Interactions of Short-Chain Alcohols with Dimyristoylphosphatidylethanolamine Bilayers: A Calorimetric and Infrared Spectroscopic Investigation†

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ABSTRACT: We have investigated the effects of methanol, ethanol, and 1-propanol on the phase transitions of L-α-dimyristoylphosphatidylethanolamine using differential scanning calorimetry and Fourier transform infrared spectroscopy. Alcohols lower the temperature of the gel (L_β) to liquid-crystalline (L_α) phase transition and also lower the temperature of the unhydrated crystalline (L_c) to liquid-crystalline phase transition. When the lipid/alcohol dispersions are incubated at 2 °C for 1–18 h, a dehydrated crystalline phase forms, which gives rise to a phase transition at about 55 °C. This dehydrated crystalline phase forms more quickly at higher alcohol concentrations. Although alcohol at low concentration lowers the enthalpy of the observed melting transition, at high concentrations 1-propanol markedly increases this enthalpy. The phase giving rise to this high-enthalpy melting process is distinct from both the unhydrated crystalline phase and the gel phase. Infrared spectra suggest that this phase contains significant amounts of alcohol in a solid solution with the lipid.

The interaction of model lipid membranes with general anesthetics, including steroids, alcohols, alkanes, and volatile compounds, has been the subject of many experimental and theoretical investigation (O'Leary et al., 1984; Ahmad & Mellors, 1978; Arrowsmith et al., 1983; Bangham et al., 1980; Barchfeld & Deamer, 1988; Biltonen, 1980; Butler, 1975; Craig et al., 1987; Franks & Lieb, 1978, 1980, 1982; Halsey, 1974; Jain & Wray, 1978; Jain et al., 1975; O'Leary, 1981, 1982, 1983, 1984; Seeman, 1972). Since the potency of many general anesthetics correlates with their lipid solubility (Janoff et al., 1981), considerable effort has been dedicated to explaining how these drugs perturb the structure of model and biological phospholipid membranes. Nevertheless, the molecular events resulting in anesthesia remain unknown. Most studies of anesthetic–lipid interaction have focused on the phosphatidylcholines (PC's).[1] Although some interactions, such as those governing the gel to liquid-crystalline phase transition temperature, are expected to be similar in all phospholipids with similar acyl chains, interactions involving changes in headgroup structure or hydration are not. Since phosphatidylethanolamines (PE's) and phosphatidylserines (PS's) are fairly abundant in presynaptic nerve terminals (Deutsch & Kelly, 1981; Tenchov, 1985), their interactions with anesthetics may be relevant to anesthesia.

Many general anesthetics lower the gel to liquid-crystalline phase transition temperature of phospholipid model membranes (Jain et al., 1975; O'Leary, 1981, 1982, 1984; Kaminoh et al., 1988). Ueda and co-workers have demonstrated in addition that alcohols cause the release of bound water from the surface of PC membranes and have suggested that this effect is related to general anesthesia (Suezaki et al., 1983). High hydrostatic pressures (>100 atm) reverse general anesthesia in vertebrates (Nosaka et al., 1988; Mountcastle et al., 1978). Although it is not proven that this effect involves the molecular site for anesthesia (Smith et al., 1986), demonstration that a membrane effect is reversible provides some support to the notion that this effect is relevant to general anesthesia. Although the lowering of the gel to liquid-crystalline phase transition temperature of phosphatidylcholines by anesthetics is reversed by pressure (Mountcastle et al., 1978), it is not known whether the release of water from the membrane surface in the presence of anesthetics is also pressure-reversible.

Unlike PC's, PE's do not spontaneously hydrate when they are added to cold water. As a result, the thermograms of PE's which have been added to cold water change significantly between the first and the second scan. For example, dimyristoylphosphatidylethanolamine (DMPE) displays a single endothermic peak with an enthalpy of approximately 18 kcal/mol at approximately 57 °C when first heated. If the sample is cooled and immediately reheated, an endotherm with an enthalpy of approximately 5.7 kcal/mol is found at approximately 50 °C, and the 57 °C peak is not present. The peak at 57 °C has been attributed to melting of an unhydrated crystalline (L_c) to form a hydrated liquid-crystalline (L_α) phase (Silvius et al., 1986). The peak which occurs at 50 °C has been attributed to the gel (L_β) to liquid-crystalline phase transition (Silvius et al., 1986). Upon prolonged incubation at low temperatures, the L_c phase re-forms (Silvius et al., 1986). Scanning calorimetry may thus be used to probe the hydration state of phosphatidylethanolamine bilayers, since the hydrated and unhydrated forms of the lipid differ significantly in both the temperature and enthalpy of melting. A scanning calorimeter modified to allow experiments under

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[1] Abbreviations: ATR, attenuated total reflection; DMPE, L-α-dimyristoylphosphatidylethanolamine; DMPC, L-α-dipalmitoylphosphatidylcholine; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PG, phosphatidylglycerol; PS, phosphatidylserine; DSC, differential scanning calorimetry; FTIR, Fourier transform infrared; L_c, unhydrated crystalline phase; L_c', dehydrated crystalline phase formed in the presence of alcohols; L_α, liquid-crystalline phase; L_β, fully hydrated gel phase; L_H, lipid phase forming in the presence of large amounts of propanol which melts at about 45 °C with an enthalpy of approximately 18 kcal/mol; ΔH, enthalpy of transition; T_m, midpoint transition temperature for the gel to liquid-crystalline transition; T_m', midpoint transition temperature of the unhydrated crystal to liquid-crystalline phase transition.

high hydrostatic pressure (Mountcastle et al., 1978) can be used to determine if increased pressure reverses drug-induced membrane changes which can be measured calorimetrically, including the hydration and dehydration of PE's discussed above.

Rowe and co-workers have reported (Rowe, 1983, 1985a,b, 1987; Nambi et al., 1988; Veiro et al., 1987, 1988) that alcohols, which gave general anesthetic properties, cause the acyl chains of phosphatidylcholines to become interdigitated. The fact that similar interdigitated phases have been demonstrated in asymmetric phosphatidylcholines (Mason et al., 1981; Hui & Huang, 1986) and phosphatidylethanolamines (Mason & Stephenson, 1989), in 1,2-dihexadecylphosphatidylcholine (DHPC) (Siminovitch et al., 1987; Ruocco et al., 1985a,b), and in phosphatidylglycerol bilayers (PG) (McDaniel et al., 1983; Boggs & Ranjaraj, 1985) suggests that such interdigitated phases may be found in natural membranes and be physiologically important. Although Rowe has presented data that suggest that alcohols do not cause interdigitation in phosphatidylethanolamine systems (Rowe, 1985), these data are limited to systems that have not been incubated at low temperatures.

Several techniques may be used to detect the presence and degree of interdigition in lipid bilayers, including scanning calorimetry (Mason et al., 1981), X-ray diffraction (Hui & Huang, 1986), NMR spectroscopy (Ruocco et al., 1985a,b), Raman spectroscopy (O'Leary & Levin, 1984), and infrared spectroscopy (Siminovitch et al., 1987). Infrared spectroscopy and differential scanning calorimetry together are useful tools with which to investigate the effects of perturbants on phospholipid systems, since together they provide both the thermodynamic and structural information required to characterize lipid conformation and intermolecular interactions.

In this paper, we present results of a calorimetric and spectroscopic investigation of DMPE–alcohol interactions. We present data concerning the effect of methanol, ethanol, and propanol on the dehydration of DMPE membranes, and on the effect of increased hydrostatic pressure on the alcohol–DMPE interaction. In addition, we present both calorimetric and spectroscopic data regarding the structure of DMPE dispersions in the presence of high concentrations of 1-propanol. We then discuss these data and their relevance to possible molecular mechanisms of general anesthesia.

Experimental Procedures

Chemicals. L-α-Dimyristoylphosphatidylethanolamine (DMPE) and L-α-dimyristoylphosphatidylcholine (DMPC) were obtained from Sigma Chemical Co. (St. Louis, MO). Each lipid was recrystallized 3 times from chloroform and dried under vacuum for 48 h until a white crystalline powder was formed. The purity of each lipid was verified by the presence of a single spot following thin-layer chromatography (eluted with methanol–chloroform, 80:20) and by a full width at half-maximum of 0.5–0.6 °C for the gel to liquid-crystalline phase transition. Methanol, ethanol, 1-propanol, and 2-propanol were obtained from Aldrich Chemical Co. (Milwaukee, WI).

Calorimetry. Calorimetric measurements were performed by using a Hart Scientific 7707 series (Provo, UT) differential scanning calorimeter. For experiments under increased hydrostatic pressure, a special cell was fabricated which could be attached to a helium tank by stainless-steel high-pressure liquid chromatography tubing. The system pressure was measured with an Omega Engineering (Stamford, CT) PX-105 transducer with a Model DP-354 indicator. Experiments performed on dipalmitoylphosphatidylcholine liposomes to verify the performance of this apparatus yielded results similar to those obtained by Mountcastle, Biltonen, and Halsey, (Mountcastle et al., 1978), but with flatter base lines and more accurate enthalpy measurements.

Unhydrated crystalline DMPE samples were prepared by adding 500 mg of cold deionized water to 6 mg of dry lipid. Alcohol was then added to yield final alcohol concentrations ranging from 0.0 to 2.1 M. The amount of lipid in the calorimeter cell was determined by weighing the cell both before and after the addition of dry lipid. Heating and cooling thermograms were obtained between 2 and 70 °C at a rate of 14.2 K/h. To ensure a uniform distribution of alcohol throughout the sample, the calorimetric cell was heated and cooled 10 times between 65 and 30 °C at a scan rate of 60 K/h immediately after initial heating. Then a cooling (from 65 to 2 °C) scan was obtained. Afterward the lipid/alcohol dispersion was incubated at 2 °C for 18–24 h, and heating and cooling thermograms were again obtained. Base lines were corrected by subtracting water thermograms obtained under the same thermal conditions. Enthalpies of transition and excess heat capacities were obtained by using software provided with the instrument. Enthalpies are reported to within ±5%. Midpoint temperatures are reported to within ±0.1 °C relative to each other. Liquid-crystalline phase/water partition coefficients were calculated from the midpoint temperatures of cooling thermograms using the approach outlined by Rowe (1983).

Infrared Spectroscopy. Phospholipid dispersions were loaded in a vacuum-tight cylindrical attenuated total reflection cell (Circle Cell) with a ZnSe crystal (Spectra-Tech, Stanford, CT). Attenuated total reflection (ATR) was used in preference to transmission spectroscopy because our ATR cell could be maintained in a vacuum. The cell was mounted in a thermal jacket and maintained at a predetermined temperature between 0 and 72 °C with a Neslab water circulator equipped with a digital temperature controller. The ATR cell was utilized in an evacuated Bomem DA3.02 Fourier transform infrared spectrometer equipped with a liquid nitrogen cooled narrow-band mercury cadmium telluride detector. Each spectrum reported here was the result of 1500 interferograms apodized with a Bartlet function. The spectral resolution was 4 cm^{-1}. Frequencies were determined by using the peak-finding routine provided by Bomem. No smoothing or deconvolution procedures were employed.

Results and Discussion

Calorimetry. The enthalpy and transition temperature for the unhydrated crystalline (L_c) to liquid-crystalline (L_α) phase transition of DMPE in the presence of various alcohols were determined from heating thermograms obtained immediately after adding anhydrous polycrystalline DMPE to cold alcohol–water solutions. Representative thermograms obtained in pure water and in the presence of 0.4 and 1.2 M alcohol are shown in Figure 1. Alcohols at low concentration caused no qualitative change in the shapes of the excess heat capacity curves, although propanol caused a small reduction in the melting temperature and all alcohols caused a small increase in the melting enthalpy. At high concentrations (greater than 1.0 M), propanol caused asymmetry to appear in the excess heat capacity curve. The effects of various concentrations of alcohols on the L_c to L_α temperature and enthalpy are summarized in Table I. The decrease in transition temperature caused by propanol was significant, and was approximately linear with alcohol concentration. The effects of ethanol and methanol were small and inconsistent. An increase in the enthalpy was seen in the presence of alcohols; the effect in-

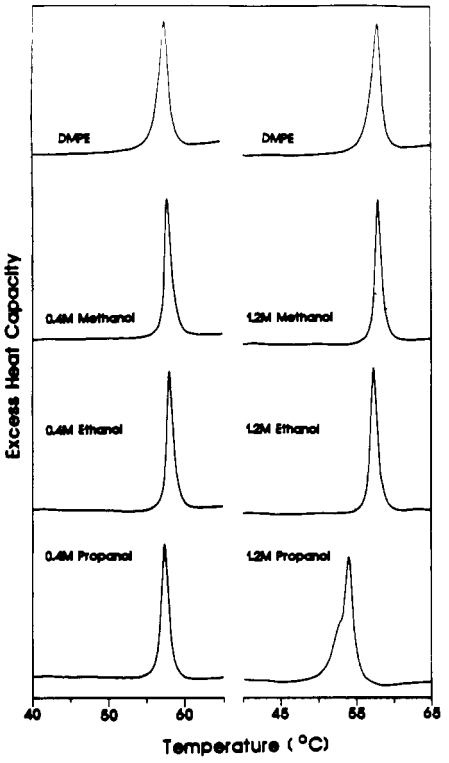

FIGURE 1: Representative thermograms for the DMPE $L_c \rightarrow L_\alpha$ phase transition in the presence and absence of alcohols.

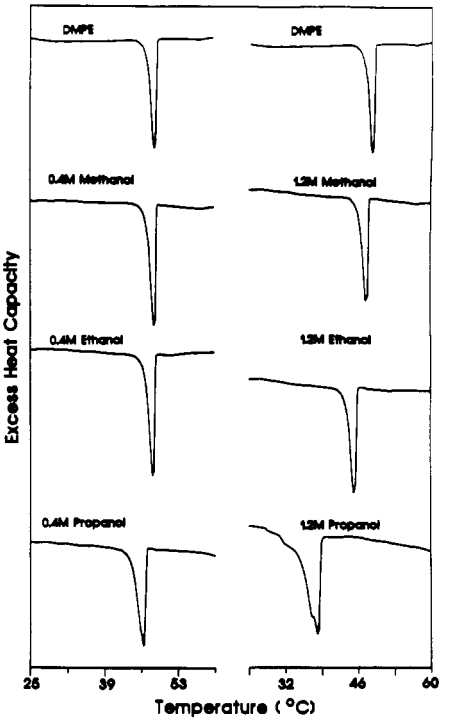

FIGURE 2: Representative thermograms for the liquid-crystalline (L_α) to gel (L_β) phase transition in the presence and absence of alcohols.

Table I: Midpoint Temperature (T_m) and Enthalpy (ΔH) for the Crystalline to Liquid-Crystalline Phase Transition Obtained from the First Heating Scan

alcohol	concn (M)	T_m (°C)	ΔH (kcal/mol)
	0.0	58.0	17.8
methanol	0.2	58.0	17.7
methanol	0.4	58.0	18.1
methanol	0.6	57.5	18.3
methanol	0.8	57.7	18.8
methanol	1.0	57.6	18.4
methanol	1.2	58.0	19.0
methanol	1.4	58.1	18.8
methanol	1.6	58.3	18.2
methanol	1.8	58.4	19.0
ethanol	0.2	57.8	18.3
ethanol	0.4	57.3	19.2
ethanol	0.6	57.0	19.3
ethanol	0.8	57.1	19.4
ethanol	1.0	57.3	19.7
ethanol	1.2	57.5	19.7
ethanol	1.4	57.4	19.8
ethanol	1.6	57.2	20.1
ethanol	1.8	57.0	20.2
1-propanol	0.2	57.1	18.1
1-propanol	0.4	56.5	19.3
1-propanol	0.6	55.4	19.4
1-propanol	0.8	54.8	20.1
1-propanol	1.0	54.2	20.9
1-propanol	1.2	54.0	21.2
1-propanol	1.4	53.4	20.4
1-propanol	1.6	53.2	20.3
1-propanol	1.8	52.6	22.3
1-propanol	2.0	52.5	22.6

creased with concentration and also with the alcohol chain length.

The effects of alcohols on the temperature and enthalpy of the liquid-crystalline to gel phase transition were obtained from cooling thermograms obtained immediately after completion of the heating thermograms described above. Representative cooling thermograms obtained in the absence of alcohol and in the presence of 0.4 and 1.2 M alcohol are shown in Figure

Table II: Midpoint Temperature (T_m) and Enthalpy (ΔH) for the Liquid-Crystalline to Gel Phase Transition Obtained from Cooling Scans

alcohol	concn (M)	T_m (°C)	ΔH (kcal/mol)
	0.0	48.5	5.57
methanol	0.2	48.4	5.78
methanol	0.4	48.2	5.80
methanol	0.6	47.9	5.96
methanol	0.8	47.6	5.99
methanol	1.0	47.5	5.95
methanol	1.2	47.4	6.04
methanol	1.6	47.1	6.18
methanol	1.8	46.4	6.42
ethanol	0.2	47.9	6.00
ethanol	0.4	47.6	6.12
ethanol	0.6	46.8	6.39
ethanol	0.8	46.2	6.58
ethanol	1.0	45.6	6.91
ethanol	1.2	45.0	6.94
ethanol	1.6	44.2	7.03
ethanol	1.8	43.7	7.49
1-propanol	0.2	46.1	5.92
1-propanol	0.4	45.2	6.57
1-propanol	0.6	42.5	7.3
1-propanol	0.8	40.6	7.93
1-propanol	1.0	39.3	8.83
1-propanol	1.2	37.7	8.88
1-propanol	1.6	35.3	10.20
1-propanol	1.8	34.1	11.17

2. The alcohols reduced the transition temperature, increased the transition enthalpy, and slightly broadened the excess heat capacity curve; this broadening was particularly pronounced in dispersions containing 1-propanol. The effects on transition temperature and enthalpy are summarized as a function of alcohol concentration in Table II. The effects of alcohols on the L_α to L_β transition are more pronounced for the longer alcohols, and increase with alcohol concentration. These results are similar to those which have been reported by Rowe (1985). Heating scans obtained immediately after cooling scans (data not shown) demonstrated the gel to liquid-crystalline transition to be reversible; the temperature at which the transition begins

Table III: Liquid-Crystalline Phase/Water Partition Coefficients for Alcohols in Dimyristoylphosphatidylethanolamine

methanol	1.68
ethanol	4.35 (4.8)[a]
propanol	12.88

[a] Value in parentheses is taken from Rowe (1985).

was identical for both the heating and cooling scans, and the enthalpies obtained in these scans were within a few percent of each other. The transition midpoint temperatures were slightly lower for cooling scans than for heating scans.

The reduction in phase transition temperatures in the presence of alcohol is readily attributed to preferential partitioning of the alcohol into the liquid-crystalline phase rather than the unhydrated crystalline or gel phases. Partition coefficients are summarized in Table III. The liquid-crystalline phase/water partition coefficients increase as the alcohol chain length increases, in keeping with the increasingly hydrophobic nature of these compounds. The values for these partition constants are remarkably similar to those reported for partitioning of methanol, ethanol, and propanol into dipalmitoylphosphatidylcholine (Rowe, 1985).

Representative heating thermograms obtained after incubating the samples at 2 °C for 18 h in pure water, and in 0.4 and 1.2 M alcohol solutions, are shown in Figure 3. For DMPE dispersed in pure water, in methanol and ethanol solutions, and in less than 0.6 M propanol, the major excess heat capacity peak was at or lower than 50 °C, and had an enthalpy of about 6 kcal/mol. These values are typical of the gel to liquid-crystalline phase transition. The onset of this transition occurred at the same temperature in both heating and cooling scans. The only effects on this transition resulting from methanol, ethanol, and propanol at less than 0.6 M were small decreases in the transition temperatures accompanied by small increases in the melting enthalpies. The data for samples incubated at 2 °C are summarized in Table IV.

In 0.4 and 1.2 M 1-propanol, and 1.2 M methanol and ethanol solutions, a second transition with a midpoint at approximately 55 °C was seen in the thermograms from the second heating (Figure 3). For convenience, we will refer to this transition as the 55 °C transition, although the precise temperature of the midpoint depends upon alcohol species and concentration. Although the 55 °C transition temperature was somewhat lower than the temperature observed for the L_c to L_α transition, it is substantially higher than the temperature of the gel to liquid-crystalline transition in either the presence or the absence of alcohol. A transition at approximately 55 °C is also seen in some preparations of unhydrated poly-

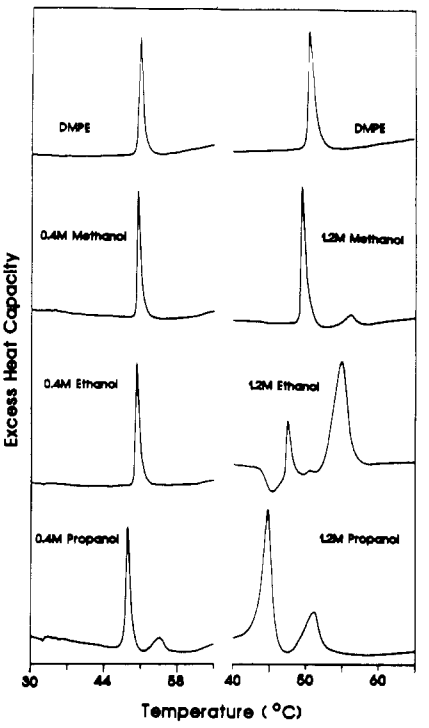

FIGURE 3: Representative heating thermograms obtained after incubating samples at 2 °C for 18 h in pure water and in 0.4 and 1.2 M alcohol solutions.

Table IV: Midpoint Temperatures (T_m) and Enthalpies (ΔH) for the Lower and Upper Transitions Observed in the Second Heating Scan

alcohol	concn (M)	T_m(lower) (°C)	ΔH(lower) (kcal/mol)	T_m(upper) (°C)	ΔH(upper) (kcal/mol)
	0.0	51.0	5.8		
methanol	0.2	50.4	5.8		
methanol	0.4	50.4	5.8		
methanol	0.6	49.7	5.8		
methanol	0.8	49.5	5.7	56.3	0.1
methanol	1.0	49.4	5.5	56.3	0.7
methanol	1.2	49.5	5.4	56.2	0.9
methanol	1.4	49.4	4.5	56.2	2.5
methanol	1.6	49.2	4.1	56.2	4.8
methanol	1.8	48.7	2.7	56.4	11.0
ethanol	0.2	50.3	6.0		
ethanol	0.4	50.0	6.0		
ethanol	0.6	49.0	6.0	56.0	1.4
ethanol	0.8	48.1	4.5	55.0	2.6
ethanol	1.0	47.8	3.8	55.0	7.7
ethanol	1.2	47.5	1.9	55.0	11.5
ethanol	1.4	47.1	0.9	55.0	13.4
ethanol	1.6	46.7	0.8	55.0	14.4
ethanol	1.8	46.3	1.1	55.0	14.9
1-propanol	0.2	48.7	5.7	54.8	1.2
1-propanol	0.4	47.3	4.6	53.7	5.2
1-propanol	0.6	46.3	1.4	53.0	12.9
1-propanol	0.8	45.3	8.9	51.8	7.9
1-propanol	1.0	45.0	13.4	52.0	5.7
1-propanol	1.2	44.5	16.2	51.4	5.7
1-propanol	1.4	44.5	17.6	50.6	3.3
1-propanol	1.6	44.0	18.0	50.3	2.8
1-propanol	1.8	43.5	18.5	49.6	2.4
1-propanol	2.0	43.0	18.5	49.0	1.2

crystalline DMPE (unpublished data). The total enthalpy of both excess heat capacity peaks (50 and 55 °C) together in these thermograms is higher than the enthalpy of transition found in cooling thermograms. Such a high total enthalpy is also seen in thermograms taken at higher alcohol concentrations. Cooling thermograms obtained immediately after the second heating thermogram are identical with those obtained after the first heating thermogram; only the liquid-crystalline to gel transition is seen regardless of the amount or species of alcohol present.

To estimate the molar enthalpy of the 55 °C transition, we observe that only lipid molecules which do not participate in the gel to liquid-crystalline transition may participate in the 55 °C transition. The fraction of such lipid may in turn be estimated from the difference between the entropy of the L_α to L_β transition, calculated from the cooling thermogram, and that of the L_β to L_α transition, calculated from the heating thermogram. The resulting estimate of the enthalpy of the 55 °C transition is found to be approximately 18 kcal/mol, similar to that of the L_c and L_α transition. Symmetric PE's may crystallize in several different forms with slightly different melting temperatures and molecular structures (Mantsch et al., 1983), and we attribute the 55 °C transition in our samples to melting of a dehydrated crystalline phase based upon four similarities to the ordinary L_c to L_α transition: (1) the melting temperature; (2) the high enthalpy; (3) the absence of this transition in cooling scans; (4) the long incubation times required for the appearance of this transition in previously melted samples. Since it is apparent from the melting temperature that the crystalline phase formed in the presence of alcohols which gives rise to the 55 °C transition is not quite identical with that of the unhydrated crystalline lipid melting at 57–58 °C, we will for convenience designate this as the L_c' phase. We were unable to detect production of either the L_c or the L_c' phase in pure DMPE dispersions allowed to incubate in the calorimeter at 2 °C for 18–24 h after initial hydration. Some L_c lipid was observed after incubation of 50 h or more (data not shown). Some of the evidence that the L_c phase is less well hydrated than the gel phase is discussed by Silvius et al. (1986).

To determine whether increased hydrostatic pressure affects formation of the L_c' phase, calorimetric experiments were performed following incubation of lipid in the presence of 0.4 M 1-propanol for 9–24 h under 100 atm of helium pressure. Pressure had relatively little effect on the formation of the L_c' phase; increased pressure appeared to cause only a very small increase in the fraction of lipid converted to this phase. This result contrasts with results which have been reported for the spontaneous dehydration of phosphatidylcholines to form the subgel phase, which is apparently inhibited by increased hydrostatic pressure (Wu et al., 1985).

Because lipid in the L_c' phase does not participate in the gel to liquid-crystalline phase transition, the fraction of lipid remaining in the gel phase may be estimated from the ratio of the enthalpy obtained from cooling scans and that of the lower temperature peak in the heating thermograms. The amount of lipid remaining in the gel phase after 24 h of incubation decreases as the amount of alcohol increases; the effect of alcohol increases with the alcohol chain length. In the case of propanol solutions, this ratio exhibits a dramatic and abrupt increase at approximately 0.6 M propanol. Examination of the thermogram presented in Figure 4 demonstrates that the excess heat capacity peak seen at approximately 45 °C in the presence of high propanol concentrations is not characteristic of the gel to liquid-crystalline phase

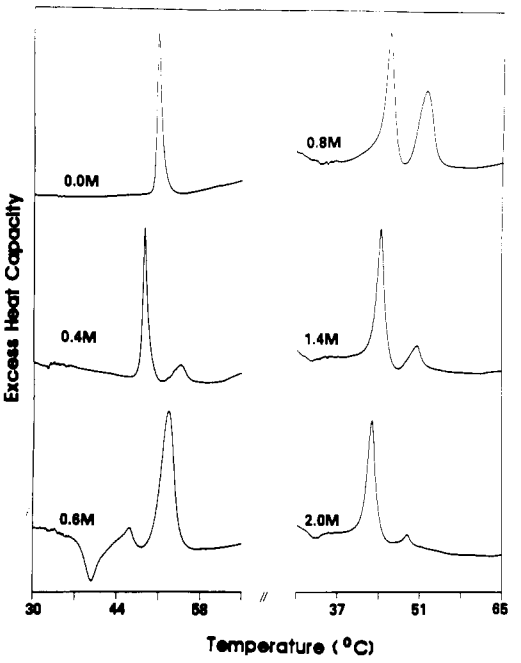

FIGURE 4: Representative heating thermograms obtained after incubating samples at 2 °C for 18 h at various concentrations of 1-propanol.

transition. It is broader and exhibits an enthalpy of approximately 16 kcal/mol, much larger than that of the gel to liquid-crystalline transition. Although the onset of this transition is at approximately the same temperature as the onset observed in the cooling scan, the enthalpy is substantially higher. Thus, the transitions observed in the heating and cooling scans are different. Since the cooling scan immediately followed the heating scan, the liquid-crystalline phase must be the same in both. Hence, the more ordered phase observed in the heating scan (which we designate the L_H phase for convenience) must differ in some way from the ordinary gel phase. A L_c' phase can still form, as evidenced by a peak at 51.4 °C; this peak is smaller than that seen in 0.6 M propanol samples, however.

The calorimetric measurements provide some clues to the nature of the L_H phase. The high enthalpy of melting strongly suggests a highly ordered acyl chain structure, similar to that of the L_c' phase. In addition, the high enthalpy of transition, the lack of reversibility, and the knowledge that other lipids can form interdigitated phases in the presence of alcohol suggest the possibility that this may represent an interdigitated phase.

In summary, calorimetric results show that DMPE dispersed in alcohols can exist in a variety of forms. In addition to the oridinary gel, liquid-crystalline, and unhydrated crystalline phases, a dehydrated crystalline phase which we denote L_c' may be formed in the presence of alcohols. The L_c' phase forms more quickly in solutions containing higher alcohol concentrations, and in solutions containing longer chain alcohols. In addition, DMPE bilayers which have been incubated in propanol solutions of greater than 0.6 M form a phase, which we have designated as L_H, which melts at a temperature similar to that of the gel to liquid-crystalline phase transition, but which has a significantly higher enthalpy. Our data do not provide evidence that this phase is, in fact, lamellar; use of this designation only indicates that this phase is distinct from others which we have observed in DMPE. At high propanol concentration, formation of the L_H polymorph is preferred over formation of the L_c' polymorph; prolonged incubation results in a far smaller endotherm at 53 °C at 2.0 M propanol than

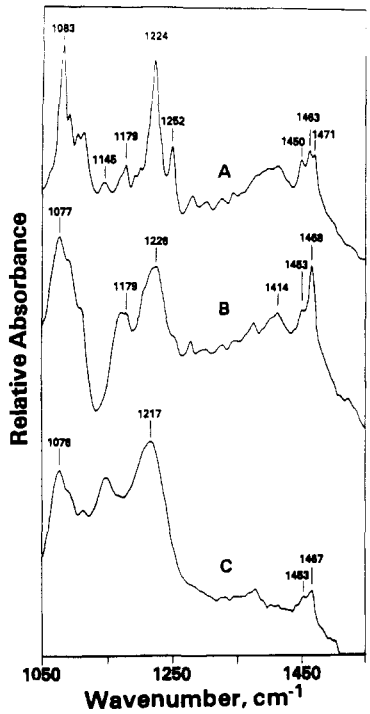

FIGURE 5: Infrared spectra of DMPE–water dispersions in the 1050–1550 cm^{-1} region. Trace A shows the spectrum obtained at 25 °C of unhydrated crystalline lipid dispersed in cold water. Trace B shows the spectrum of hydrated lipid obtained at 25 °C after incubation at 2 °C for 18 h in water. Trace C shows the spectrum of liquid-crystalline lipid (58–60 °C) in water.

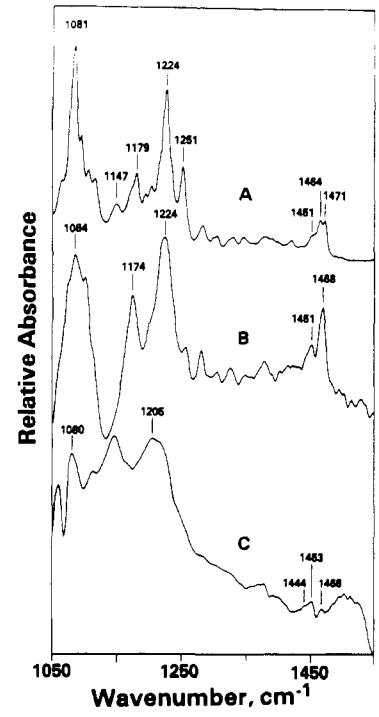

FIGURE 6: Infrared spectra of DMPE–water dispersions in the 1050–1550 cm^{-1} region. Trace A shows the spectrum obtained at 25 °C of unhydrated crystalline lipid dispersed in 1.4 M 1-propanol. Trace B shows the spectrum of hydrated lipid obtained at 25 °C after incubation at 2 °C for 18 h in 1.4 M 1-propanol. Trace C shows the spectrum of liquid-crystalline lipid (58–60 °C) in 1.4 M 1-propanol.

at 0.6 M propanol (data not shown). At concentrations lower than 0.6 M propanol, formation of the L_c' polymorph is preferred. Between these two extremes, both forms are found.

In order to better characterize the L_H polymorph, a series of infrared spectroscopic experiments on lipids incubated in the presence of 1.4 M 1-propanol for 24 h were performed.

Infrared Spectroscopy. Structural alterations associated with the addition of small amounts of alcohol to lipid bilayers are well understood. In addition, the natures of L_α, L_β, L_c, and related crystalline phases have been well characterized. For this reason, infrared spectroscopic studies were performed solely to characterize DMPE dispersions incubated in the presence of high concentrations of alcohol (the L_H phase). The thermodynamic behavior of DMPE at these high alcohol concentrations differs quantitatively and qualitatively from that of phosphatidylcholines and of DMPE in the presence of small amounts of alcohol.

Acyl chain methylene stretching mode features reflect changes in chain conformation resulting from gauche rotations (Mendelsohn & Mantsch, 1986; Cameron et al., 1980). Ordered acyl chains have lower symmetric and asymmetric stretching mode frequencies than disordered chains. Temperature profiles for the L_H to L_α transition and for the L_β to L_α transition, constructed from the asymmetric and symmetric CH_2 stretching mode frequencies of DMPE, show the liquid-crystalline phase CH_2 stretching frequencies to be the same in the presence or absence of alcohol, and the frequencies of the L_β and the L_H phases also to be identical. Thus, it appears that the hydrocarbon chain order is similar whether or not 1.4 M propanol is present.

The CH_2 deformation (scissoring motion) modes, which appear from 1400 to 1500 cm^{-1} (Mendelsohn & Mantsch, 1986; Cameron et al., 1980; Snyder, 1967; Snyder et al., 1978), are sensitive to lipid acyl chain packing. Lipids with acyl chains packed in an orthorhombic or monoclinic crystal lattice subcell are easily identified by site group splitting of the CH_2 deformation bands; hexagonal or quasi-hexagonal packing is characterized by a single CH_2 scissoring mode (Snyder et al., 1978). The magnitude of the splitting is correlated with the degree of interchain packing; i.e. a larger splitting reflects tighter interchain packing (Snyder, 1967, 1979; Snyder et al., 1978, 1980; Snyder & Schachtschneider, 1963). The splitting decreases with increasing temperature, indicating loosening of acyl chain packing (Snyder, 1979). Figures 5 and 6 show spectra of DMPE–water and DMPE–propanol dispersions in the region from 1050 to 1550 cm^{-1}. Site group splitting with frequencies at 1471 and 1464 cm^{-1}, characteristic of an orthorhombic or monoclinic packing, is present in spectra of unhydrated DMPE in both the presence and absence of alcohol. Only one methylene deformation mode feature, with a frequency of 1468 cm^{-1}, is found in spectra of gel, L_H phase, or L_α DMPE in either the presence or the absence of alcohol.

Siminovitch and co-workers (Siminovitch et al., 1987) have suggested that methylene deformation features may be used to determine whether or not lipids are interdigitated. They reason that interdigitated lipids are more tightly packed than noninterdigitated lipids and that they therefore will exhibit features characteristic of this tighter packing, in particular increased splitting of the methylene rocking and scissoring modes (Siminovitch et al., 1987). Our spectra of the L_H phase do not demonstrate this feature. However, the utility of this feature for demonstrating interdigitation has been established only for a single class of lipids. In addition, correlation field splitting may not be an adequate indicator of acyl chain packing density; although various crystalline polymorphs of dilauroylphosphatidylethanolamine presumably have tightly packed acyl chains (on the basis of their high enthalpies of melting), only one exhibits splitting of the deformation mode region (Mantsch et al., 1983). Hence, we do not believe that the spectroscopic characteristics of the methylene deformation

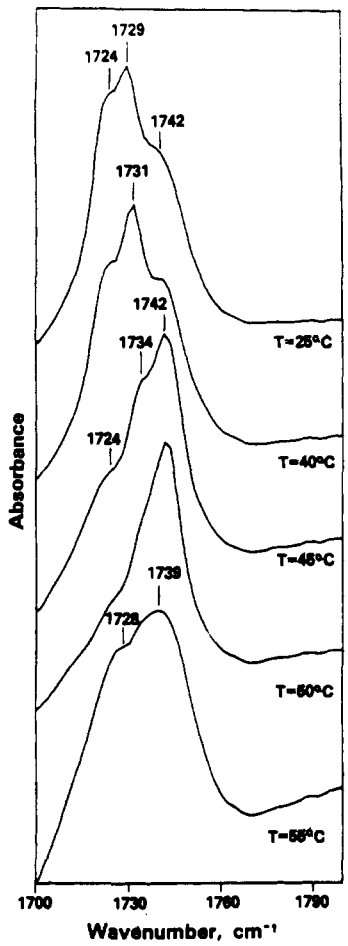

FIGURE 7: Infrared spectra showing the C=O stretching mode region of the L_H phase (T = 25 °C, T = 40 °C), the phase transition region (T = 45 °C, T = 50 °C), and the liquid-crystalline phase (T = 55 °C).

mode region resolve the question of whether or not the L_H phase is interdigitated.

The temperature dependence of the headgroup phosphate bands is illustrated in Figures 5 and 6 for unhydrated DMPE–water in the absence and presence of alcohol, and for the fully hydrated gel and liquid-crystalline phases. In the L_c phase, the various spectroscopic features, including both the symmetric (1085 cm^{-1}) and asymmetric (1225 cm^{-1}) phosphate modes, are sharp and well-resolved; in contrast, these features are quite broad in L_α phase lipids. Although in the gel phase the features have narrower bandwidths than in the liquid-crystalline phase, they still have greater bandwidths than in the unhydrated crystalline phase. Similarly, in the L_H phase formed in the presence of propanol, the features remain relatively broad compared to those of unhydrated crystalline lipids. This suggests that the L_H phase has relatively mobile headgroups in comparison with the L_c phase and that in this respect it more closely resembles the ordinary gel phase than an unhydrated crystalline phase.

The carbonyl stretching modes of model phospholipid membranes serve as useful indicators of bilayer phase transitions and lipid–protein interactions. The effects of alcohol and temperature on the carbonyl stretching modes of fully hydrated gel phase DMPE–alcohol dispersions are illustrated in Figure 7. In the absence of alcohol, gel phase DMPE exhibits a single band with a maximum at 1742 cm^{-1}; upon melting, this band broadens, and the maximum shifts to approximately 1738 cm^{-1}. Only one component is observed in either the gel or the liquid-crystalline state (spectra not shown). In the L_H phase, three distinct C=O components with frequencies at 1742 cm^{-1} (shoulder), 1729 cm^{-1}, and 1724 cm^{-1} (shoulder) are seen. Although the precise frequencies and relative intensities of these components change somewhat as the temperature is raised, all three remain until the melting transition is observed. There are two possible explanations for the appearance of multiple C=O components in the L_H phase. Multiple components could arise from either hydrogen bonding between the acyl chain carbonyl and alcohol hydroxyl groups (Vinogradov & Linnell, 1971) or, possibly, the occurrence of unusual acyl chain rotational isomers (Mushayakarara & Levin, 1982). We believe hydrogen bonding to be the more likely of the two possibilities. Since it appears that the perturbed carbonyl frequency is 1742 cm^{-1}, we infer that the *sn*-1 ester C=O group is the preferred site for this hydrogen bonding interaction. This in turn suggests penetration of the alcohol hydrocarbon chains into the hydrocarbon chain region of the lipid bilayer. As the temperature increases, the C=O stretching band at 1728 cm^{-1} increases monotonically in frequency, while the shoulder at 1742 cm^{-1} increases steadily in intensity. At 45 °C, the temperature at which the DMPE–propanol gel to liquid-crystalline phase transition exhibits its maximal excess heat capacity, the 1742 cm^{-1} component has recovered about 93% of its intensity, while the 1728 cm^{-1} line has become a shoulder and shifted to 1734 cm^{-1}. Above the gel to liquid-crystalline phase transition temperature, the intensity of the 1742 cm^{-1} line is completely restored, and the full width at half-height (FWHH) has decreased from 32 cm^{-1} (trace B) to 18 cm^{-1}, characteristic of a non-hydrogen-bonded carbonyl. Interestingly, slightly above T_m' (at T = 55 °C), the C=O shifts to 1739 cm^{-1} with a concomitant increase of the FWHH to 35 cm^{-1}. This may possibly reflect an interaction between the solvent and the lipid C=O group. The presence of a strong hydrogen bonding interaction between alcohol and lipid carbonyl or, alternatively, the induction of unusual acyl chain rotational isomers provides strong evidence that the L_H phase differs both from the usual gel phase and from the L_c phase, which does not demonstrate such hydrogen bonding in either the presence or the absence of alcohol. In addition, these data suggest the possibility that the L_H phase represents a solid solution containing both lipid and alcohol.

In summary, the infrared spectroscopic data demonstrate that the L_H phase is distinct from the gel, L_c, and L_c' phases. They show that in the L_H phase, the lipid headgroups are more mobile than in the unhydrated crystalline phase and show differences in the acyl chain carbonyl region between L_H, L_c, and gel phases. These difference in carbonyl region spectra suggest the possibility that the L_H phase represents a solid solution of alcohol and lipid. The spectroscopic measurements do not resolve the question of whether or not this phase is interdigitated.

SUMMARY

In addition to the well-known lowering of DMPE gel to liquid-crystalline phase transition temperatures, short-chain alcohols alter the phase behavior of DMPE in several ways. First, the alcohols promote formation of a dehydrated crystalline phase (L_c') upon incubation at 2 °C; the effect is larger at higher alcohol concentrations and increases with the alcohol chain length. Dehydration was not inhibited by hydrostatic pressures of 100 atm. Second, high concentrations of 1-propanol result in the formation of a phase, which we have called an L_H phase, with a melting temperature slightly lower than that of the gel phase but with a much higher melting enthalpy. This phase is characterized by greater headgroup mobility than the L_c phase. Alcohol may be incorporated into

this phase in the form of a solid solution. Although it is tempting to speculate that this phase may be interdigitated based on comparison to the PC's, we have no direct evidence to support this hypothesis.

It seems unlikely that the effect of alcohols either on dehydration or in promoting the formation of the L_H phase is relevant to the mechanism of general anesthesia. The concentrations of alcohol required to exert an observable effect are much higher than the concentrations required for anesthesia, and the effects are not reversible by application of hydrostatic pressure. Whether or not either of the effects described in this paper is of any biological importance remains undetermined.

References

Ahmad, P., & Mellors, A. (1978) *J. Membr. Biol. 41*, 235–247.

Arrowsmith, M., Hadgraft, J., & Kellaway, I. W. (1983) *Biochim. Biophys. Acta 750*, 149–156.

Bangham, A. D., Hill, M. W., & Mason, W. T. (1980) in *Molecular Mechanisms of Anesthesia* (Fink, B. R., Ed.) pp 69–77, Raven Press, New York.

Barchfeld, G. L., & Deamer, D. W. (1988) *Biochim. Biophys. Acta 944*, 40–48.

Biltonen, R. L. (1980) in *Molecular Mechanisms of Anesthesia* (Fink, B. R., Ed.) Raven Press, New York.

Boggs, J. M., & Ranjaraj, G. (1985) *Biochim. Biophys. Acta 816*, 221–233.

Butler, K. W. (1975) *J. Pharm. Sci. 64*, 497–501.

Cameron, D. G., Casal, H. L., & Mantsch, H. H. (1980) *Biochemistry 19*, 3665–3672.

Craig, N. C., Bryant, G. J., & Levin, I. W. (1987) *Biochemistry 26*, 2449–2458.

Deutsch, J. W., & Kelly, R. B. (1981) *Biochemistry 20*, 378–385.

Franks, N. P., & Lieb, W. R. (1978) *Nature 274*, 339–342.

Franks, N. P., & Lieb, W. R. (1980) *Nature 292*, 248–251.

Franks, N. P., & Lieb, W. R. (1982) *Nature 300*, 487–493.

Halsey, M. J. (1974) in *Anesthetic Uptake and Action* (Eger, E. I., Ed.) Williams and Wilkins, Baltimore.

Hui, S. W., & Huang, C. (1986) *Biochemistry 25*, 1330–1335.

Jain, M. K., & Wray, L. V. (1978) *Biochem. Pharmacol. 27*, 1294–1295.

Jain, M. K., Wu, N. Y., & Wray, L. V. (1975) *Nature 255*, 494–496.

Janoff, A. S., Pringle, M. J., & Miller, K. W. (1981) *Biochim. Biophys. Acta 649*, 125–128.

Kaminoh, Y., Tashiro, C., Kamaya, H., & Ueda, I. (1988) *Biochim. Biophys. Acta 946*, 215–220.

Mantsch, H. H., Hsi, S. C., Butler, K. W., & Cameron, D. G. (1983) *Biochim. Biophys. Acta 728*, 325–330.

Mason, J. T., & Stephenson, F. A. (1990) *Biochemistry 29*, 590–598.

Mason, J. T., Huang, C., & Biltonen, R. L. (1981) *Biochemistry 20*, 6086–6092.

McDaniel, R. V., McIntosh, T. H., & Simon, S. A. (1983) *Biochim. Biophys. Acta 731*, 97–108.

Mendelsohn, R., & Mantsch, H. H. (1986) in *Progress in Protein–Lipid Interactions* (Watts, A., & De Pont, J. J. H. H. M., Eds.) pp 103–146, Elsevier, Amsterdam.

Mountcastle, D. B., Biltonen, R. L., & Halsey, M. J. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 4906–4910.

Mushayakarara, E., & Levin, I. W. (1982) *J. Phys. Chem. 86*, 2324–2327.

Nambi, P., Rowe, E. S., & McIntosh, T. J. (1988) *Biochemistry 27*, 9175–9182.

Nosaka, S., Kamaya, H., & Ueda, I. (1988) *Anesth. Analog (N.Y.) 67*, 988–992.

O'Leary, T. J. (1981) *Biophys. Chem. 13*, 315–323.

O'Leary, T. J. (1982) *Biophys. Chem. 15*, 299–310.

O'Leary, T. J. (1983) *Biochim. Biophys. Acta 731*, 47–53.

O'Leary, T. J. (1984) *Biochim. Biophys. Acta 769*, 197–200.

O'Leary, T. J., & Levin, I. W. (1984) *Biochim. Biophys. Acta 776*, 185–189.

O'Leary, T. J., Ross, P. D., & Levin, I. W. (1984) *Biochemistry 23*, 4636–4641.

Rowe, E. S. (1983) *Biochemistry 22*, 2299–3305.

Rowe, E. S. (1985a) *Alcohol (N.Y.) 2*, 173–176.

Rowe, E. S. (1985b) *Biochim. Biophys. Acta 813*, 321–330.

Rowe, E. S. (1987) *Biochemistry 26*, 46–51.

Ruocco, M. J., Makriyannis, A., & Siminovitch, D. (1985a) *Biochemistry 24*, 4844–4851.

Ruocco, M. J., Siminovitch, D. G., & Griffin, R. G. (1985b) *Biochemistry 24*, 2406–2411.

Seeman, P. (1972) *Pharmacol. Rev. 24*, 583–655.

Silvius, J. R., Brown, P. M., & O'Leary, T. J. (1986) *Biochemistry 25*, 4249–4258.

Siminovitch, D. J., Wong, P. T. T., & Mantsch, H. H. (1987a) *Biophys. J. 51*, 465–473.

Siminovitch, D. J., Wong, P. T. T., & Mantsch, H. H. (1987b) *Biophys. J. 51*, 465–473.

Smith, E. B., Browser-Riley, F., & Daniels, S. (1986) in *Molecular and Cellular Mechanisms of Anesthetics* (Roth, S. H., & Miller, K. W., Eds.) pp 341–353, Plenum, New York.

Snyder, R. G. (1967) *J. Chem. Phys. 47*, 1316–1360.

Snyder, R. G. (1979) *J. Chem. Phys. 71*, 3229–3235.

Snyder, R. G., & Schachtschneider (1963) *Spectrochim. Acta 19*, 85–116.

Snyder, R. G., Hsu, S. L., & Krimm, S. (1978) *Spectrochim. Acta, Part A 34A*, 395–406.

Snyder, R. G., Scherer, J. R., & Gaber, B. P. (1980) *Biochim. Biophys. Acta 601*, 47–52.

Suezaki, Y., Kaneshina, S., & Ueda, I. (1983) *J. Colloid Interface Sci. 93*, 225–234.

Tenchov, B. G. (1985) *Prog. Surf. Sci. 20*, 378–385.

Veiro, J. A., Nambi, P., Herold, L. L., & Rowe, E. S. (1987) *Biochim. Biophys. Acta 900*, 230–238.

Veiro, J. A., Nambi, P., & Rowe, E. S. (1988) *Biochim. Biophys. Acta 943*, 108–111.

Vinogradov, S. N., & Linnell, R. H. (1971) *Hydrogen Bonding*, pp 147–175, Van Nostrand Reinhold, New York.

Wu, W., Parkson, L. C., & Huang, C. (1985) *Biophys. J. 47*, 237–242.