Biochemistry

 Volume 22, Number 14 July 5, 1983

Articles

Lipid Chain Length and Temperature Dependence of Ethanol–Phosphatidylcholine Interactions†

Elizabeth S. Rowe*

ABSTRACT: The effects of ethanol on the thermotropic properties of the series of saturated phosphatidylcholines having acyl chain lengths from 14 to 21 carbons have been systematically investigated. Spectrophotometric measurements were used to follow the gel to liquid-crystal phase transitions. Ethanol has a biphasic effect on the phase transition temperatures of each of these lipids, which can be interpreted in terms of two independent ethanol–lipid interactions. The relationship between these two effects changes systematically with increasing acyl chain length and increasing transition temperature. The primary ethanol–lipid interaction, which occurs at relatively low ethanol concentration, involves the partitioning of ethanol preferentially into the fluid-phase lipid, thus lowering the phase transition temperature. In this ethanol concentration range, there is no change in transition cooperativity due to ethanol. Thermodynamic analysis of the lowering of the transition temperature provides estimates of the membrane–buffer partition coefficients for ethanol in each of the lipids at its transition temperature. The variation of partition coefficient with temperature (and acyl chain length) is treated thermodynamically to estimate free energy, enthalpy, and entropy for the transfer of ethanol from aqueous solution into the lipid. The secondary ethanol–lipid interaction, which occurs at higher ethanol concentration, stabilizes the gel-phase lipid, shifting the transition temperature toward higher temperature and enhancing the cooperativity of the phase transition. This interaction appears to involve the specific binding of ethanol at sites on the surface of the bilayer. There is a variation in the ethanol concentration at which this effect becomes dominant which correlates with temperature (and acyl chain length). It is concluded that this variation is a temperature effect.

The role of lipid composition in the function of membranes is not known. It is clear that membrane lipid compositions are closely regulated and finely tuned. Moreover, lipid compositions have been shown to change to adapt to changes in growth temperatures of *Escherichia coli* (McElhaney, 1974) or to the chronic presence of ethanol in mice (Chin et al., 1978; Littleton & John, 1977; Waring et al., 1981). Evidently, a physical property of the lipids which depends on composition is critical for membrane function; however, this specific property has not yet been identified. An important property of pure lipids and simple lipid mixtures is their ability to exist in several different phases, depending upon temperature, lipid class, and the length and degree of saturation of their acyl chains [for a review, see Lee (1977a,b) and Chapman (1975)]. The role of phase equilibria of the lipids and lateral phase separations of lipids of differing composition or phase state has been proposed to be important by a number of authors (Trudell, 1977; Mountcastle et al., 1976; Overath et al., 1975; Linden et al., 1973; Verkleij et al., 1972; Melchior & Steim, 1979; Racker et al., 1975; Overath & Thilo, 1978).

General anesthetics including ethanol apparently work by dissolving in the lipids of membranes, changing their physical properties and altering the membrane function [see Seeman (1972)]. From correlations of the physical effects of these perturbants on the lipids with the anesthetic effects, several theories of the mechanism of anesthesia have been developed, each based on a particular lipid physical property as being the critical property (Pang et al., 1980; Trudell, 1977; Mountcastle et al., 1976). We are studying the interactions of alcohols, particularly ethanol, with individual pure membrane lipids in order to elucidate the possible role of lipid specificity in alcohol–membrane interactions. We have previously shown that ethanol decreased the phase transition temperature of

† From the Veterans Administration Medical Center, Kansas City, Missouri 64128, and the Biochemistry Department, University of Kansas Medical School, Kansas City, Kansas 66103. *Received September 27, 1982.* Supported by the Veterans Administration and by the National Institute of Alcoholism and Alcohol Abuse (Grant AA05371-01).

* Address correspondence to this author at the Veterans Administration Medical Center, Kansas City, MO 64128.

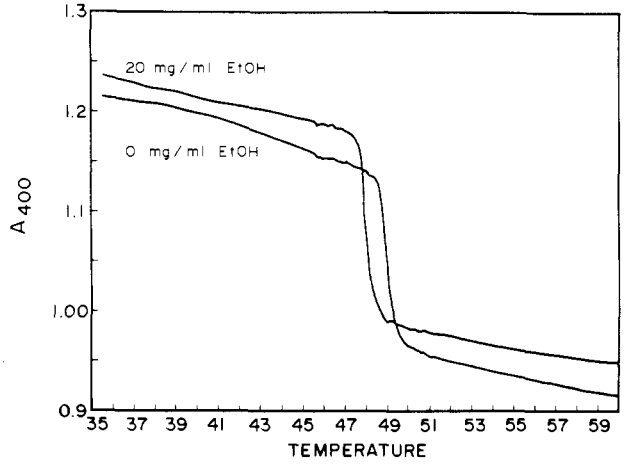

FIGURE 1: Absorbance at 400 nm of PC(17:0) as a function of temperature in the presence and absence of 20 mg/mL ethanol.

dihexadecanoylphosphatidylcholine [PC(16:0)][1] and PC(14:0) with no increase in transition width up to a concentration of 50 mg/mL ethanol in the aqueous phase (Rowe, 1981, 1982a). This report describes the results of measurements of the effect of ethanol on the gel to liquid-crystal phase transition of the series of saturated phosphatidylcholines with acyl chains from 14 to 21 carbons long.

Materials and Methods

Lipids. Lipids were purchased from Avanti, Inc., Birmingham, AL. Lipid purity was checked by thin-layer chromatography. Lipid suspensions were hand-shaken multilamellar liposomes prepared as described previously (Bangham et al., 1967; Rowe, 1982a). The samples contained 16% sucrose, added to reduce settling and to improve optical data (Rowe, 1982b). The lipid concentrations used were in the range of 0.25–0.6 mg/mL. Phosphorus concentrations were determined according to Bartlett (1957).

Spectrophotometry. The phase transitions were followed by the change in lipid optical density at 400 nm, as described previously (Rowe, 1982a). The Cary 219 spectrophotometer is equipped with a temperature readout accessory and interfaced to an Apple II microcomputer. The temperature and optical density data were read directly into the computer, where they could then be stored on floppy disks, and analyzed and plotted as required. Data averaging was accomplished by averaging data over temperature intervals of 0.02 °C. The accumulation of spectrophotometric data from the Cary 219 spectrophotometer and its subsequent storage and manipulation were carried out by using our own Basic software. The routine for least-squares variable-point smoothing with derivative calculation was based on the Savitzky & Golay (1964) method and used a BASIC algorithm generously provided by Dr. Raja Khalifah. Temperature was controlled by water circulated through jacketed cuvettes from a refrigerated bath, and the temperature was monitored by the built-in thermistor which was immersed in a jacketed cuvette hooked in series with the sample cuvette. Reversibility of the transitions was established as described previously (Rowe, 1982a) for the conditions under which thermodynamic calculations are made, and the data were collected during heating curves for the reasons described therein. A heating rate of approximately 0.3 °C/min was used.

[1] Abbreviations: PC, phosphatidylcholine; PC(14:0), ditetradecanoylphosphatidylcholine; PC(15:0), dipentadecanoylphosphatidylcholine; PC(*x*:*y*), phosphatidylcholine with acyl chains of *x* length containing *y* double bonds.

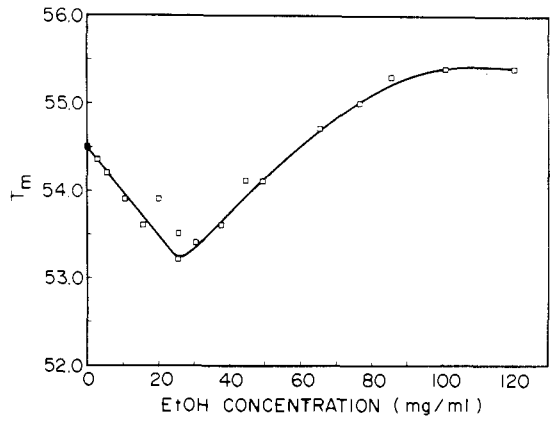

FIGURE 2: Transition midpoint of PC(18:0) as a function of aqueous ethanol concentration.

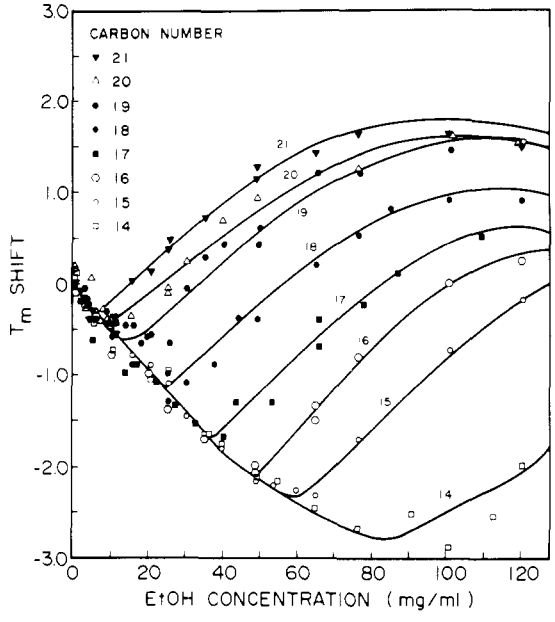

FIGURE 3: Shift in transition midpoint temperature from T_0 as a function of ethanol concentration for the series of saturated PC's with indicated acyl chain lengths. The T_0 for each lipid is given in Table I.

Results

Effect of Ethanol on Transition Temperature. Figure 1 shows a typical transition curve as observed by following the change in optical density at 400 nm as a function of temperature. The change in optical density which accompanies the gel to liquid-crystal transition of phosphatidylcholines (PC's) is a light-scattering change due to the change in lipid density and the resulting change in the index of refraction increment (Yi & MacDonald, 1973). The particular data shown in Figure 1 is for PC(17:0), and it is very similar in appearance to the data obtained for each of the saturated PC's having acyl chains from 14 to 21 carbons long. This figure also shows a transition curve in the presence of ethanol, showing that the transition is shifted to lower temperature by the addition of ethanol.

Transition curves similar to that shown in Figure 1 were obtained for each of the lipids studied. The transition midpoints were measured as a function of ethanol concentration for each of the lipids. Figure 2 shows a summary plot of these measurements for a representative lipid, PC(18:0). Figure 3 shows a summary for all of the lipids studied of the transition temperature as a function of ethanol concentration plotted as the temperature shift from the transition midpoint in the ab-

sence of ethanol. It is clear from these plots that there is a biphasic effect of ethanol concentration on the phase transition temperature of these lipids. At low concentration, there is a linear decrease of transition temperature with increasing ethanol concentration. An initial negative slope was seen with each of the lipids studied. The shift of the phase transition to lower temperature indicates that at the lower ethanol concentration the ethanol interacts preferentially with the fluid-phase lipids. As discussed below, this effect is due to the dissolving of ethanol into the fluid hydrophobic region of the bilayer.

At the higher ethanol concentrations, there is a reversal of the effect of ethanol on the transition temperature, and the transition temperature begins to increase with increasing ethanol concentration; in some cases, the transition temperature exceeds the transition temperature in the absence of ethanol. This result indicates that at the high ethanol concentration there is a secondary ethanol–lipid interaction in which ethanol is bound preferentially to the gel phase, stabilizing this phase. In contrast to the low concentration effect, the gel-stabilizing effect is nonlinear, giving the appearance of a hyperbolic binding curve which approaches saturation. Since this secondary interaction stabilizes the gel phase, it is likely that it represents an interaction of the ethanol with the head-group region of the bilayer in which more than one lipid molecule is involved in an individual binding site, thus stabilizing the intermolecular interactions in the crystalline gel state.

Acyl Chain Length Effects. It is clearly seen in Figure 3 that while all of the lipids show biphasic effects, there is a systematic variation in the ethanol concentration at which the high concentration effect becomes dominant. This indicates that as temperature increases or as chain length increases, there is a change in the relative affinities of the two ethanol–lipid interactions. As pointed out above, since the high concentration effect stabilizes the gel-phase lipids, it seems probable that the ethanol is interacting with the lipid bilayer surface, perhaps by binding at sites which involve two or more lipid molecules, thus enhancing the gel-state intermolecular interactions and stabilizing the gel state. If this is the case, it would be difficult to rationalize an effect of acyl chain length on this interaction. On the other hand, since each set of data shown in Figure 3 is at a different temperature, increasing with increasing chain length, the trend seen there is a function of temperature as well as chain length. It appears probable that the secondary high concentration ethanol–lipid interaction is affected by increasing temperature rather than by chain length.

Transition Cooperativity. The transition cooperativity is related to the size of the clusters of lipid molecules in either phase under the conditions where the two phases coexist. At least one current theory about anesthetic mechanisms is based on a broadening of the phase transitions, i.e., a reduction in cluster size, as the crucial anesthetic effect (Mountcastle et al., 1976). We have previously studied the transition cooperativity of PC(16:0) and found no broadening of the transition due to ethanol (Rowe, 1982a). In the present investigation, with improved temperature resolution, data acquisition, and data manipulation methods, we have investigated the transition cooperativity of the series of saturated PC's.

For examination of the transition cooperativity, first derivatives of the transitions were calculated for each transition curve, and the width at half-height was measured graphically. Figure 4 shows a representative example of the results obtained. Data for one lipid are shown; however, the results were similar for all of the lipids studied. In each of these studies,

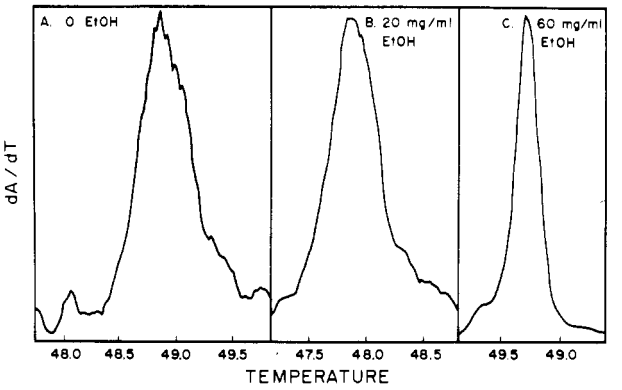

FIGURE 4: First derivative of transition curves of PC(17:0) in the presence of (A) no ethanol, (B) 20 mg/mL ethanol, and (C) 60 mg/mL ethanol. The first-derivative curves were determined as described in the text, by using a nine-point smoothing procedure, with measurements averaged over 0.05 °C.

there was no broadening of the transition curve by ethanol in the region of ethanol concentration where the ethanol reduces the melting temperature, that is, at the low concentration end of the range. The transition width was the same with and without ethanol in this region and was in the range of 0.4–0.6 °C for all of the lipids. In contrast, in the ethanol concentration range in which the ethanol effect is to raise the transition temperature, the transition width is seen to decrease to approximately 0.2 °C.

Membrane–Buffer Partition Coefficients. The shift in transition temperature at low ethanol concentration is linear with respect to ethanol concentration for each PC. This is characteristic of a nonspecific partitioning phenomenon in which the ratio of ethanol concentrations in solvent and lipid is a constant independent of overall ethanol concentration. The shift in phase equilibria due to solute can be expressed thermodynamically as

$$\frac{-\Delta H}{R}\left(\frac{1}{T_m}-\frac{1}{T_0}\right)=\ln\frac{X_{l,f}}{X_{l,g}}=\ln\frac{1-X_{s,f}}{1-X_{s,g}} \qquad (1)$$

where $X_{l,f}$ and $X_{l,g}$ are the mole fractions of lipid in the fluid and gel phases, respectively, and $X_{s,f}$ and $X_{s,g}$ are the mole fractions of solute in each phase so that $X_{l,f} + X_{s,f} = 1$ and $X_{l,g} + X_{s,g} = 1$ (Lee, 1977b). ΔH is the enthalpy of the phase transition, and T_m and T_0 are the transition midpoints in the presence and absence of solute, respectively. It is assumed that ΔH is independent of solute. If $X_{s,f}$ and $X_{s,g}$ are small relative to $X_{l,f}$ and $X_{l,g}$, respectively, we have

$$\frac{-\Delta H}{R}\left(\frac{1}{T_m}-\frac{1}{T_0}\right)= \ln(1-X_{s,f})-\ln(1-X_{s,g})\simeq -X_{s,f}+X_{s,g} \qquad (2)$$

If we let $K_g = X_{s,g}/X_{s,f}$, where the equilibrium constant K_g expresses the ethanol partitioning between the gel and fluid lipids, eq 2 can be rearranged and simplified to give

$$\frac{\Delta H \Delta T}{RT_0^2} = X_{s,f}(1-K_g) \qquad (3)$$

It is clear from this expression that if the interactions of ethanol with the lipid are the same for fluid- and gel-phase lipids ($K_g = 1$), there will be no transition temperature shift. On the other hand, if it is assumed that K_g is very small, i.e., no ethanol partitions into the gel phase, then we have the familiar freezing point depression expression. For further analysis, the data have been treated as though there is no significant partitioning into the gel-phase lipids, i.e., $K_g \ll 1$. As seen in eq 3, partitioning into the gel would reduce the transition shift by a constant

Table I

PC	T_0 (°C)	ΔH^a (kcal/mol)	K_p (unitary)	K_w (wt fractions)
14:0	24.5	5.8	3.0	0.079
15:0	34.2	6.4	3.7	0.093
16:0	41.6	7.2	4.3[b]	0.10
17:0	48.8	8.8	5.1	0.12
18:0	54.5	9.2	7.0	0.16
19:0	59.2	12.7	3.7	0.079
20:0	64.4	12.2		
21:0	68.9			

[a] R. N. McElhaney (personal communication). [b] This value is different from our previously published value because we have used a different value of ΔH for the phase transition.

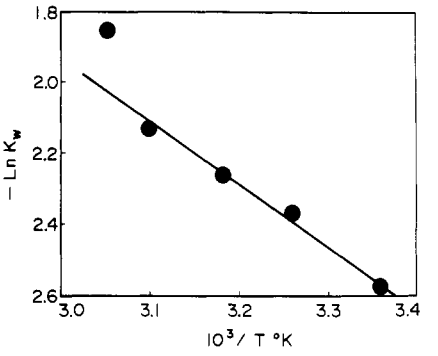

FIGURE 5: Temperature dependence of weight fraction partition coefficients.

factor which would lead to an underestimation of the amount of ethanol dissolved in the fluid-phase lipid.

The membrane–buffer partition coefficient for the fluid lipid can be calculated by substituting eq 3 into the partition coefficient expression and rearranging:

$$K_p = \frac{X_{s,f}}{X_{s,soln}} = \frac{-\Delta H}{RT_0^2}\left(\frac{-\Delta T}{X_{s,soln}}\right) \quad (4)$$

where $X_{s,soln}$ is the mole fraction concentration of ethanol in the aqueous phase and the quantity in parentheses is the slope of the plots such as shown in Figure 2 with the aqueous concentration of ethanol expressed in mole fraction units. In this analysis, it is assumed that the solvent phase is in excess over lipid so that the concentration of ethanol in the aqueous phase remains constant throughout the transition.

Each of the PC's exhibited a linear dependence of the transition temperature on ethanol concentration in the low concentration range. Table I shows the transition temperatures, enthalpy values, and partition coefficients calculated from eq 4 by using the low concentration slope. For the longest chain lipids, the region of the plot in which the transition temperature is depressed is too narrow for an accurate determination of the slope. Also in the table are the partition coefficients expressed in terms of weight fractions, included for the purposes of discussion below. It may be noted that the accuracy of the partition coefficients is dependent upon the values of the enthalpy for the phase transition, taken from the literature. Because of the variation in ΔH values in the literature for those lipids that have been most thoroughly studied [see review by Silvius (1982)], we have chosen to use values from a single laboratory (R. N. McElhaney, personal communication) in order to maximize the precision of the lipid comparisons. However, it should be noted that the absolute accuracy of the values is less than the precision. In addition, if there is significant partitioning of ethanol into the gel phase, these partition coefficients are low by a constant factor which can be assumed to be the same for each lipid.

Temperature Dependence of Membrane–Buffer Partition Coefficients. The thermodynamic quantities pertaining to the partitioning of ethanol between the buffer and the lipid can be estimated from the data given in Table I. The free energy of transfer of ethanol from water to the lipid is given by the relation $\Delta G = -RT \ln K_p$ where K_p is the partition coefficient expressed in unitary (mole fraction) units. Since

$$\Delta G = \Delta H - T\Delta S \quad (5)$$

then the enthalpy of the interaction can be obtained from

$$\frac{d(\Delta G/T)}{d(1/T)} = \Delta H = -RT\frac{d \ln K_p}{d(1/T)} \quad (6)$$

The entropy change is obtained from eq 5 by the difference. It may be noted that while the free energy and the entropy depend upon the concentration units chosen (i.e., the reference state), the enthalpy is independent of the concentration units used.

The variation in partition coefficients seen in Table I from the top to the bottom of the list of lipids is subject to two effects, the change in temperature and the change in acyl chain length. In the case of an ideal solute–solute system, no effect of the molecular size of the solvent on the partition coefficient (in unitary units) is expected. This point of view suggests that a valid estimate of the thermodynamic properties can be obtained by treating the data in Table I as though each solvent (lipid) is the same. Alternatively, because of the relative size of the solvent (lipid) molecules compared to the solute molecules, and the flexibility of the solvent molecules, a more valid assumption may be that a principle of additivity holds with respect to the solvent, in which case the different lipids need to be normalized with respect to their molecular weights before the temperature dependence is analyzed. This is equivalent to assuming that each segment of the acyl chain acts independently in solubilizing the solute. We have chosen to normalize the molecular weights by plotting ln K_w vs. $1/T$ as seen in Figure 5. The slope of this plot gives $\Delta H = +3.2$ kcal/mol; without molecular weight normalization, the enthalpy would be +5.6 kcal/mol. For purposes of discussion, the normalized value will be used; however, it should be noted that the general conclusions are the same for both of these small positive enthalpy values. Using eq 5, for PC(16:0) with $\Delta H = +3.2$ kcal/mol, we have $\Delta G = -0.91$ kcal/mol and $\Delta S = 13$ cal/(deg·mol).

Discussion

Biphasic Ethanol–PC Interaction. A major finding of this investigation is that ethanol has a biphasic effect on PC phase transition temperatures and thus is interacting with PC bilayers by two mechanisms. The primary interaction involves preferential interactions with the fluid phase at low ethanol concentration, and at higher ethanol concentration, a secondary lower affinity interaction becomes important which favors the gel phase. These two interactions are probably independent of each other. The curvature of the plots in Figure 3 indicates that the two interactions have different orders with respect to ethanol concentration, so that as the concentration is varied, the relationship between the two effects changes. The secondary interaction is thus negligible at low ethanol concentration but becomes dominant at high ethanol concentration.

The secondary interaction of ethanol with the bilayer appears to involve the binding of ethanol at the head-group region of the crystalline gel-phase bilayer, at discrete sites which

involve more than one lipid molecule. The conclusion that this interaction involves the binding of ethanol preferentially to the gel-phase bilayer comes from the fact that this interaction shifts the phase equilibrium toward higher temperatures. The nonlinear shape of the curves in Figure 3 at the high concentration end has the appearance of a saturable binding curve which approaches an asymptotic value. The suggestion that the site of this secondary interaction occurs at the head-group region of the bilayer comes from consideration of the nature of the gel-phase state of the lipid; in order for a binding interaction to stabilize this phase, it must enhance the intermolecular lipid interaction. Additional evidence for the enhancement of these interactions comes from the observation that this interaction increases the cooperativity of the transition; i.e., it increases the size of the cooperative lipid clusters. The most obvious location where such binding of ethanol could enhance the lipid–lipid interactions is the head-group region; the alternative site in the crystalline acyl chain region seems highly improbable.

Effect of Chain Length on Ethanol–Lipid Interactions. The data summarized in Figure 3 show that there is a systematic change in the melting temperature vs. ethanol concentration profile with increasing acyl chain length and increasing transition temperature. The effect of chain length and temperature in the low concentration range is interpreted thermodynamically as a temperature effect and is discussed below. The change in shape of the curves of Figure 3 is primarily due to a change in the secondary ethanol–lipid interaction, which becomes dominant at lower ethanol concentration as the chain length and transition temperature increases. It is not possible to separate the effect of temperature and the effect of acyl chain length because for each lipid chain length there is data at only one temperature, the temperature of the phase transition. However, since the secondary ethanol–lipid interaction appears to involve the head-group region of the lipid, it appears most palusible that the observed variation reflects the effect of temperature on this interaction.

Thermodynamic Analysis of Transition Temperature Depression. The thermodynamic treatment of the transition temperature to estimate the amount of ethanol dissolved in the membrane at low ethanol concentration is similar to the standard methods used in analytical chemistry to determine the purity of solids calorimetrically (Johnston & Giaque, 1947; Tunnicliff & Stone, 1955; Mastrangelo & Dornte, 1955). This treatment has been used in the analysis of the effect of small molecules on the phase transitions of lipids suspended in aqueous solutions by a number of authors (Hill, 1974; Kamaya et al., 1979; Vanderkooi et al., 1977; Rowe, 1981, 1982a). The analysis of the freezing point depression for the lipids in suspension is simplified relative to the analysis of solids by the presence of an excess of the aqueous phase containing the dissolved solute. Provided one is using solute concentrations well below the aqueous solubility of the solute, as in the present study, it can be assumed that the mole fraction of solute in the fluid phase is constant throughout the transition and is governed by the membrane–buffer partition coefficient. This is in contrast to the treatment of calorimetric data on solids where it is assumed that the mole fraction of solute dissolved in the melted phase is a function of the fraction melted, since the total amount of solute must be dissolved in the fluid phase unless solid solutions are formed (Mastrangelo & Dornte, 1955). Sturtevant (1982) has analyzed some solute–membrane interactions in terms of the effects of solid solutions on the transition shape. However, under the conditions used in the present study, in which it may be assumed that the solute concentration in the fluid phase is constant during the transition, Sturtevant's model reduces to one in which no information concerning solid solutions can be obtained from the transition shape. In this case, the effect of ethanol solubility in the gel phase would be only to reduce the transition temperature shift with no change in the transition sharpness, according to eq 3, and introduce a systematic error into the calculated partition coefficient. This would not affect our overall conclusions, which are general and are primarily based on the relative rather than the absolute values of the partition coefficients.

Our results on the transition temperature shift to lower temperature with no transition broadening are thus consistent with the thermodynamic treatment. Further support for this treatment is provided by the agreement between the membrane–buffer partition coefficients obtained by this method and the results obtained by other methods (Katz & Diamond, 1974) and by extrapolation of data from the longer chain alcohols (Seeman, 1972).

Thermodynamics of Transfer of Ethanol from Aqueous Solution to Lipid. The free energy of transfer of ethanol from aqueous solution to the fluid lipid for PC(16:0) is −0.91 kcal/mol in unitary (mole fraction) units. It is seen that the free energy is small and is negative when expressed in these units. In spite of this negative value of unitary free energy, however, on a volume or weight fraction concentration scale there is a higher concentration of ethanol in the aqueous solution than in the lipid. These values can be compared with −0.82 kcal/mol for ethanol transfer into DPPC (Kamaya et al., 1981). One can also look at aqueous solubility data. For example, Tanford (1980) gives a value of 0.76 kcal/mol for the free energy of transfer of ethanol from pure ethanol to aqueous solution. Hill has explored the use of the principles of aqueous solubility of organic compounds to estimate the membrane–buffer partition coefficients, with some success for the small organic and amphipathic compounds (Hill, 1974). The assumption inherent in this analysis is that the solute–solute interactions in the pure solute are similar to the interaction between the solute and the acyl chains making up the membrane interior. All of these considerations suggest that the partitioning of ethanol into the bilayer has the character of a nonspecific solubilization of the solute into the hydrophobic interior of the membrane.

The value obtained for the enthalpy of transfer of ethanol from aqueous solution to the lipid interior is +3.2 kcal/mol. This value may be compared with +2.7 kcal/mol determined by Katz & Diamond (1974) for the partitioning of ethanol into PC(14:0) bilayers, determined by van't Hoff plots of direct binding measurements by using the centrifugal method. Another relevant comparison is the value of the ΔH of transfer of ethanol from water to pure ethanol of +2.4 kcal/mol given by Tanford (1980). Our value is within reasonable agreement with these values, considering the approximations used and the uncertainty in the data.

The thermodynamic parameters obtained here are typical of hydrophobic interactions where the driving force is the entropy change associated with the removal of the hydrophobic moiety from the water phase. Typically, ΔH is not a constant due to a change in heat capacity for the hydrophobic process [Tanford, 1980; e.g., see Simon et al. (1979)]. Unfortunately, we could not obtain data over a sufficiently wide range to detect curvature, because the secondary ethanol–lipid interaction precluded good partition coefficient measurements above 55 °C. However, the small positive enthalpy value is typical of the partitioning of small hydrocarbons or amphiphiles be-

tween water and organic solvents or of the transfer of these small molecules from water to the pure organic compound (Tanford, 1980).

Units. The problem of units to be used is an important one, because the choice of units can affect the conclusions drawn in thermodynamic studies. The question of what units are to be preferred for studies of solute–membrane partitioning has been discussed by several authors (Katz & Diamond, 1974; Kamaya et al., 1981; Miller et al., 1977). The thermodynamically significant units are the unitary (mole fraction) units. In an ideal solution, it is the mole fraction which governs the macroscopic behavior, not the volume or weight concentration. For example, the solubilities of CO_2 in vinyl chloride, chloroform, carbon tetrachloride, and toluene are quite similar when expressed by mole fraction units, but not in terms of weight or volume fractions (Denbigh, 1971). On the other hand, for nonideal situations in which the solvent molecules are large and flexible compared to the solute molecules, this ideal result is not realized. Nevertheless, when the thermodynamic quantities free energy and entropy are discussed, the unitary quantities are preferable because they give information only about the solute–solvent interactions, without complications from the cratic contributions determined by relative size and location of the molecules. [For a discussion of unitary free energies, see Gurney (1953), Tanford (1970), and Kauzmann (1959).] It may be noted that the enthalpy determined from the van't Hoff plot is independent of the units used for the partition coefficients.

Biological Mechanisms. Meyer and Overton first observed that there was a correlation between anesthetic potency and olive oil solubility, and this correlation has stood the test of time (Meyer, 1899; Overton, 1901; Seeman, 1972; Janoff et al., 1981). The present theories of general anesthesia are predicated upon the effects that these molecules have on the lipid properties when they are dissolved into the membrane interior. The several theories differentiate among several physical effects of the anesthetic on the lipid properties, including microviscosity (Pang et al., 1980), changes in phase distributions or lateral phase separations (Trudell, 1977), and cooperative fluctuation between phases by critical clusters of lipids (Mountcastle et al., 1976). The latter proposal holds that a reduction in cooperativity of phase equilibria by anesthetic molecules is the primary anesthetic event. The data presented here indicate that there is no reduction in transition cooperativity of any of the PC's examined by the presence of ethanol. Thus, with respect to the PC's, these data are consistent with the Trudell theory but not the fluctuation theory.

The high degree of specificity and control of lipid compositions in biological membranes provides strong circumstantial evidence that lipid composition is critical to membrane function. There is also evidence that lipid composition is important for the biological effects of ethanol, since the lipid composition of membranes varies with chronic ethanol exposure in experimental animals (Chin et al., 1978; Littleton & John, 1977; Waring et al., 1981). Also, there is growing evidence that the membrane solubility of anesthetics varies (within a relatively narrow range) with lipid composition (Smith et al., 1981; Korten et al., 1980; Miller et al., 1977; Simon et al., 1979; Rottenberg et al., 1981). Our results show that lipid solubility of ethanol is dependent upon the physical state of the lipid. Thus, it is clear that composition-dependent physical properties are crucial. Among the possibilities, phase distributions and lateral phase separations appear to have the potential for more functional consequences than does membrane fluidity. It is also possible that nonbilayer phases play a role (Cullis & De Kruijff, 1979). Thus, it appears that studies on the effects of ethanol on different lipid classes, on lipid mixtures including cholesterol, on phase distributions, and on protein–lipid interactions are necessary to provide information concerning the role of lipid specificity in membrane function and the mechanisms of general anesthesia.

Acknowledgments

I thank Dr. Ronald N. McElhaney for providing his calorimetric enthalpy data prior to publication. The expert technical assistance of James W. Klein is appreciated.

Registry No. PC(14:0), 13699-48-4; PC(15:0), 67896-63-3; PC(16:0), 2644-64-6; PC(17:0), 67896-64-4; PC(18:0), 4539-70-2; PC(19:0), 85800-07-3; PC(20:0), 71259-34-2; PC(21:0), 85800-08-4; ethanol, 64-17-5.

References

Bangham, A. D., DeGier, J., & Greville, G. D. (1967) *Chem. Phys. Lipids 1*, 115–145.
Bartlett, G. R. (1957) *J. Biol. Chem. 234*, 466.
Chapman, D. (1975) *Q. Rev. Biophys. 8*, 185–235.
Chin, J. H., Parson, L. M., & Goldstein, D. B. (1978) *Biochim. Biophys. Acta 513*, 358–363.
Cullis, P. R., & De Kruijff, B. (1979) *Biochim. Biophys. Acta 559*, 399–420.
Denbigh, K. (1971) *The Principles of Chemical Equilibrium*, 3rd ed., Cambridge University Press, Cambridge, England.
Gurney, R. W. (1953) *Ionic Processes in Solution*, McGraw-Hill, New York, Reprinted 1962 by Dover Publications, New York.
Hill, M. (1974) *Biochim. Biophys. Acta 356*, 117–124.
Janoff, A. S., Pringle, M. J., & Miller, K. W. (1981) *Biochim. Biophys. Acta 649*, 125–128.
Johnston, H. L., & Giauque, W. F. (1947) *J. Am. Chem. Soc. 51*, 3194–3214.
Kamaya, H., Ueda, I., Moore, P. S., & Eyring, H. (1979) *Biochim. Biophys. Acta 550*, 131–137.
Kamaya, H., Kaneshina, S., & Ueda, I. (1981) *Biochim. Biophys. Acta 646*, 135–142.
Katz, Y., & Diamond, J. M. (1974) *J. Membr. Biol. 17*, 101–120.
Kauzmann, W. (1959) *Adv. Protein Chem. 14*, 1–47.
Korten, K., Sommer, T. J., & Miller, K. W. (1980) *Biochim. Biophys. Acta 599*, 271–279.
Lee, A. G. (1977a) *Biochim. Biophys. Acta 472*, 237–281.
Lee, A. G. (1977b) *Biochim. Biophys. Acta 472*, 285–344.
Linden, C. D., Wright, K. L., McConnell, H. M., & Fox, C. F. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 2271.
Littleton, J. M., & John, G. J. (1977) *J. Pharm. Pharmacol. 29*, 579–580.
Mastrangelo, S. V. R., & Dornte, R. W. (1975) *J. Am. Chem. Soc. 77*, 6200–6201.
McElhaney, R. N. (1974) *J. Mol. Biol. 84*, 145–157.
Melchior, D. G., & Steim, J. M. (1979) *Prog. Surf. Membr. Sci. 13*, 211.
Meyer, H. (1899) *Arch. Exp. Pathol. Pharmakol. 42*, 109–118.
Miller, K. W., Hammond, L., & Porter, E. G. (1977) *Chem. Phys. Lipids 20*, 229–241.
Mountcastle, D. B., Biltonen, R. L., & Halsey, M. J. (1976) *Proc. Natl. Acad. Sci. U.S.A. 75*, 4906–4910.
Overath, P., & Thilo, L. (1978) *Int. Rev. Biochem. 19*, 1–44.
Overath, P., Brenner, M., Gulik-Krzywicki, T., Schechter, E., & Letellier, L. (1975) *Biochim. Biophys. Acta 389*, 358.
Overton, E. (1901) *Studen ubr die Narkose Zugleich ein Beitrag zur allgemeinen Pharmakologie*, Verlog von Gustav

Fischer, Jena, West Germany.
Pang, K.-Y., Braswell, L. M., Chang, L., Sommer, T. J., & Miller, K. W. (1980) *Mol. Pharmacol. 18*, 84–90.
Racker, E., Knowles, A. F., & Dytar, E. (1975) *Ann. N.Y. Acad. Sci. 264*, 17–33.
Rottenberg, H., Waring, A., & Rubin, E. (1981) *Science (Washington, D.C.) 213*, 583–585.
Rowe, E. S. (1981) *Alcohol.: Clin. Exp. Res. 5*, 159–263.
Rowe, E. S. (1982a) *Mol. Pharmacol. 22*, 133–139.
Rowe, E. S. (1982b) *Biochim. Biophys. Acta 685*, 105–108.
Savitzky, A., & Golay, M. J. E. (1964) *Anal. Chem. 36*, 1627–1639.
Seeman, P. (1972) *Pharmacol. Rev. 24*, 583–654.
Silvius, J. R. (1982) in *Protein-Lipid Interactions* (Jost, P., & Griffiths, O. H., Eds.) Vol. II, pp 239–278, Wiley, New York.
Simon, S. A., McIntosh, T. J., Bennett, P. B., & Shrivastav, B. B. (1979) *Mol. Pharmacol. 16*, 163–170.
Smith, R. A., Porter, E. G., & Miller, K. W. (1981) *Biochim. Biophys. Acta 645*, 327–338.
Sturtevant, J. M. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 3963–3967.
Tanford, C. (1970) *Adv. Protein Chem. 24*, 1–95.
Tanford, C. (1980) *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, 2nd ed., Wiley, New York.
Trudell, J. R. (1977) *Anesthesiology 46*, 5–10.
Tunnicliff, D. D., & Stone, H. (1955) *Anal. Chem. 27*, 73–80.
Vanderkooi, J. M., Landesberg, R., Selick, H., II, & McDonald, G. G. (1977) *Biochim. Biophys. Acta 464*, 1–16.
Verkleij, A. D., Ververgaert, P. H. J., van Deenen, L. L. M., & Elbers, P. F. (1972) *Biochim. Biophys. Acta 288*, 326.
Waring, A. J., Rottenberg, H., Ohnishi, T., & Rubin, E. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 2582–2586.
Yi, P. N., & MacDonald, R. C. (1973) *Chem. Phys. Lipids 11*, 114–134.

Phase Transition Kinetics of Phosphatidic Acid Bilayers. A Pressure-Jump Relaxation Study†

Khalid Elamrani and Alfred Blume*

ABSTRACT: The phase transition kinetics of unilamellar vesicles of dilauroyl-, dimyristoyl-, and ditetradecylphosphatidic acid were investigated by the pressure-jump technique with optical detection. At neutral pH, phosphatidic acids display three relaxation processes within the temperature range of the phase transition. The time constants of these processes are in the 1–10-, 10–100-, and 100–1000-ms ranges, respectively. They exhibit distinct maxima at the midpoint of the phase transition as determined by differential scanning calorimetry. The temperature dependence of the relaxation times and amplitudes indicates that these processes are of a cooperative nature and thus directly related to the phase transition. The cooperative units calculated from the relaxation amplitudes agree well with those determined from the differential scanning calorimetry curves. Whereas changes in head group structure generally have only small effects on the thermodynamic properties of the transition, the kinetics of the transition are changed drastically. Phosphatidic acids show relaxation times more than 1 order of magnitude slower than those observed for phosphatidylcholines. Increasing the negative charge of the phosphatidic acid head group leads to a decrease in the relaxation times by more than 1 order of magnitude. Addition of 0.1 M NaCl has the opposite effect. Compared with these large changes in the relaxation behavior due to variations in the head group interactions, changes in hydrocarbon chain length have no effect. Thus, the phase transition kinetics of phosphatidic acids are dominated by the strength of the interactions between the polar head groups.

The thermodynamic aspect of the gel to liquid-crystalline phase transition of phospholipid bilayers has been a field of intensive research over the past years. Several theories have been developed that try to describe the equilibrium properties of the transition on the basis of statistical-mechanical or mean-field calculations [for a review, see Nagle (1980)]. Compared with the large amount of data available for the thermodynamic properties of the lipid phase transition, the kinetic aspects have received relatively little attention. Up to now most kinetic investigations focused on the behavior of PC,[1] applying different kinetic methods like ultrasonic and dielectric relaxation or the temperature-jump method (Träuble, 1971a; Tsong, 1974; Tsong & Kanehisa, 1977; Kaatze et al., 1975; Eggers & Funck, 1976; Gamble & Schimmel, 1978; Mitaku et al., 1978; Gruenewald et al., 1981; Gruenewald, 1982; Mitaku & Date, 1982). Recently, we have reported on the application of the pressure-jump method using optical detection in studying the transition kinetics of PC vesicles and liposomes (Gruenewald et al., 1980). This technique has the advantage over the temperature-jump method that no orientation effects on the lipid head groups and no dielectric breakdown of the bilayer due to the large electric fields can occur (Zimmermann et al., 1974; Shepherd & Büldt, 1978). This method is also suitable for the investigation of charged phospholipids as, in contrast to the temperature-jump experiments, there is no need for the addition of salt to obtain sufficient conductivity. This is important because changes in the ionic strength have large effects on the phase transition characteristics of charged lipids (Träuble & Eibl, 1974; Träuble et al., 1976; Jähnig, 1976).

† From the Institut für Physikalische Chemie II, D-7800 Freiburg, West Germany. *Received November 3, 1982.* This work was supported by the Deutsche Forschungsgemeinschaft.

[1] Abbreviations: DLPA, dilauroylphosphatidic acid; DMPA, dimyristoylphosphatidic acid; DTPA, ditetradecylphosphatidic acid; DPPC, dipalmitoylphosphatidylcholine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PA, phosphatidic acid; DPH, 1,6-diphenylhexatriene; T_m, transition temperature; DSC, differential scanning calorimetry; NMR, nuclear magnetic resonance.

0006-2960/83/0422-3305$01.50/0