Interdigitated Structure of Phospholipid-Alcohol Systems Studied by X-Ray Diffraction

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ABSTRACT In the interdigitated structure of phosphatidylcholine/alcohol systems, the one-dimensional electron density profile in the direction normal to the membrane surface is generated from the x-ray diffraction pattern. The membrane thickness for these systems is expressed by the sum of the hydrocarbon chain lengths of phosphatidylcholine and alcohol molecules. For this study, various sets of phosphatidylcholines and 1-alcohols were used; a phosphatidylcholine has a carbon number from 14 to 18 in a hydrocarbon chain, and an alcohol has a carbon number from 1 (methanol) to 4 (1-butanol). Based upon the results, we propose a model for the interdigitated structure in which 1) two alcohol molecules occupy a volume whose surface is surrounded interstitially by the headgroups of phosphatidylcholine molecules, and 2) the methyl ends of both hydrocarbon chains in alcohol and phosphatidylcholine molecules face each other at the bottom of the volume.

INTRODUCTION

For more than a decade, many reports have pointed to the existence of the interdigitated state under a variety of experimental conditions. Mixed-chain phosphatidylcholine (PC) gives rise to chain interdigitation in bilayers, e.g., PC with 18 carbons in the *sn*-1 chain and 10 carbons in the *sn*-2 chain (18:10 PC), 12:18 PC, etc. (McIntosh et al., 1984; Boggs and Mason, 1986; Hui and Huang, 1986; Mattai et al., 1987; Ali et al., 1989). In the structure of the fully interdigitated layer, the methyl ends of the hydrocarbon chains of PC molecules (PCs) on one surface are close to the polar headgroups on the opposite surface. Such a structure is found when the PC headgroup is connected with the *sn*-2 position (Serrallach et al., 1983) and in lysophospholipid (Pascher and Sundell, 1985). Dihexadecylphosphatidylcholine (DHPC) bilayers also form a fully interdigitated structure in excess water (Ruocco et al., 1985a,b; Laggner et al., 1987). Other fully interdigitated structures take place in phospholipids with symmetrical hydrocarbon chains in solution by addition of some amphiphilic molecules such as polymyxin B (Ranck and Tocanne, 1982; Theretz et al., 1983), choline and acetylcholine (Ranck and Tocanne, 1982), alcohol (Rowe, 1983, 1985; Kamaya et al., 1984; Simon and McIntosh, 1984; Nambi et al., 1988; Boggs et al., 1989; Ohki et al., 1990; Rowe and Cutrera, 1990; Roth and Chen, 1991), buffer molecules (Wilkinson et al., 1987), and anions (Cunningham and Lis, 1986). In addition, high pressure-induced interdigitation in PC bilayers has been reported (Kaneshina et al., 1992).

Many small molecules cause the interdigitation in PC. Among those molecules, alcohol is one of the best known. There is a biphasic effect of alcohol concentration on the main transition temperature of PCs. The addition of 1-alcohol series to PCs depresses the transition temperature at the low concentration of alcohol, stabilizing the liquid crystalline phase. On the other hand, it elevates the transition temperature at high concentrations of alcohol, destabilizing the liquid crystalline phase (Rowe, 1983). Furthermore, the 1-alcohol series induces the fully interdigitated structure below the liquid crystalline phase (Nambi et al., 1988). The alcohol concentration, where the transition temperature is minimal, decreases systematically with a carbon number of alcohol molecules (Rowe, 1985) and with the carbon number of the hydrocarbon chain in PCs (Rowe, 1983).

From a systematic x-ray diffraction study on the lamellar structure of various sets of PC and alcohol molecules, this paper aims to propose a model of the interdigitated structure based upon the membrane thickness obtained as a function of the carbon number of both PC and alcohol molecules.

MATERIALS AND METHODS

Phospholipids, 1,2-dimyristoyl-*sn*-3-phosphatidylcholine (DMPC), 1,2-dipalmitoyl-*sn*-3-phosphatidylcholine (DPPC), and 1,2-distearoyl-*sn*-3-phosphatidylcholine (DSPC) were obtained from Avanti Polar Lipids, Inc. (Alabaster, AL). All materials were used without further purification. Methanol, ethanol, 1-propanol, and 1-butanol with 99.5% pure were obtained from Katayama Chemical Ltd. (Osaka, Japan).

A desired amount of lipid was dispersed in a water solution of alcohol; the concentrations were chosen so that the PC/alcohol systems might undergo interdigitated phase transition and lie close to the minimum temperature on each main transition line (Rowe, 1983, 1985). All the samples were incubated in the liquid crystalline phases above the main transition temperature for about 1 h with vortexing and then cooling to the gel phases. The details of the conditions are listed in Table 1.

X-ray diffraction experiments were carried out at station 15A of the Photon Factory in National Laboratory for High Energy Physics in Tsukuba, Japan (Amemiya et al., 1983). X-ray diffraction patterns were recorded with a storage phosphor detector called an imaging plate (Type BA-III, Fuji Photo Film, Tokyo, Japan) (Amemiya and Miyahara, 1988). The sample-to-detector distance was about 200 mm, and the diffraction spacing was calibrated with a powder pattern of synthetic fluorophlogopite mica (National Bureau of Standards, Washington, DC). The Debye-Scherrer rings on the

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TABLE 1 Experimental conditions for samples used

Lipid	Alcohol	Concentration		Temperature (°C)		
		Alcohol (mg/ml)	Lipid (wt%)	Main transition*	Incubation	Experiment
DMPC	Propanol	198		23.5	40	5
	Methanol	106				
		120				
	Ethanol	80				
DPPC		152		41.4	55	25
	Propanol	60				
		198				
	Butanol	50	40			
	Methanol	106				
		120				
	Ethanol	80				
DSPC		152		55.1	70	35
	Propanol	60				
		198				
	Butanol	50				

*In the case without existence of alcohol (Marsh, 1990).

imaging plate were transformed into one-dimensional data as follows. First, the intensity with a particular diffraction angle was obtained by adding all the intensity data at the same distance from the center of the direct beam on the imaging plate, and second, it was divided by the number of data points at the same distance from the center. Before analysis, the contribution of water and polyimide films to the background was subtracted. Details of experiments and analyses were described elsewhere (Tenchov et al., 1989; Takahashi et al., 1991, 1992).

RESULTS

Wide-angle x-ray diffraction

The wide-angle x-ray diffraction patterns of pure DPPC and DPPC/ethanol systems are shown in Fig. 1, *A* and *B*, respectively. A reflection appears at 0.42 nm with a broad shoulder around 0.41 nm in pure DPPC at a lipid concentration of 4 mg/10 mg water, indicating that this sample is in the gel ($L_{\beta'}$) phase at 25°C. In the DPPC/ethanol system at a lipid concentration of 4 mg/10 mg of solution in which the ethanol concentration is 80 mg/ml, a single sharp reflection appears at 0.41 nm. This is indicative of an interdigitated gel ($L_{\beta I}$) phase at 25°C. The same reflection appears in the other PC/alcohol systems (data not shown). The sharp and symmetrical single peak indicates that the hydrocarbon chains are packed in a hexagonal lattice, and the direction of chains is normal to the membrane surface. These profiles agree with the results of Simon and McIntosh (1984) and Nambi et al. (1988).

Small-angle x-ray diffraction

The small-angle x-ray diffraction pattern of the DPPC/ethanol system recorded at 25°C is shown in Fig. 2 as a lipid concentration of 4 mg/10 mg of the solution in which the ethanol concentration is 80 mg/ml. The lamellar repeat period is 4.89 nm. The sharp lamellar reflection peaks appear and higher-order lamellar reflections up to the fifth order are detectable. There are two additional broad background humps to the background of water and polyimide films. One lies between the first and the second peaks, and the other lies beneath the third peak. In the patterns of the other PC/alcohol systems, we could observe sharp higher order lamellar reflections up to the fifth order, but only the values of the

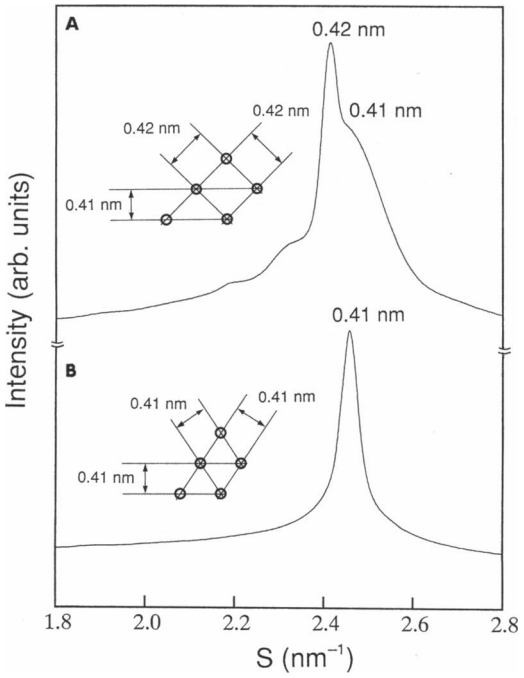

FIGURE 1 (*A*) Wide-angle x-ray diffraction pattern of DPPC in water, and (*B*) that in ethanol solution of 152 mg/ml. These patterns were recorded at 25°C with lipid concentration of 4 mg lipid/10 mg of the solution. In *A*, there is a reflection at 0.42 nm with a broad shoulder around 0.41 nm. In *B*, there is a single sharp peak at 0.41 nm. The inserts are hydrocarbon chain packing lattices: *A* indicates distorted triangular lattice, and *B* indicates right hexagonal lattice. *S* is defined as 2 $\sin\theta/\lambda$.

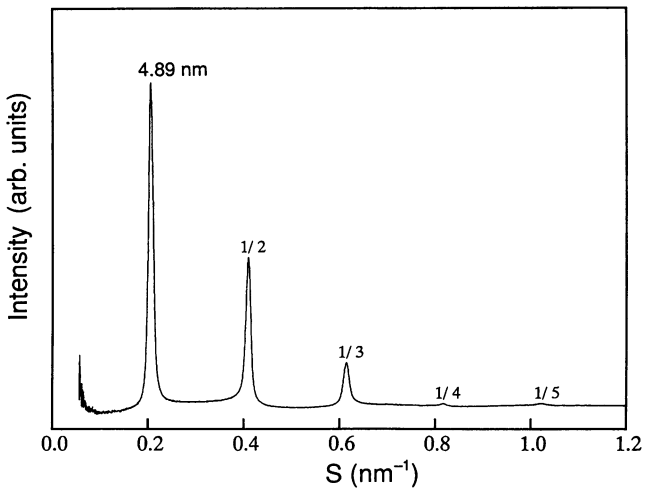

FIGURE 2 Small-angle x-ray diffraction pattern of DPPC at 25°C with lipid concentration of 4 mg lipid/10 mg of the solution in which ethanol concentration is 80 mg/ml. In this pattern, reflections up to the fifth order can be observed. The lamellar repeat period is 4.89 nm.

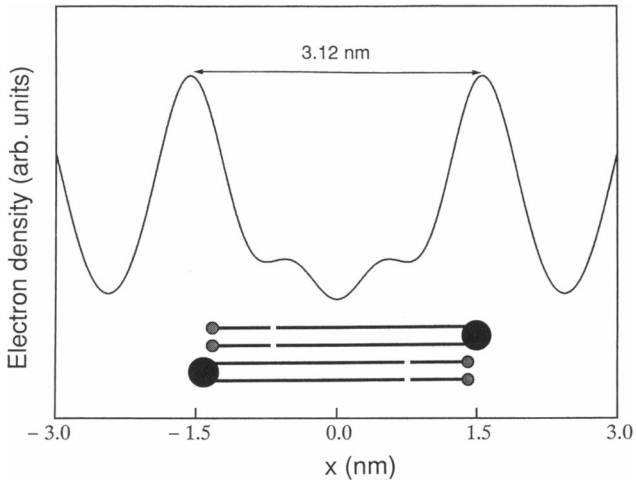

FIGURE 3 The one-dimensional electron density profile generated from the diffraction pattern given in Fig. 2. The adopted phases of the first to the fifth orders are (π,π,0,π, and π) (see text). A schematic view of the structure in the $L_{\beta I}$ phase is inserted, with the black dots representing the lipid headgroups and the lines representing the lipid hydrocarbon chains.

lamellar repeat periods are different between PCs. In the following, we treat the DPPC/ethanol system as a representative of the PC/alcohol systems, but the same analyses were performed to the other PC/alcohol systems.

Electron density

For the DPPC/chlorpromazine system that undergoes an interdigitated phase, McIntosh et al. (1983) have performed a series of swelling experiments to determine the phase angles from the first to the third order reflections in the phase. Based upon the result, for the DPPC/ethanol system Nambi et al. (1988) have used the same phase set and have obtained a reasonable electron density profile. Then, we adopted the same phase set from the first to the third order reflections in the DPPC/ethanol system. Furthermore, a phase set of the fourth and the fifth order reflections was taken so as to get the flat profile around the portion of the hydrocarbon chains region as (π,π), respectively. Fig. 3 shows the one-dimensional electron density profile of the DPPC/ethanol system at 25°C in the interdigitated phase that was generated from the diffraction pattern given in Fig. 2, where the one-dimensional direction is normal to the membrane surface. The electron density profiles for the other PC/alcohol systems were calculated under the same phase set as for the DPPC/ethanol system. The electron density profiles of all the PC/alcohol systems studied take a characteristic shape in an interdigitated phase. A highest peak in the electron density profile of PC/alcohol systems shows PC headgroups. We can estimate the separation of PC headgroups at both surfaces of the membrane, and then the distance of the separation is the membrane thickness. For example, in the DPPC/ethanol system, the spacing of 3.12 nm indicates the separation of PC headgroups at both surfaces of the membrane, i.e., the membrane thickness. The spacing of 1.77 nm indicates the separation of the adjacent membranes, i.e., the thickness of a water layer.

As a result, the dependence of the membrane thickness on the carbon number of alcohol is plotted in Fig. 4. The two lines for DSPC systems and DPPC systems have slopes of 0.08 ± 0.02 nm and 0.10 ± 0.02 nm per hydrocarbon chain unit, respectively. Here, one hydrocarbon chain unit indicates one —CH_2— in a hydrocarbon chain. Fig. 5 shows the dependence of the membrane thickness on the sum of the carbon numbers of lipid and alcohol in PC/1-alcohol systems. The slope of the line is 0.08 ± 0.01 nm per hydrocarbon chain unit.

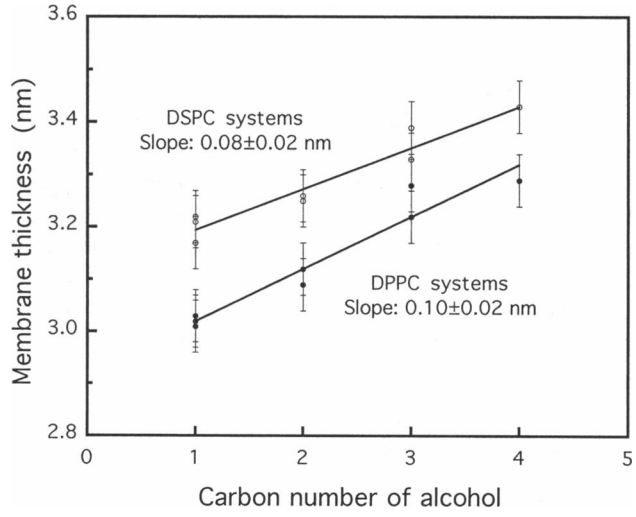

FIGURE 4 The dependence of the membrane thickness on the carbon number of alcohol (○, DSPC/alcohol systems; ●, DPPC/alcohol systems). The hydrocarbon number in DPPC is 16 and that in DSPC is 18. These lines have almost a similar slope (for DSPC, 0.08 ± 0.02; for DPPC, 0.10 ± 0.02 per one hydrocarbon chain unit in units of nm).

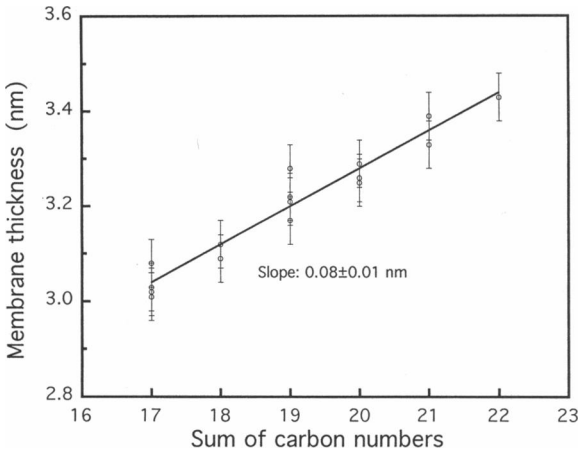

FIGURE 5 The relationship between the membrane thickness in the $L_{\beta I}$ phase and the sum of carbon numbers of hydrocarbon chain of PC and alcohol molecules. The thickness is proportional to the total length of the hydrocarbon chains. The slope, 0.08 ± 0.01/one hydrocarbon chain unit, corresponds almost to the length of one hydrocarbon unit in units of nm.

DISCUSSION

In the PC/alcohol systems, the interdigitated structures have been studied intensively (McDaniel et al., 1983; Simon and McIntosh, 1984; Nambi et al., 1988; Tenchov et al., 1989), and furthermore, the electron density profiles have been obtained (McDaniel et al., 1983; Simon and McIntosh, 1984; Nambi et al., 1988). However, the position of the alcohol molecules in the interdigitated structure is still not clear. In this paper, the interdigitated structure of the PC/alcohol systems is systematically analyzed by x-ray diffraction. From the present results we propose a model of the interdigitated structure indicating the position of the alcohol molecules as shown in Fig. 6. Alcohol molecules occupy a volume surrounded interstitially by the headgroups of PCs, and therefore, the methyl ends of both hydrocarbon chains of alcohol and PCs face each other. From the dependence of the membrane thickness on the carbon number of the alcohol, the membrane thickness increases by about 0.1 nm per one hydrocarbon chain unit in alcohol molecules. Furthermore, the membrane thickness also increases by about 0.1 nm per hydrocarbon chain unit of a PC. Therefore, from both the slopes of the results in Fig. 5 and 6, it follows that almost the same increase in membrane thickness occurs when one hydrocarbon chain unit is added in either the lipid or alcohol hydrocarbon chain. The above results are explained in terms of the length of one —CH_2— unit. It is 0.1 nm in the stretched chain based on a molecular model of alkane (Pauling, 1960). Thus the increase of the membrane thickness is consistent with the length of one —CH_2—. However, the incremental increase of the membrane thickness, somewhat less than 0.1 nm, is less than the length of hydrocarbon unit for a stretched chain. This might be due to fluctuations of the hydrocarbon chain. The above results indicate that two alcohol molecules can occupy a volume surrounded by the PC headgroups of one side of the layer and also by the methyl ends of the PC hydrocarbon chains of the other side.

It is worthwhile to point out that the proposed model is consistently interpreted in terms of the structural parameters in the gel phase of DPPC as follows. First, from the membrane thickness in the gel phase of DPPC (Simon and McIntosh, 1984), a half thickness of the membrane, 2.43 nm, is estimated by assuming that the tilt angle (Wiener et al., 1989) of the hydrocarbon chain is zero. Here, from the hexagonal packing lattice of hydrocarbon chains shown in Fig. 1, we have assumed that the tilt angle is 0. This gives the length of the phospholipid part as seen in Fig. 7. Second, we obtain the distance, twice 0.16 nm, between the methyl ends of the

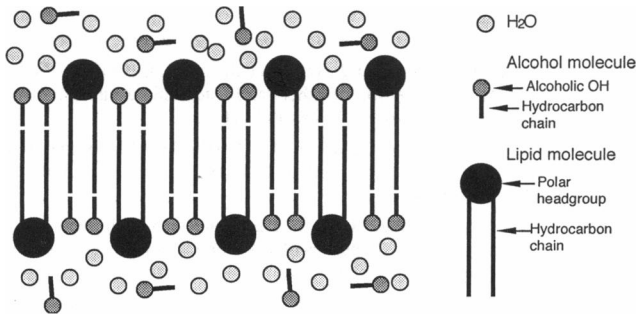

FIGURE 6 The model of the interdigitated structure for the PC/alcohol systems. Two alcohol molecules are filled in a volume surrounded interstitially by the headgroups of PCs. The hydroxyl groups of alcohol molecules face the water layer. Black dots represent the PC headgroups, and lines represent the hydrocarbon chains in both PC and alcohol molecules.

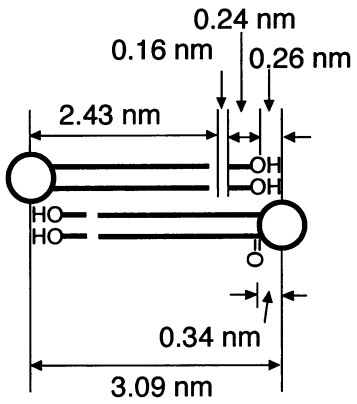

FIGURE 7 The numerical parameters of model of the interdigitated structure in DPPC/ethanol systems. From the results of neutron scattering experiments (Zaccai et al., 1979) the following distance was estimated: half the distance between methyl ends of an acyl chain and an alcohol molecule is 0.16 nm. From the results of x-ray diffraction for the gel phase of DPPC (Simon and McIntosh, 1984; Wiener et al., 1989), the distance was estimated as follows: half the bilayer thickness is 2.43 nm. Finally, the distance between an oxygen atom in an alcohol molecule and a phosphorus atom in a headgroup was determined to be 0.26 nm using the above estimated distance together with the thickness, 3.09 nm, of an interdigitated membrane that was obtained from the present experiment. On the other hand, from the results of neutron-scattering experiments (Zaccai et al., 1979) the following distance was estimated: the distance between a phosphorus atom in a headgroup and the position of carbonyl on an acyl chain is 0.34 nm. The position of the oxygen atom of an alcohol molecule stands close to the position of the carbonyl of DPPC; i.e., the difference between the two positions is only about 0.08 nm (=0.34–0.26 nm).

bilayer hydrocarbon chain from the neutron scattering data for the gel phase of DPPC (Zaccai et al., 1979). This distance is applied to the estimation for the distance between the methyl ends of PC and alcohol molecules. The length of an alcohol molecule, 0.24 nm, is calculated by the values both of the atomic bond length and angle data. The distance between the phosphorus atom and the carbon atom of carbonyl, 0.34 nm, is given by 2.43 nm (given above) − 2.09 nm. The latter is obtained from the neutron scattering data (Zaccai et al., 1979) together with both molecular bond length and angle data. Based upon the above parameters, we can consider the boundaries between the hydrophobic and hydrophilic regions for both ethanol and DPPC molecules in the interdigitated gel phase. It is concluded that the position of hydroxyl on an alcohol molecule stands close to the position of carboxyl on a DPPC molecule as seen in Fig. 7. This is relevant to the amphiphilic nature of phospholipid molecules and alcohol molecules.

In the small-angle x-ray diffraction of the PC/alcohol systems, we observed that there were two background humps; one lies between the first and second order peaks, and the other lies beneath the third. In these profiles, the backgrounds of water and polyimide films have already been subtracted from the raw data. We calculate an electron density function of a single bilayer vesicle in the DPPC/ethanol systems. In this calculation, we assumed that the electron density of the polar headgroup had a Lorentzian shape, and we connected hydrocarbon chain level and water level with an arc tangent function. Furthermore, we used the values of the electron density of the headgroup, methylene region, and water region reported by Wiener et al. (1989). Then we could draw the background humps from these values as follows. We calculated the Fourier transform of the electron density function for the single bilayer structure, i.e., the structure factor, and then obtained the square of the structure factor. It gives the two humps, whose spacings almost correspond to the background humps in the present experiment. The results indicate that the suspensions of the PC/alcohol systems are composed not only of multilamellar vesicles but also the single bilayer and the minor disarrays in the multilamellar structures.

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