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Thermodynamic reversibility of phase transitions. Specific effects of alcohols on phosphatidylcholines

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The gel-to-fluid phase transitions of several phosphatidylethanolamines (PE's) and phosphatidylcholines (PC's) have been investigated in the presence of three short-chain alcohols. The effects of the alcohols on the thermodynamic reversibility of these transitions was studied and it was found that the transitions for PC's are not thermodynamically reversible at relatively high alcohol concentrations. The PE transitions are thermodynamically reversible for all alcohol concentrations, and the PE's do not exhibit the biphasic effects of alcohol on the transition temperature previously reported for the PC's (Rowe, E.S. (1983) Biochemistry 22, 3299–3305). The biphasic transition temperature effects and the thermodynamic irreversibility of PC transitions at high alcohol concentrations appear to be correlated with the induction of a fully interdigitated gel phase recently reported in the literature (Simon, S.A. and McIntosh, T.J. (1984) Biochim. Biophys. Acta 773, 169–172). The biological significance of these findings is discussed.

Introduction

The role of lipid physical properties in the function of membranes is not understood. Membranes are regulated with respect to lipid composition, and lipid compositions have been shown to change to adapt to changes in growth temperatures of *Escherichia coli* [1]. Lipid compositions also respond to the chronic presence of alcohol in mice [2–4]. However, a general molecular explanation of the role of lipid properties in the function of membranes has not been elucidated. An important property of pure lipids and lipid mixtures which depends on composition is their ability to exist in several different phases, depending on temperature, lipid class, and the length and degree of saturation of their acyl chains (for review, see Refs. 5–7). In addition to the well-characterized fluid and gel phase states, it is now recognized that many lipids can exist in several bilayer states, including a fully interdigitated gel state [8,9], and in some cases, in non-bilayer states [10]. Phase equilibria of the lipids and lateral phase separations of lipids of differing composition or phase state has been proposed by a number of authors [11–16] to be important for membrane function.

The action of general anesthetics provides some insight into these questions. It has long been known that the anesthetic potency of many small structurally unrelated organic compounds have anesthetic potencies which are related to their membrane : buffer partition coefficients or their lipid solubilities (for review see Ref. 17). It is

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Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PE (x : y) and PC (x : y), PC or PE with acyl chains of x length having y unsaturations.

generally believed that these anesthetics work by dissolving in the membrane lipids and altering their physical properties and therefore the membrane function. Thus the study of the effects of general anesthetics such as alcohols on lipid physical properties can provide information pertaining to the role of these properties in function.

A great deal of information about membranes has been obtained through the study of the thermotropic properties of pure synthetic lipids and simple lipid mixtures. In general the phase behavior of these lipids is studied using classical thermodynamic approaches (e.g., Refs. 5, 6). These approaches have the implicit assumption that the processes examined are thermodynamically reversible; however, in many cases the thermodynamic reversibility of the particular system is not established. In our investigations we have found that the assumption of thermodynamic reversibility of phase 'equilibria' is not always justified; it must be demonstrated under the conditions of interest before any valid thermodynamic analysis can be made.

We have been systematically investigating the physical interactions of synthetic lipids with alcohols in order to elucidate the role of lipid physical properties in the mechanisms of general anesthesia and/or intoxication, and to gain some insight into the role of the lipid properties and lipid composition in membrane function. In a previous publication we showed that ethanol has a biphasic effect on the gel-to-liquid crystal phase transition of phosphatidylcholines, indicating two independent interactions of ethanol with PC's [18]. Recently it has been shown by X-ray diffraction that the secondary high concentration effect of alcohol on the transition temperature of PC(16 : 0) correlates with the induction of the newly discovered interdigitated gel state [19]. In the current investigation we have studied the reversibility of the gel-to-fluid phase transitions of PC's and PE's as a function of alcohol concentration with the unexpected result that under some conditions, alcohols have a profound effect on the thermodynamic reversibility of these transitions. In addition, the specificity of the two alcohol lipid interactions are investigated with respect to both alcohol and lipid. The biological implications of these results are also discussed.

Methods

Lipids

The lipids were obtained from Sigma or Avanti, and used as supplied after verification of purity by thin-layer chromatography; the lipids were of the L-α configuration. The samples were hand shaken multilamellar vesicles prepared as described previously by the method of Bangham et al. [20]. The concentration of lipid in the suspensions was approximately 0.6 mg/ml. The samples contained 16.6% sucrose added to reduce settling and improve optical data [21].

Spectrophotometry

The phase transitions were followed by the change in absorbance at 400 nm of the lipid suspensions, as described previously [18,21,22]. The change in absorbance which occurs during the gel-to-liquid crystal phase transition is a light-scattering change due to the change in refractive index increment of the lipid as the lipid density changes during melting [23]. Some changes in the anisotropy of the bilayer surface may also contribute to this light-scattering change [23]. The absorbance method of following phase transitions has the advantage over other optical methods, which involve probe molecules, that it follows a property of the lipid itself, and does not rely on a probe molecule which could perturb the property of interest or require assumptions concerning its location. This method has been previously widely used for following the phase transitions of PC's (e.g., Refs. 23–26). The present study confirms that the method also is suitable for following the transitions of PE's.

The phase transitions were followed by the change in absorbance at 400 nm, using the Cary 219 spectrophotometer interfaced with the Apple II microcomputer system. The Cary 219 is equipped with built-in thermistor and cell programmer. The absorbance and temperature data were read directly into the computer, stored on floppy disks, and analyzed and plotted as required. The temperature is controlled by water circulated through three jacketed cuvettes connected in series with a programmed bath circulator. Two cuvettes contained samples and the thermistor was placed in the third cuvette at the depth

of the light beam. The accumulation of data into the computer and its subsequent manipulation and storage was carried out using our own BASIC software. The routine for least squares variable-point smoothing and derivative calculation was based on the Savitzky-Golay method [27], and used a BASIC algorithm generously provided by Dr. Raja G. Khalifah.

Reversibility experiment

In order to demonstrate the thermodynamic reversibility of a process it is necessary to show that the state of the material is dependent only on the final conditions, and not on the pathway of achieving those conditions. The reversibility experiments were designed to compare the states of two identical samples simultaneously under identical conditions, which had been subjected to different pathways. The steps of the experiments were as follows. (1) A double-sized sample was prepared as usual, including the addition of the desired amount of ethanol. (2) The sample was then divided in two, and one half was incubated above the transition temperature (55°C) and the other below the transition (20°C) for 30 min. (3) Meanwhile, the two sample cuvettes in the Cary were equilibrated at the selected mid-transition temperature. (4) The first sample was then added to the Cary cuvette, and the absorbance was followed until apparently stable (5 min). The second sample was then placed in the Cary and followed for 5 min. (5) Using the cell programmer, the two samples were then followed simultaneously (i.e., alternating at 30-s intervals) for at least 30 min. Some samples were followed for 17 h. (6) Finally, the two samples were then followed simultaneously through a cooling-heating-cooling cycle.

Results

Effects of different alcohols on lipid melting temperature

The effect of methanol, ethanol, and propanol on the apparent transition temperature of PC(16:0) as measured during heating scans are shown in Fig. 1. The effect of ethanol on the apparent melting temperature of three PE's as measured during heating scans are shown in Fig. 2. As seen here, methanol and propanol give a biphasic effect on PC similar to that exhibited by ethanol for PC(16:0), and previously demonstrated for PC's of varying acyl chain lengths [18]

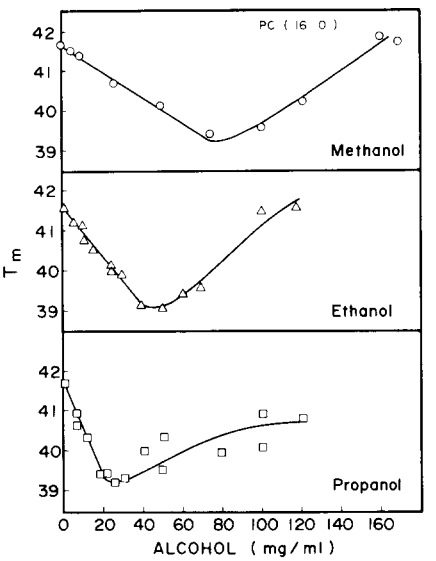

Fig. 1. Dependence of the transition midpoint temperature of PC(16:0) on alcohol concentration for methanol (○), ethanol (△), and propanol (□) as determined by absorbance at 400 nm.

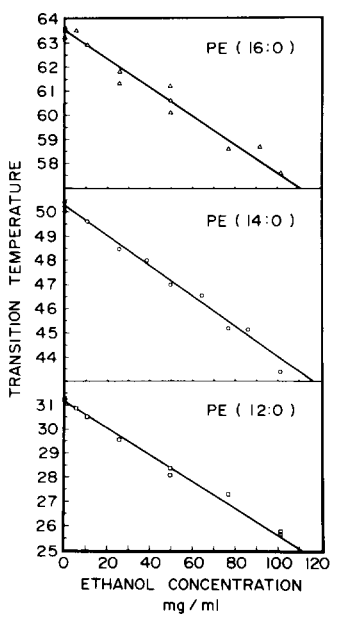

Fig. 2. Dependence of the transition midpoint temperatures of PE(12:0), PE(14:0), and PE(16:0), as a function of ethanol concentration, as followed by absorbance at 400 nm.

and for short-chain alcohols in PC(16 : 0) [28]. At relatively low alcohol concentrations the effect of the alcohol is to reduce linearly the apparent transition temperature, indicating preferential interactions of alcohol with the fluid-phase lipid. The effect of ethanol on the PE's appears to be similar to that of the alcohols on PC's in the low concentration range.

At high alcohol concentrations, the apparent transition temperature of the PC's begins to increase and eventually levels out. This result indicates that there is a second alcohol-lipid interaction with PC's stabilizing the gel phase relative to the fluid phase. It appears saturable, and must involve additional binding of the alcohol to the lipid. It has been shown that, at 60 mg/ml ethanol, ethanol induces an interdigitated gel phase in PC(16 : 0) below the gel-to-liquid crystal phase transition [19]. Thus the biphasic effect of alcohols on PC's is apparently the result of the transition of the gel phase to the interdigitated gel phase upon the addition of the alcohol. This effect does not occur with the PE's.

Thermodynamic reversibility of phase transitions

The definition of thermodynamic reversibility is that the state of the material is dependent only on the conditions of temperature, pressure, composition, etc., and not on the history of the sample or the pathway of arriving at those conditions. A system in which two phases are in equilibrium is a dynamic one in which the forward and reverse reactions have the same rate. In order for such a system to respond to changes in conditions and reach a new state of equilibrium, for example during a temperature scan, these forward and reverse rates must be reasonably rapid. If they are not, then the 'transition curve' obtained is not an equilibrium curve.

The thermodynamic reversibility of the gel-to-fluid phase transition of PC under various conditions was tested as described in the Methods section by dividing the sample in two. The two halves were then treated differently, and brought by different pathways to the final temperature; the phase state was then examined simultaneously under a particular condition to determine whether they were identical.

Fig. 3 shows the results for PC(16 : 0) in the presence of 25 mg/ml ethanol and in the absence of ethanol. The solid curves with arrows indicate the pathway that the lipid followed upon heating and cooling; as seen here, there is a difference of approx. 0.5°C in the midpoints obtained in heating and cooling curves. The points represent the results of the reversibility experiment in which a single sample was divided into halves, and one half being taken to the midpoint temperature from the high temperature (fluid state) side of the transition, and the other half being taken to the midpoint temperature from the gel state. The absorbance of both members of the pair was then followed simultaneously at that constant temperature until they reached equilibrium, or stopped changing. The arrows indicate the progress of the members of the pair over time as they were held at this temperature. There is only one point there to indicate that both members of the pair did reach the same absorbance. The conclusion that they had both reached the same phase state was verified by taking both members of the pair simultaneously through a heating-cooling-heating cycle, and showing that they were identical in their behavior. From these results it can be concluded that this phase transition is thermodynamically reversible, and that the curve shown is nearly the true equlibrium curve. (The true equilibrium curve is the one that would have been obtained if the experiment described above were done at every

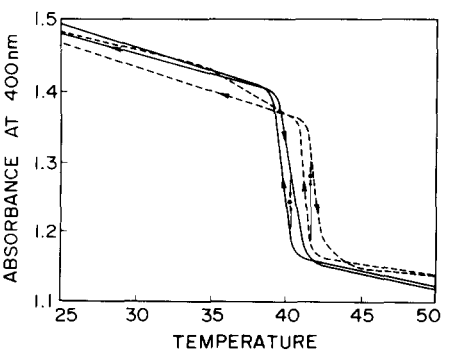

Fig. 3. Reversibility experiment in the presence of no (broken line) and 25 mg/ml (solid line) ethanol for PC(16 : 0), performed as described in the text. The points indicate the final values of absorbance of the solution pairs after 30 min at the indicated temperatures. The curves represent the heating and cooling curves subsequently followed by both samples in the respective pair.

point on the curve; thus the true curve must lie between the observed heating and cooling curves.)

Fig. 4 shows the results of the kinetic part of the experiment. In this example, the two identical samples reached their final equilibrium value in approx. 2 min; the rates appear similar for the sample which is approaching the center of the transition from the gel state and the one approaching the center from the fluid state. The control experiment represents a measure of the kinetics of the equilibration of the temperature itself for these experiments. In these experiments the temperature itself takes approx. 1 min to equilibrate, so it is probable that the apparent mild hysteresis seen in Fig. 3 is due to the lag in the temperature equilibration.

Fig. 5 shows a similar reversibility experiment on PC(16:0) in the presence of 100 mg/ml ethanol. As seen here there is a 4 deg. C discrepancy between the heating and cooling curves when run at a heating and cooling rate of 0.75 deg/min. As described above, reversibility experiments were performed on the halves of samples which were brought to the temperature from either side of the transition. The two open circles at 39.1°C represent the 'final' values of the absorbances of this pair of samples after 17 h of incubation at 39.1°C. A similar experiment was performed at 40.5°C. The arrows indicate the progress of each pair member approaching the transition from the gel state (20°C) or the fluid state (55°C). It is seen that, even after 17 h of incubation, the two samples of each pair were far from being in the same physical state. In order to establish further the state of the samples after the 17 h equilibration, the sample pair was taken simultaneously through a cooling-heating-cooling scan. The initial cooling process is indicated by the dotted lines; for all four of the samples, the subsequent heating and cooling followed the solid lines.

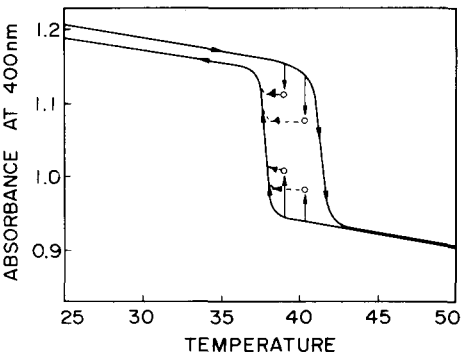

Fig. 5. Reversibility experiment of PC(16:0) in the presence of 100 mg/ml ethanol, as described in the text. The open circles represent the values of absorbance achieved after 17 h equilibration at the indicated temperature. The broken lines with arrows indicate the initial progress of the cooling cycle initiated after 17 h; the solid curves represent the subsequent cooling and heating cycle of both samples in the pair, which were superimposable.

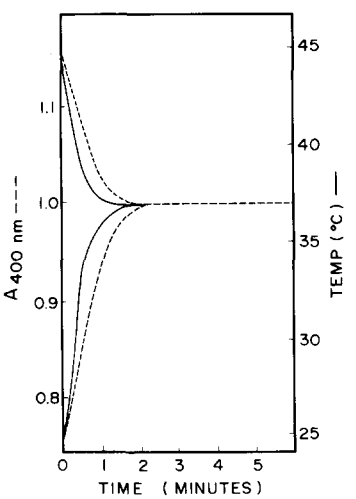

Fig. 4. Results of the kinetic part of the experiment shown in Fig. 3, with no ethanol. Dashed lines represent the equilibration of the absorbance of the members of the solution pair as a function of time. The solid lines represent the equilibration of the solution temperature after the solution is added to the pre-equilibrated cuvettes in the Cary sample compartment.

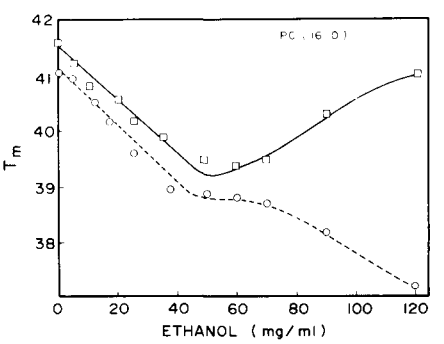

Fig. 6. Apparent transition midpoints of PC(16:0) for heating and cooling scans as a function of ethanol concentration.

These results demonstrate that the transition curves obtained for PC(16 : 0) at high alcohol concentrations are not thermodynamically reversible. The state of the lipid at temperatures near the transition temperature is metastable; i.e., instead of being in a state of dynamic equilibrium between the two states, with relatively rapid forward and reverse reactions going on, the lipid molecules are kinetically 'frozen' in whatever state they happen to be.

Fig. 6 shows the apparent transition midpoint for heating and cooling scans of PC(16 : 0) as a function of ethanol concentration. It is seen that the onset of hysteresis is correlated with the beginning of the upward curvature of the alcohol dependence of the apparent melting temperature curves. Similar results were obtained in the following systems: ethanol-PC(18 : 0), methanol-PC(16 : 0), and propanol-PC(16 : 0). In each of these systems there was only a small rate-dependent hysteresis in the low alcohol concentration region, and increasingly pronounced hysteresis in the high alcohol concentration range. From these findings it is concluded that it is the high-concentration PC gel-alcohol state, that is, the putative interdigitated state, for which the transition to and from the fluid state is not thermodynamically reversible.

The reversibility of the PE phase transition as a function of ethanol concentration was also examined. Fig. 7 shows the apparent heating and cooling transition midpoints for PE(12 : 0) as a function of ethanol concentration. Here it is seen that high concentrations of ethanol do not lead to significant hysteresis in this system, indicating that the transition of PE is thermodynamically reversible throughout the alcohol concentration range studied.

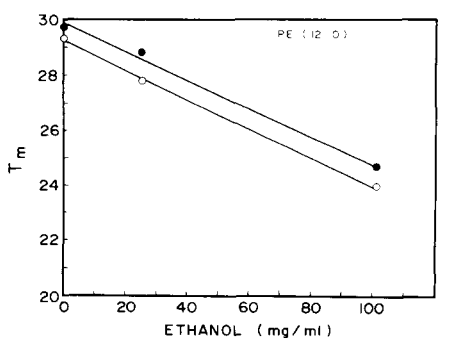

Fig. 7. Apparent transition midpoint of PE(12 : 0) for heating and cooling scans as a function of ethanol concentration.

Alcohol and lipid specificity

As seen in Figs. 1 and 2, at low alcohol concentration, the T_m is lowered for both PC and PE. The membrane : buffer partition coefficients were calculated from the initial slopes using the thermodynamics of freezing point depressions as described in detail previously [18], as originally described by Hill [29]. The membrane : buffer partition coefficients are listed in Table I, along with the enthalpy of the transition used in the calculation. It is seen that for ethanol, the partition coefficients for the PE's and PC's are very similar. The partition coefficients for the three alcohols into PC(16 : 0) vary with the alcohol chain length as expected on the basis of their increasing hydrophobicity. The partition coefficients for the three alcohols are in good agreement with literature values [17,30].

The high concentration effect which leads to the formation of the interdigitated state for PC's does not occur with the PE's. This could indicate that there is a specific interaction between the alcohol and the PC headgroup. However, a more plausible interpretation of this difference in the lipids is that the ability or tendency to form the interdigitated phase is dependent on the lipid class.

TABLE I

MEMBRANE : BUFFER PARTITION COEFFICIENTS

Membrane : buffer partition coefficients, in dimensionless mole fraction units, for the indicated alcohol-lipid pairs, were calculated using the melting point depression in the low concentration region as described previously [18]. The enthalpies used in the calculations are also shown.

Lipid	Alcohol	T_m	ΔH	K (mol/mol)
PE(12 : 0)	ethanol	31.1	4.0 [a]	3.0
PE(14 : 0)	ethanol	50.2	6.4 [a]	4.8
PE(16 : 0)	ethanol	63.2	8.5 [a]	5.7
PC(16 : 0)	methanol	41.8	7.2 [b]	2.3
PC(16 : 0)	ethanol	41.8	7.2 [b]	4.3
PC(16 : 0)	propanol	41.8	7.2 [b]	17.5

[a] Ref. 45.
[b] Ref. 18.

TABLE II

THRESHOLD ALCOHOL CONCENTRATIONS FOR BIPHASIC EFFECT IN PC's

Alcohol concentrations at the threshold of the biphasic effect. The partition coefficients shown here were calculated as described in the text for propanol and methanol, and the ethanol values are from Ref. 18.

Alcohol	PC	T_m	K_p (mol/mol)	Aqueous alcohol (mol/l)	Membrane alcohol (mole fract.)
Ethanol	14:0	24.0	3.0	1.84	0.10
	15:0	34.0	3.7	1.10	0.08
	16:0	41.8	4.3	1.02	0.08
	17:0	48.7	5.1	0.74	0.07
	18:0	54.4	7.0	0.54	0.07
	19:0	59.2	3.7	0.43	0.05
Methanol	16:0	41.8	2.3	2.5	0.10
Propanol	16:0	41.8	17.5	0.33	0.11

Alcohol concentration effects

Table II shows the threshold alcohol concentrations for the apparent transition to the interdigitated phase for the series of PC's studied previously [18] and for the three alcohols studied in the current investigation. Both the solution and mole fraction membrane alcohol concentrations, calculated from the partition coefficients, are given. As seen here, there is a wide variation in the solution alcohol concentrations, but the threshold membrane alcohol concentrations are very similar for all the examples studied. (The PC(19:0) is the exception, but the error in the partition coefficient for this lipid is unusually large because of the high temperature and the short region of linearity in the T_m depression [18]). The similarity of the membrane alcohol concentrations over a wide range of lipids and alcohols suggests that the mechanism of the transition to the interdigitated phase may involve the membrane-dissolved alcohol in the fluid phase.

Discussion

Thermodynamic reversibility

Perhaps the most important finding of the present investigation is the demonstration that alcohol induces a lipid state which is not thermodynamically reversible under some conditions. The fact that such a condition can occur emphasizes the importance of testing each system studied for thermodynamic reversibility before performing extensive thermodynamic analyses of the transition curves obtained. Indeed, it is also important to point out again the qualitative difference between a mid-transition condition in which half of the lipid is kinetically trapped in one state, and the other half in the other, as compared with an equilibrium mid-transition state in which there is a dynamic exchange between the two phases. It seems that much confusion exists in the literature over the meaning of the word 'equilibrium'. As noted below, there is an increasing number of recognized lipid systems, both natural and synthetic, which exhibit hysteresis due to metastable states.

Hysteresis of phase transitions due to metastable states has been observed in a number of lipids. For example, such anomalous thermotropic behavior has been reported for cerebrosides and for certain sphingomyelins [31–35]. In these examples, the metastability is attributed to hydrogen-bonded networks in the headgroup region of the lipids. Anomalous thermotropic behavior including hysteresis has also been observed in phosphatidylcholines with nitroxide labels on the fatty acyl chains buried deep in the bilayer [36,37], and on PC(16:0) with difluoro substituents on various positions on the acyl chains, again deep in the bilayer [38]. In the systems studied here, it was found that irreversibility was correlated with the interdigitated gel state for the PC's. The PE's did not show

biphasic effects with alcohols, or irreversibility, so it is suggested that the PE's do not form the interdigitated state under these conditions. The question can be raised as to whether other instances of irreversibility or metastability, such as those cited above, can be due to new physical states such as the interdigitated phase.

Interdigitated gel states have been described for PC's and phosphatidylglycerols (PG's) in the presence of several amphipathic molecules [8,9], and for mixed-chain PC's [39]. In addition, it has been shown that the antibiotic polymyxin B induces an interdigitated gel state in dipalmitoylphosphatidylglycerol [40], and in binary mixtures of PG and PC [41]. No direct studies of the melting thermodynamics of this phase have been reported, but there has been a suggestion that the enthalpy of this melting transition is higher than that of the usual gel phase [42]. The possibility that the enthalpy of melting is affected by the new phase, and the finding that this process is not thermodynamically reversible, suggests that analysis of the curve shapes or even the temperature of melting, in terms of thermodynamic models, is not necessarily valid.

Extremely slow kinetics of equilibration under intermediate conditions, with rapid formation of products under the more extreme conditions, has been observed in other systems, for example, in the equilibration of the denaturation and dissociation equilibria of a cooperative two-subunit protein, the Fab fragment of IgG [43]. In that instance it was concluded that the extremely slow kinetics were due to the fact that a bimolecular reaction involved folded species that were themselves not stable as monomers. In the present case, also, it appears that in the region of temperature where the hysteresis occurs there are two stable phases, the interdigitated gel phase-alcohol complex and the fluid phase. If the usual gel phase with no bound alcohol is an intermediate in the interconversion of these two phases, then under conditions where this phase is not stable, the kinetics of conversion can be very slow because the amount of the intermediate state is very low. In going from the fluid phase to the gel-alcohol complex, one or more alcohol molecules must interact with a nucleating cluster of gel phase lipids. At temperatures where the gel phase is not stable unless the alcohol is bound, these clusters are rare. Thus the kinetics of formation of this state are slow. In the other direction, one or more alcohol molecules must dissociate from several gel phase lipid molecules simultaneously for the lipid to melt; at temperatures where the interdigitated gel-alcohol complex is marginally stable, this is a relatively rare event. Thus, under these intermediate conditions the interconversion of the two states is extremely slow. In addition, if there is a conformational change in the gel state prior to melting, such as a conversion from the interdigitated to the bilamellar state, one or more of the individual kinetic constants can be quite slow, also.

Alcohol and lipid specificity

The results reported here, combined with our previous results, and the discovery of the interdigitated phase, provide new insight into the specificity, and possibly the mechanism, of the biphasic effects of alcohols on PC phase transitions. As discussed above, it appears that the transition to the interdigitated gel state for PC's is the explanation for the biphasic effect of alcohols on PC phase transitions. The lipid specificity of this effect is therefore most likely a reflection of the relative tendencies of the different lipid classes to form the interdigitated state. The role of the alcohol in the transition to the interdigitated state is to stabilize the interdigitated state by binding to the lipid surface and interacting with the hydrophobic terminal methyl groups which are near the surface of the lipid. As cited above [8,9], there is a variety of amphipathic compounds which stabilize the interdigitated state in PC's and PG's. Thus there is no need to invoke specific alcohol-PC headgroup interactions to explain the biphasic effect.

New perspective on the acyl chain length and/or temperature effect on the threshold of the biphasic effect reported previously [18] has also been provided by the current results. It has been shown that the threshold membrane alcohol concentrations are very similar for each system, when expressed as mole fraction alcohol in the fluid lipid. Thus the apparent wide variation in threshold aqueous alcohol concentrations with acyl chain length and/or temperature, and with alcohol chain

length, turns out to be a reflection of the variations of membrane: buffer partition coefficients. We previously interpreted the variation of partition coefficients for ethanol in the series of saturated PC's as a thermodynamic temperature effect [18], but it could also be a reflection of a more direct effect of acyl chain length on the partition coefficients.

Biological considerations

The biological implications of the work reported here may be significant. The two issues to be considered are the possibility that fully interdigitated states occur in biological systems, either as a normal state of affairs or as a drug- or alcohol-induced state, and the existence of kinetically frozen states in biological membranes.

The 'high concentration' state of the lipid has been tentatively identified as being a fully interdigitated gel state, which is metastable under some conditions. Since there are some naturally occurring lipids, as noted above, which also exhibit metastable states, the question is raised whether the interdigitated state is a possibility for these lipids, also. Also, it has been shown that an antibiotic, polymyxin B, induces a fully interdigitated gel state in PG bilayers [40,41]. The possibility of interdigitated states in biological membranes would have very great significance, and it is hoped that experiments on naturally occurring lipids to consider this question will be done in the near future. If regions of interdigitated lipids occur in biological membranes, this is a new dimension in the variability of membranes which is dependent on lipid composition. The demonstration of such phases, particularly if interdigitated and non-interdigitated regions exist in a single membrane, would be particularly interesting from the point of view of the properties of the membrane at interfaces or boundaries between the different phases. One obvious role that interdigitation could play in biological membranes is the coupling of the two 'leaflets' of the bilayer, which are very poorly coupled in the classical bilayer (e.g. Ref. 44).

The alcohol-induced interdigitated state occurs at lethal alcohol concentrations in the examples studied here. However, it is possible that for some other lipids or other combinations of acyl chains interdigitated states could be induced at biologically possible alcohol concentrations. It has been shown that the induction of the interdigitated state is correlated with the membrane: buffer partition coefficient, which is a characteristic of the mechanism of anesthesia. Thus it cannot be ruled out that the interdigitated state plays a role in intoxication or anesthesia.

The demonstration of the existence of the metastable states may in itself also have important biological implications. In view of the possible long lifetimes of metastable states, it is quite possible that the functional distributions and states of lipids in biological membranes are not necessarily thermodynamically determined, but are determined by the synthetic pathway by which they are developed.

Acknowledgments

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