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Diacylglycerols, Lysolecithin, or Hydrocarbons Markedly Alter the Bilayer to Hexagonal Phase Transition Temperature of Phosphatidylethanolamines†

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ABSTRACT: The bilayer to hexagonal phase transition temperatures of dielaidoylphosphatidylethanolamine and 1-palmitoyl-2-oleoylphosphatidylethanolamine are 65.6 and 71.4 °C, respectively. Using high-sensitivity differential scanning calorimetry, I have shown that these transition temperatures are extremely sensitive to the presence of small amounts of other lipid components. For example, at a mole fraction of only 0.01, dilinolenin lowers the bilayer to hexagonal phase transition temperature of 1-palmitoyl-2-oleoylphosphatidylethanolamine by 8.5 °C. Other diacylglycerols have similar effects on this transition temperature, although the degree of unsaturation of the acyl chains has some effect, with distearin being less potent. In comparison, the 20-carbon alkane eicosane lowers this transition temperature by 5 °C, while palmitoyllysolecithin raises it by 2.5 °C. Similar effects of these additives on the bilayer to hexagonal phase transition temperature are observed with dielaidoylphosphatidylethanolamine. At these concentrations of additive, there is no effect on the gel-state to liquid-crystalline-state transition temperature. The observed shifts in the temperature of the bilayer to the hexagonal phase transition can be qualitatively interpreted in terms of the effects of these additives on the hydrophilic surface area and on the hydrophobic volume. Substances expanding the hydrophobic domain promote hexagonal phase formation and lower the bilayer to hexagonal phase transition temperature. The sensitivity of the bilayer to hexagonal phase transition temperature to the presence of additives is at least as great as that which has been observed for any other lipid phase transition.

Phospholipids in biological membranes are organized predominantly as bilayers although NMR studies have provided evidence for the presence of nonbilayer phases in sarcoplasmic reticulum vesicles (Davis & Inesi, 1971; Cullis & de Kruijff, 1979). A substantial fraction of the phospholipids which occur in biological membranes can readily be converted into a hexagonal phase when they are in pure form. Phosphatidylethanolamines containing an alkenyl ether bond in position 1 of glycerol undergo a bilayer to hexagonal phase transition close to physiological temperatures (Lohner et al., 1984). There is evidence that the formation of a hexagonal phase can have a marked effect on biological phenomena (Verkleij, 1984; Rilfors et al., 1984; Gordon-Kamm & Steponkus, 1984) including the functioning of the Ca^{2+}-ATPase of sarcoplasmic reticulum (Navarro et al., 1984). It is therefore of importance to determine the factors which modulate the bilayer to hexagonal phase transition. Using high-sensitivity differential scanning calorimetry, we can demonstrate that the temperature at which phosphatidylethanolamines undergo a bilayer to hexagonal phase transition is markedly sensitive to the presence of certain minor impurities.

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EXPERIMENTAL PROCEDURES

Materials. Dielaidoylphosphatidylethanolamine (DEPE),[1] 1-palmitoyl-2-oleoylphosphatidylethanolamine (POPE), and 1-palmitoyllysolecithin were obtained from Avanti Polar Lipids. The phosphatidylethanolamines exhibited a sharp gel to liquid-crystalline phase transition, indicating a high degree of purity. DEPE had a transition at 37.2 °C with a van't Hoff enthalpy of 1800 kcal/mol while the transition for POPE was at 24.8 °C with a van't Hoff enthalpy of 950 kcal/mol. Both gel to liquid-crystalline phase transitions show a small degree of asymmetry, with a shoulder on the low-temperature side of the transition peak, a characteristic of gel to liquid-crystalline phase transitions of phosphatidylethanolamines (Chowdhry et al., 1984).

Eicosane, a 20-carbon saturated alkane, eicosene, an analogue with one double bond, and several diacylglycerols (all the 1,2-isomers) were obtained from Nu Chek Prep. These products were all chromatographically purified to greater than 99%.

Sample Preparation. The phospholipid and additive were dissolved together in a solution of chloroform and methanol (2:1 v/v). The solvent was evaporated with a stream of dry nitrogen so as to deposit the lipid as a film on the walls of a glass test tube. Last traces of solvent were removed into a liquid nitrogen trap by placing the samples in a vacuum oven at 40 °C. The apparatus was maintained under high vacuum for at least 90 min. It is possible that some eicosene (which is a liquid in pure form at room temperature) is lost during this procedure. This lipid film was then suspended in a pH 7.40 buffer of 20 mM Pipes, 1 mM EDTA, 150 mM NaCl, and 0.02 mg/mL NaN_3 by warming the tube to about 45 °C and vortexing vigorously for about 30 s. The buffer and lipid suspensions were degassed under vacuum before being loaded into the calorimeter.

Differential Scanning Calorimetry (DSC). Lipid suspension or buffer was loaded into the sample or reference cell, respectively, of an MC-2 high-sensitivity scanning calorimeter (Microcal Co., Amherst, MA). Samples were maintained for at least an hour below the bilayer to hexagonal phase transition temperature before being scanned. The scan rate was generally 0.9 K min^{-1}, but similar results were obtained, in both the presence and absence of additives, with a 3 times slower scan rate. The faster scan rate was routinely used to achieve higher sensitivity because of the low enthalpy of the transition, to avoid degradation as a result of exposing the lipid to high temperatures for a prolonged time, and to save time because of the large number of scans and wide temperature range used for this study. Second heating scans on the same sample were essentially superimposable on the first scan except for pure lipid samples which were heated above 80 °C. These latter samples exhibited an increase of approximately 0.1 °C in the bilayer to hexagonal phase transition temperature. The calorimeter was calibrated electrically. The transition enthalpy was calculated from the areas of the peaks which were obtained by cutting and weighing. There was no marked or consistent effect of the additives on the transition enthalpy. However, the enthalpy for the bilayer to hexagonal phase transition could not be determined to a precision better than ±35% for an individual measurement. This poor precision results from the fact that the enthalpy for this transition is only about 400 cal/mol, the transition becomes broader in the presence of additive, and the lipid samples with additive tended to clump, making it more difficult to obtain a uniform suspension. Previously, we used a 3 times more concentrated suspension of lipid to obtain a more accurate estimate of the transition enthalpy for pure DEPE and POPE (Epand, 1985). This was not done in this work to conserve the lipid sample, since it was clear that there was no large effect on the transition enthalpy. At a higher mole fraction of additive, where a larger effect on the transition enthalpy might be expected, the bilayer to hexagonal phase transition becomes too broad to accurately determine the enthalpy.

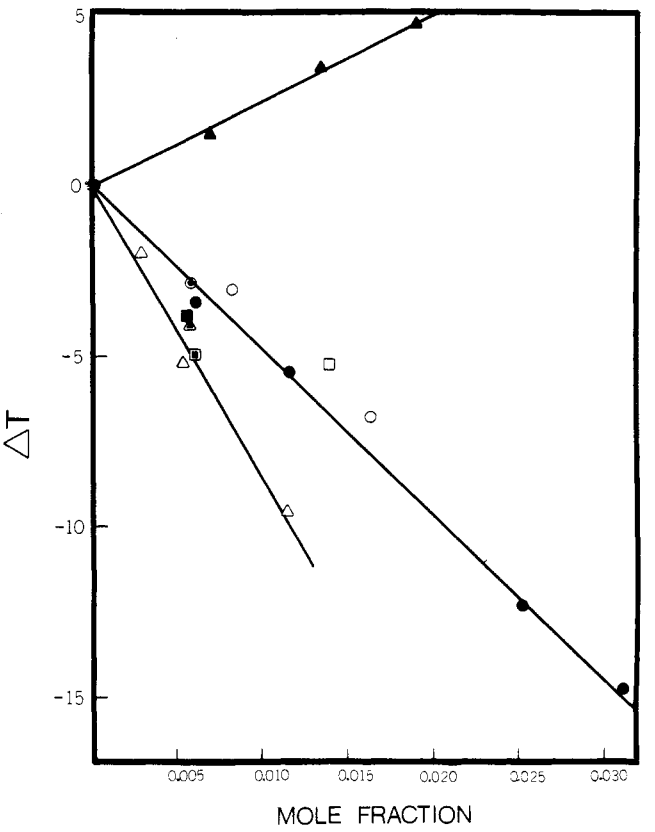

FIGURE 1: Shift of the bilayer to hexagonal phase transition temperature in the presence of additives. ΔT is the change in transition temperature from 65.6 °C for DEPE or 71.4 °C for POPE. Phospholipid concentration 4 mM in a pH 7.4 buffer of 20 mM Pipes, 1 mM EDTA, 150 mM NaCl, and 0.02 mg/mL NaN_3. (●) Eicosane + POPE; (○) eicosane + DEPE; (⊙) distearin + DEPE; (■) distearin + POPE; (□) eicosene + DEPE; (closed square inscribed in open square) diolein + POPE; (closed triangle inscribed in open triangle) dilinolein + POPE; (△) dilinolenin + POPE; (▲) 1-palmitoyllysolecithin + POPE.

^{31}P NMR. Lipid was deposited on the wall of a 10-mm NMR tube. Last traces of solvent were removed under high vacuum for 6 h. The lipid was suspended in Pipes buffer to give a final concentration of DEPE of 90 mM. Spectra were obtained with a Bruker WM-250 NMR spectrometer operating at 101.2 MHz. Broad-band proton decoupling was employed. A spectral bandwidth of 30 KHz was used with an acquisition time of 0.28 s, a relaxation delay of 0.3 s, and a pulse width of 25 μs (90°). Typically, accumulated free induction decays were obtained from 800 transients to obtain an adequate signal to noise ratio.

RESULTS

The temperature of the bilayer to hexagonal phase transition of phosphatidylethanolamines is very sensitive to the presence of several substances. The temperature at which pure DEPE is converted to the hexagonal phase is 65.6 °C and is 71.4 °C

[1] Abbreviations: DEPE, dielaidoylphosphatidylethanolamine; POPE, 1-palmitoyl-2-oleoylphosphatidylethanolamine; DSC, differential scanning calorimetry; EDTA, ethylenediaminetetraacetic acid; Pipes, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid).

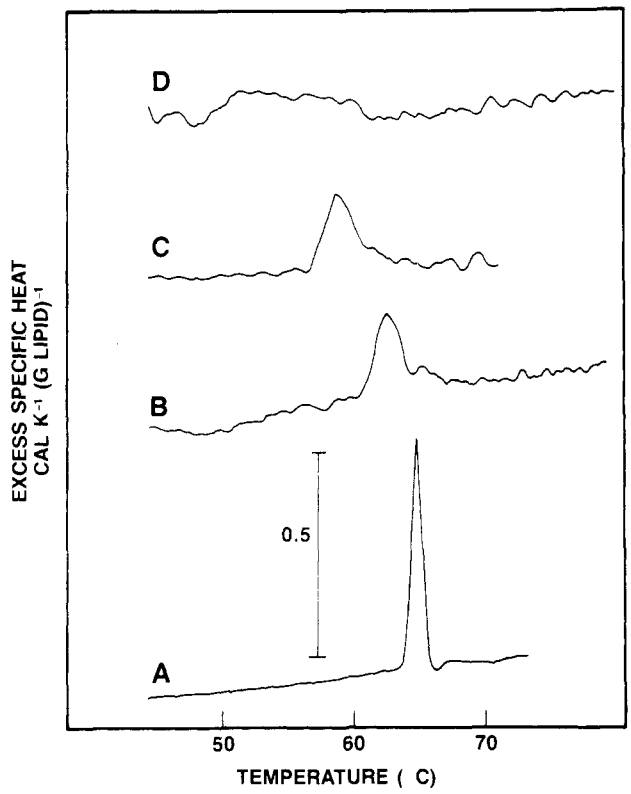

FIGURE 2: Differential scanning calorimetry curves for DEPE in the presence of increasing concentrations of eicosane. Scan rate 0.9 K min^{-1}. Buffer: 20 mM Pipes, 1 mM EDTA, and 0.15 M NaCl containing 0.02 mg/mL NaN_3, pH 7.40. DEPE concentration 3.7 mM. Mole fraction of eicosane: (A) 0.000; (B) 0.0083; (C) 0.016; (D) 0.032.

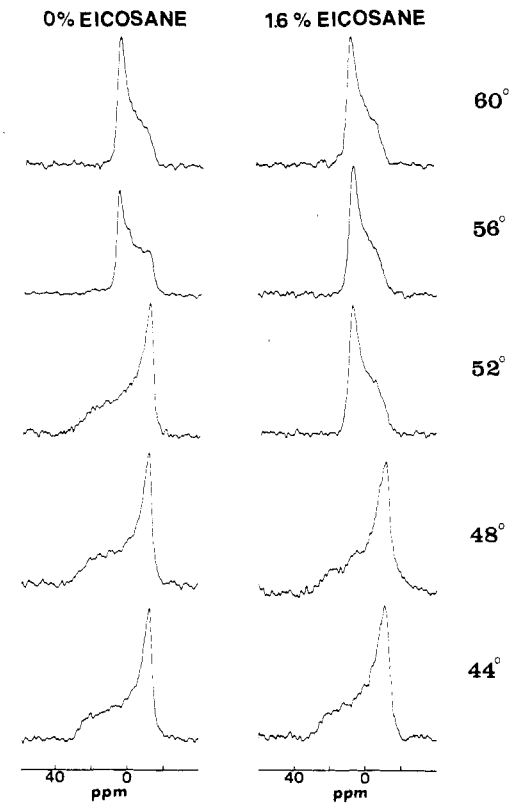

FIGURE 3: ^{31}P NMR spectra of DEPE and DEPE in the presence of 1.6 mol % eicosane (0.6 wt %) as a function of temperature. The chemical shift is given with respect to phosphoric acid equal to 0.

for POPE. The extent to which this temperature is lowered by alkanes or diacylglycerols or is raised by lysolecithin is proportional to the mole fraction of additive (Figure 1). An example of a series of DSC scans is given for DEPE in the presence of increasing amounts of eicosane (Figure 2). In the presence of 99% phosphatidylethanolamine and only 1% additive, the bilayer to hexagonal phase transition is altered by several degrees centigrade. The effects of eicosane on DEPE and POPE are similar. Dilinolenin is the most effective substance on a molar basis, of those tested, in lowering the temperature of the transition to the hexagonal phase. On a weight basis, dilinolenin has an effect comparable to that of eicosane. The other diacylglycerols are of intermediate effect. They are less effective than eicosane on a weight basis but more effective on a molar basis. Distearin is the least effective of the diacylglycerols. However, the effect of introducing one, two, or three double bonds into the 18-carbon acyl chains, i.e., converting to diolein, dilinolein, or dilinolenin, has only a small effect on the ability of the diacylglycerol to lower the transition temperature for hexagonal phase formation. The same order of effectiveness of the diacylglycerols or 1-palmitoyllysolecithin was observed with DEPE as with POPE, but all of the data obtained are not presented in Figure 1 for clarity.

The bilayer to hexagonal phase transition can also be determined from the anisotropy of the ^{31}P NMR spectra. Our results again demonstrate the lowering of the temperature at which this transition takes place upon addition of low concentrations of eicosane (Figure 3). At 52 °C, for example, the presence of only 1.6% eicosane can covert the bilayer arrangement of DEPE to one giving NMR spectra representative of the hexagonal phase. The alterations in the NMR spectra were reversible upon recooling the samples. The temperature at which the bilayer-type NMR spectrum converts to an NMR spectrum representative of the hexagonal phase is several degrees lower than that which would be anticipated on the basis of the results from differential scanning calorimetry. This could result from the molecular motions of the head groups being altered to resemble those which occur in the hexagonal phase while the temperature is still below the bilayer to hexagonal phase transition temperature. It has been previously demonstrated that phospholipids in bilayer arrangement can give ^{31}P NMR spectra characteristic of the hexagonal phase (Noggle et al., 1982).

The gel to liquid-crystalline phase transition temperatures of DEPE and POPE are 37.2 and 24.8 °C, respectively. These temperatures are not altered by more than 0.2 °C in the presence of any of the additives used at mole fractions below 0.03, compared to a change in the bilayer to hexagonal phase transition temperature of 5–15 °C.

Discussion

There have been many studies on the effects of additives on the properties of the gel to liquid-crystalline transition of phospholipid bilayers. One of the most dramatic changes in the temperature of this phase transition occurs upon the addition of calcium to phosphatidylserine (Newton et al., 1978). The sensitivity of the bilayer to hexagonal phase transition temperature of phosphatidylethanolamines to the presence of alkanes, diacylglycerols, or lysolecithin is at least as great as the sensitivity of any gel to liquid-crystalline phase transition to the presence of an additive.

Many substances have been shown to promote or inhibit the formation of the hexagonal phase (Verkleij, 1984; Rilfors et al., 1984; Killian et al., 1985). It was suggested that diacylglycerols can activate phospholipase activity through the formation of nonbilayer phases (Dawson et al., 1983). This could lead to a feedback mechanism by which a product of phospholipase activity, lysolecithin, could inhibit the action

of this enzyme by favoring a hexagonal to bilayer phase transition. While the current study was in progress, Das & Rand (1984) presented evidence to show that diacylglycerols can induce hexagonal phase formation in phospholipid bilayers. Recently, Valtersson (1985) demonstrated the induction of the hexagonal phase by dolichols. Factors which have been shown to be of importance in determining whether a bilayer or hexagonal phase is formed are the shape of the molecule (Cullis & de Kruijff, 1979; Israelachvili et al., 1980; Wieslander et al., 1980), the degree of hydrogen bonding of the head groups (Hitchcock et al., 1974; Boggs, 1984), or the hydration of the head group (Seddon et al., 1983). These factors are not all mutually independent. Our results can most directly be interpreted in terms of the marked sensitivity of the bilayer to hexagonal phase transition temperature to the molecular shape. This dependence of lipid morphology on molecular shape can be expressed in terms of the relationship v/al where v is the volume of the hydrophobic portion of the bilayer, l is the length of the hydrophobic portion in a direction perpendicular to the plane of the bilayer, and a is the hydrophilic surface area (Israelachvili et al., 1980). The higher the value of v/al, the more likely the lipid will be converted to the reversed hexagonal, H_{II}, phase. A cone-shaped molecule like diacylglycerol with a small head group (i.e., a low value of a) stabilizes the hexagonal phase as do pure hydrocarbons which partition exclusively into the hydrophobic domain (i.e., increase v). In contrast, lysolecithin with a large head group (i.e., a large value of a) and a small (only one acyl chain) hydrophobic portion (i.e., small v) stabilizes the bilayer phase.

The ability of such a small fraction of the total lipid composition to markedly alter the temperature at which the bilayer phase becomes unstable suggests that substances such as alkanes and diacylglycerols can trigger biological phenomena by inducing the formation of a hexagonal phase. Alkanes can arise in vivo as a result of lipid oxidation or can be given as general anaesthetics while diacylglycerols are potent activators of protein kinase C. The importance of hexagonal phase formation to the mechanism of action of these substances remains to be directly demonstrated. The ability of such low concentrations of these substances to markedly alter the bilayer to hexagonal phase transition temperature makes such a possibility more likely.

Added in Proof

The tendency to form nonbilayer phases has recently been described in terms of bilayer curvature and hydrocarbon packing strains (Gruner, 1985).

Acknowledgments

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Registry No. DEPE, 16777-83-6; POPE, 10015-88-0; eicosane, 112-95-8; distearin, 1188-58-5; eicosene, 27400-78-8; diolein, 2442-61-7; dilinolein, 2442-62-8; dilinolenin, 35098-84-1; 1-palmitoyllysolecithin, 14863-27-5.

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