDE BY DEUTERIUM OXIDE ON THERMOTROPIC TRANSITION BETWEEN THE INTERDIGITATED GEL PHASE AND THE RIPPLE PHASE OF DIHEXADECYLPHOSPHATIDYLCHOLINE

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SUMMARY: Thermotropic transitions of dihexadecylphosphatidylcholine (DHPC) dispersions in hydrogen oxide (^1H_2O) and deuterium oxide (^2H_2O) were investigated by differential scanning calorimetry (DSC). In DHPC dispersions, transition temperature between interdigitated gel phase (LβI) and ripple phase (Pβ') is lower in ^2H_2O than in ^1H_2O, and transition between the ripple phase (Pβ') and fluid phase (Lα) in ^2H_2O occurs at a temperature slightly higher than in ^1H_2O. In dipalmitoylphosphatidylcholine (DPPC) dispersions, on the other hand, transition temperature between lamellar gel phase (Lβ') and ripple phase is higher in ^2H_2O than in ^1H_2O. These results suggest that the interdigitated gel phase is more stable in ^1H_2O than in ^2H_2O. To account for the shift of transition temperature by the water substitution, difference of interfacial energies between these aqueous environments is discussed.

INTRODUCTION: In an interdigitated gel phase of phospholipid membranes, acyl chains of the phospholipid penetrate the apposing monolayer. Now it is known that the interdigitated structure is induced in dispersions of various phospholipid species under certain conditions. Diacyl phosphatidylcholines form the interdigitated structure in the presence of alcohol such as ethanol, methanol and so on [1]. And dihexadecylphosphatidylcholine (DHPC), an dialkyl phosphatidylcholine, exhibits the interdigitated gel phase [2] being hydration dependent [3]. In this case, phase transitions proceed in the order of interdigitated gel phase, ripple phase and fluid phase as the temperature is raised. In the recent study on DPPC and distearoylphosphatidylcholine (DSPC), we have manifested that ethanol induces interdigitated gel phase between lamellar gel and ripple phases [4]. Despite of many studies on this phase, the mechanism inducing the interdigitated gel phase is still unknown.

In the present report, in order to investigate the effect of hydrophobic interaction on the appearance of interdigitated gel phase, the phase transition temperatures of DHPC dispersions were compared between in ^1H_2O and in ^2H_2O. The transition temperature between interdigitated gel phase (LβI) and ripple phase (Pβ') was raised by the substitution of hydrogen oxide by deute-

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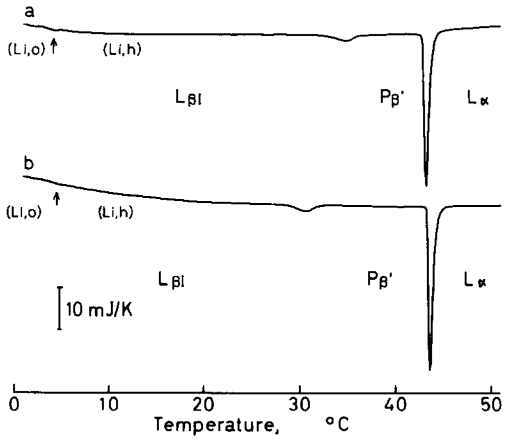
rium oxide. This result is explained by change in interfacial free energy since deuterium bond is stronger than hydrogen bond.

MATERIALS AND METHODS: Synthetic 1,2-dihexadecyl-sn-glycero-3-phosphocholine (DHPC) was purchased from Fluka Chemie AG (Buchs, Switzerland). And synthetic 1,2-dimyristoyl-sn-glycero-3-phosphocholine(DMPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) were purchased from Sigma Chemical Co. (St. Louis, MO). Pure hydrogen oxide was obtained from tap water by distilling twice, passing through an ion exchange column and filtrating with Milli-Q Labo (Millipore). Specific resistance of the purified water was 17.8 MΩ·cm. Deuterium oxide(^2H_2O, purity 99.75 %) was purchased from Wako Pure ChemicalIndustries Ltd., Osaka). Lipid dispersions were prepared by the following procedure. A chloroform solution including 0.75 μmol of phosphatidylcholine was taken into a pan (Du Pont 900796.901), and the solvent was evaporated naturally at first and then under reduced pressure overnight. Fifteen microliters of ^1H_2O or ^2H_2O was added to the phosphatidylcholine in the pan, and then the pan and a cover (Du Pont 900790.901) were sealed hermetically. In order to hydrate the phosphatidylcholine to make dispersions, it was heated up above the main transition temperature in a water bath. Calorimetric scans were performed with a SEIKO DC10 and SSC580 differential scanning calorimeter (SEIKO I&E, Tokyo), at a heating scan rate 1.0 K/min [5].

RESULTS AND DISCUSSION

It is well known that amphipathic phospholipid molecules spontaneously assemble to form membranes in aqueous environment by principally hydrophobic force. This is the most important nature of phospholipids in constructing basic structure of biomembranes [6]. And the hydrophobic interaction among the phospholipid molecules is primary based on hydrogen bonding of water molecules. By a statistical thermodynamic treatment based on the flickering cluster model, the energy required to break one mole of hydrogen bonds in the liquid has been estimated to be 1.32 kcal/mole [7] and that of deuterium bonds has been found to be 1.56 kcal/mole [8]. Therefore, substitution of hydrogen dioxide (^1H_2O) by deuterium oxide (^2H_2O) must affect phase behavior of lipid assemblies in the water. Actually, the substitution shifts phase transition temperatures of dielaidoylphospatidylethanolamine and 1-palmitoyl-2-oleoyl-phosphatidylethanolamine membranes [9].

DHPC molecules, which are hydrated enough or in excess water, give three transition troughs thus four phases in a temperature range between 0 °C and 50 °C (Fig. 1a). Laggner et al. [3] have identified these phases as an interdigitated gel phase with an orthorhombic arrangement of fatty alkyl chains, an interdigitated gel phase with a hexagonal arrangement, a ripple phase and a fluid phase, respectively, in a direction of temperature increase. In contrast to a bilayer gel structure of diacyl phosphatidylcholines, there exist interdigitated membrane structures below the ripple phase in the DHPC, a dialkyl phosphatidylcholine, dispersions. The substitution of ^1H_2O by ^2H_2O enhances the hydrogen bonding force, which is reflected differently on each



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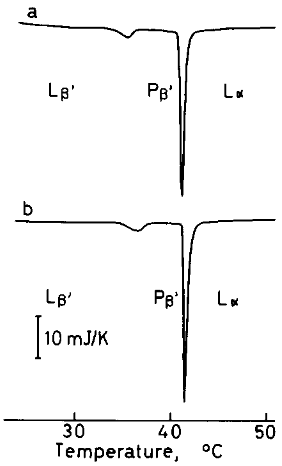

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Fig. 1. Thermograms of DHPC in (a) hydrogen oxide and (b) deuterium oxide. Fifteen microliters of 50 mM DHPC dispersions was enclosed in a DSC pan. The rate of heating scan was 1 K/min. Arrows indicate the transition trough between interdigitated gel phases with an orthorhombic arrangement (Li,o) and with a hexagonal arrangement (Li,h).
LβI, interdigitated gel phase; Pβ', ripple phase; Lα, fluid phase.

Fig. 2. Thermograms of DPPC in (a) hydrogen oxide and (b) deuterium oxide. Fifteen microliters of 50 mM DPPC dispersions was enclosed in a DSC pan. The rate of heating scan was 1 K/min.
Lβ', lamellar gel phase; Pβ', ripple phase; Lα, fluid phase.

phase transition. Although the temperatures of the orthorhombic to hexagonal and the ripple to fluid transitions are raised slightly, the transition temperature of the interdigitated to the ripple phases is remarkably lowered by the substitution (Fig. 1b).

The effect of substitution of ^1H_2O by ^2H_2O is also examined in dispersions of diacyl phosphatidylcholines. Fig. 2 shows thermograms of DPPC dispersions in ^1H_2O and ^2H_2O. The endothermic troughs indicate the pre-transition of the gel phase to the ripple phase and the main transition of the ripple phase to the fluid phase. Those transition temperatures are slightly raised by the substitution of ^1H_2O by ^2H_2O. And the same effects are also observed in other diacyl phosphatidylcholine dispersions; DMPC and DSPC dispersions.

The phase transition temperatures of the dialkyl phosphatidylcholine and three diacyl phosphatidylcholines in hydrogen oxide and deuterium oxide are summarized in Table I. The deuterium bonding of ^2H_2O lowers the transition temperature of the interdigitated to the ripple phases by 3 °C. In a theoretical consideration of lipid phase transition, an effective Hamiltonian is represented by three terms. They are interactions among the head groups, interaction between chains, and conformational energy of the chains [10]. The first term includes some effects of the aqueous medium due to hydration and hydrophobic interactions. Interaction free energy between lipid molecules in an assembly is principally composed of an attractive interaction arising from

Table I. Phase Transition Temperatures of Phosphatidylcholine Dispersions in Hydrogen Oxide and in Deuterium Oxide

	Transition temperature [°C]			
	in hydrogen oxide		in deuterium oxide	
	LβI → Pβ'	Pβ' → Lα	LβI → Pβ'	Pβ' → Lα
DHPC	34.89 ± 0.34 (4)	43.24 ± 0.00 (4)	30.85 ± 0.31 (4)	43.53 ± 0.06 (4)
	Lβ' → Pβ'	Pβ' → Lα	Lβ' → Pβ'	Pβ' → Lα
DMPC	13.74 ± 0.69 (6)	23.45 ± 0.11 (6)	15.76 ± 0.16 (4)	23.77 ± 0.06 (4)
DPPC	35.25 ± 0.49 (4)	41.18 ± 0.05 (4)	37.01 ± 0.12 (3)	41.62 ± 0.11 (3)
DSPC	50.28 ± 0.29 (5)	54.63 ± 0.09 (5)	52.18 ± 0.16 (3)	55.00 ± 0.06 (3)

Values are expressed as the mean±S.D. from 3-6 independent experiments. Figures in parentheses represent the number of experiments.

hydrophobic force or interfacial tension and a repulsive interaction arising from electrostatic head-group repulsion and steric repulsions. Thus the interfacial free energy per molecule in the assembly is represented by

$$\mu = \gamma \cdot a + C/a$$

where γ is density of hydrophobic free energy, C is free energy constant of electrostatic repulsion and a is the molecular area measured at the hydrocarbon-water interface [11]. The minimum free energy is given when the molecular area is

$$a = a_0 = \sqrt{C/\gamma} \ .$$

The density of hydrophobic free energy, γ, is greater in ^2H_2O than in ^1H_2O, which indicates that the area occupied by a phosphatidylcholine molecule on the membrane interface is smaller in ^2H_2O than in ^1H_2O. X-ray diffraction and a scanning density meter studies have revealed that the area per DHPC molecule in one side of the membrane is 77.6 Å^2 for the LβI (Li,h) phase while the area per DHPC molecule in the Pβ' phase is 40.8 Å^2 [3]. Therefore, the transition from the bilayer to interdigitated structures requires increase of the area per phospholipid chain. Greater value of γ in deuterium oxide decreases the area, which is supposed to lower the phase transition temperature of the interdigitated to the ripple phases. On the other hand, addition of ethanol induces interdigitated gel phase between lamellar gel and ripple phases in diacyl phosphatidylcholine dispersions [4]. The appearance of interdigitated gel phase might be explained by reduction of hydrophobic force in ethanol-containing water.

Transition temperatures of pre-transitions in diacyl phosphatidylcholine dispersions are raised by the substitution of ^1H_2O by ^2H_2O (Table I). On the surface of DPPC membranes, the area occupied by the phospholipid molecule is 39.1 Å^2 in the lamellar gel phase, and it increases to be 40.6 Å^2 as entering the ripple phase [12]. Thus stronger hydrogen bonding force in ^2H_2O raises the transition temperature probably because suppressing the expansion of the area occupied by the phosphatidylcholine molecule. The substitution of ^1H_2O by ^2H_2O raises the temperatures of transitions from ripple to fluid phases, or main transitions, slightly in diacyl phosphatidylcholines as well as DHPC (Table I), which suggests that the effect doesn't depend on type of linkage between glycerol and hydrocarbon chain but depends on phase structures of the transition. In phosphatidylethanolamine membranes, the substitution of ^1H_2O by ^2H_2O have raised the temperature of lamellar gel to fluid phase transition and lowered that of fluid to hexagonal II phase transition [9].

One of the most important force to form lipid membranes in the aqueous environment is hydrophobic force produced by hydrogen bonding. The present study shows that change in hydrogen bonding affects physical properties of the membranes. In particular, the transition between the interdigitated gel and the ripple phases is markedly affected by the change in hydrogen bonding force. And the result suggests that appearance of the interdigitated structure depends on the interfacial free energy.

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