PHYSICAL PROPERTIES OF PHOSPHATIDYLETHANOLAMINE AND LYSOPHOSPHATIDYLETHANOLAMINE DIFFERING IN THE DEGREE OF METHYLATION

by

Khalid Omar Abulnaja

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Approved by:

Willis E. Klopfenstein
Major Professor

TABLE OF CONTENTS:

LIST OF FIGURES

LIST OF TABLES

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INTRODUCTION

Lipids have long been known to be major components of biological membranes. In order to understand the structure and function of membranes, the structures of the lipid component and the effects which variations of the structures within this component might have on the properties of the membrane have been extensively studied. One property of polar lipids which is easily observed results from the mesomorphic nature of lipids. This is the temperature-dependent transition between the gel state and a liquid crystalline state. The gel state, in which the fatty acid chains are relatively restrained, exists at lower temperatures, while the liquid crystalline state, in which the fatty acid chains, as well as the whole lipid molecule, enjoy considerable freedom of movement, is present at higher temperatures. Passage from one state to the other is called a phase transition (1,2,3).

A wide range of physical techniques has been used to investigate the phase transitions of anhydrous and hydrated lipid systems. These include x-ray diffraction (4), fluorescence polarization, Raman spectroscopy, differential scanning calorimetry (5), and nuclear magnetic resonance (NMR) (6). Each technique has its advantages and disadvantages.

Differential scanning calorimetry is used to study the thermotrophic behavior of lipid systems, including biological membranes. The method detects and measures the heat uptake in the conversion of the system from the ordered gel state to the relatively less ordered liquid crystalline state (2). The heat uptake is the result of the melting of the hydrocarbon chains of the phospholipids. In this process the hydrocarbon chains are converted from the ordered and condensed (all trans) structure to a fluid

and more expanded (gauche) structure. Accompanying this melting of the hydrocarbon chains are an expansion of the system, (an increase in the total volume occupied by the phospholipid molecule) and a thinning of the bilayer (8). The temperature at which a pure phospholipid may undergo this thermal transition is called the thermal transition temperature (T_t). The phase transition is sometimes accompanied by a pretransition (T_p) which is a small, broad transition which occurs at a lower temperature than the main thermal transition (9,10). The temperature spread between the T_p, if observed, and the main transition is dependent upon the structure of the phospholipid being studied (10).

The transition temperature depends upon the structure of the phospholipid molecule and a variety of environmental factors. The various factors affecting T_t are summarized in Table 1.

The physical properties of the lysophosphatidylethanolamines have not been investigated to any extent. Therefore, we have undertaken to determine the effects of the degree of methylation (0 to 3 methyl groups) of phosphatidylethanolamines on their thermotropic properties. This required the synthesis of 1-palmitoyllysophosphatidyl-N-methylethanolamine and 1-palmitoyllysophosphatidyl-N-N-dimethylethanolamine. For comparison, we have also studied the analogous diacyl compounds. Thermal transitions were determined on aqueous dispersions of the lipids using differential scanning calorimetry.

Table 1

Factors Which Affect the Transition Temperatures of Phospholipids

A. Lipid Structure

1. Fatty acid structure

a. transition temperature increases with chain length. (11,12)

b. unsaturated fatty acids result in lower transition temperatures than the corresponding saturated fatty acid.

c. increasing the degree of unsaturation results in lowered transition temperatures.

d. cis unsaturation produces lower transition temperatures than trans unsaturation.

e. branching of the fatty acid chain lowers the transition temperature. The effect is greater for anteiso than for iso branched fatty acids. (13, 14)

2. Number of fatty acids esterified. Lyso compounds have lower transition temperatures than the corresponding diacyl phospholipid.

3. Nature of the polar group.

a. increasing the state of methylation from phosphatidylethanolamine to phosphatidylcholine results in decreased transition temperatures. (12)

b. other structural features which increase the bulkiness of the polar group result in lowered transition temperatures.

B. Other factors which affect transition temperatures. (15)

1. pH.

2. Ionic strength of the medium.

3. Concentration of cations, especially divalent cations.

4. Basic protein in the medium.

ABBREVIATIONS

Some common abbreviations used throughout this thesis are the following:

ΔH - enthalpy of transition

ΔS - entropy of transition

DLPC - dilauroylphosphatidylcholine

DPPC - dipalmitoylphosphatidylcholine

DPPE - dipalmitoylphosphatidylethanolamine

DPP-N-methyl E - dipalmitoylphosphatidyl-N-methylethanolamine

DPP-N-N-dimethyl E - dipalmitoylphosphatidyl-N-N-dimethylethanolamine

DSC - differential scanning calorimetry

DSPC - dislearoylphosphatidylcholine

OPPC - 1-oleoyl-2-palmitoylphosphatidylcholine

PC - phosphatidylcholine

PE - phosphatidylethanolamine

PG - phosphatidylglycerol

PLPC - palmitoyllysophosphatidylcholine

PLPE - palmitoyllysophosphatidylethanolamine

PLP-N-methyl E - palmitoyllysophosphatidyl-N-methylethanolamine

PLP-N-N-dimethyl E - dipalmitoylphosphatidyl-N-N-dimethylethanolamine

POPC - 1-palmitoyl-2-oleoylphosphatidylcholine

PS - phosphatidylserine

T_t - transition temperature

LITERATURE REVIEW

Lysophospholipids:

Lysophospholipids are physiologically important for many tissues and play an important role in the metabolism of phospholipids (16). They are intermediate compounds in the synthesis of free arachidonic in mammalian cells (Fig. 1) (17). For example, lysophosphoglycerides are intermediates in phosphoglyceride metabolism. These compounds are found in cells or tissues in very small amounts. In high concentration they are toxic or injurious to membrane and cause the cells to lyse by induced fusion (18). The adsorption and penetration of lysophospholipids into cell membranes cause a disordering of the structure of the hydrocarbon chains, changing the osmotic equilibrium inside and outside the cell, change membrane permeability, and result in loss of cellular contents (19).

Inoue and Kilagawa (20) investigated the effect of lysophosphatidylcholine on liposomal membranes and they found that the addition of cholesterol to the cells or liposomal membranes can suppress the effects of lysophosphatidylcholine which interacts with cholesterol and forms a 1:1 molecular complex.

Membrane Structure

Membranes serve a multifunction role. They not only provide charged barriers between inside and outside the cells but also form compartments within cell, control specific enzymatic activity, contain antigens, regulate the transport of various molecules and ions, and contain hormone receptors (21). The basic structure of a biological membrane is the bilayer. A good representation of the membrane structure is the fluid

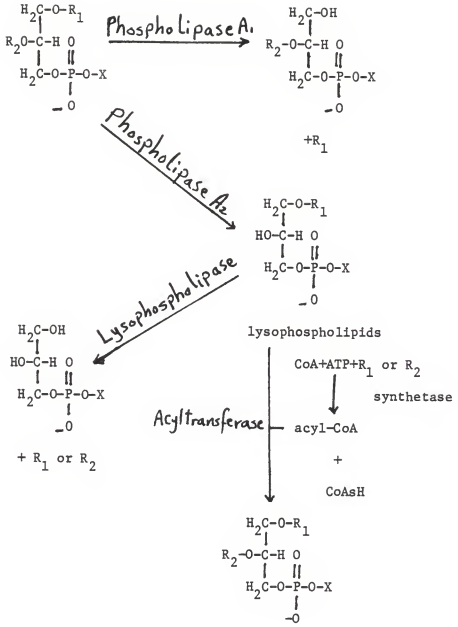


Fig. (1) Deacylation and reacylation of glycerophospholipids

X is a base (choline in PC, ethanolamine in PE and, etc.)

R_1 and R_2 are the fatty acids. R_2 is where most of the arachidonate in mammalian cells is esterified.

mosaic model of Singer and Nicolson (22) who postulate that the phospholipids of the membrane are arranged in a bilayer to form a fluid or liquid-crystalline matrix with globular protein molecules penetrating into either side or extending entirely through the membrane. The polar groups of the phospholipid molecules are localized in the outer faces of the bilayer with the hydrocarbon chain nonpolar part directed to the interior.

Most membranes contain about 40 percent lipid and 60 percent protein with some variation. The lipid composition in the biological membrane may vary by the effect of different factors. The phospholipid and the fatty acid composition in the microsomal membrane change with fatty acid composition of diet (23). Zinc deficiency causes an increase of the cholesterol:phospholipid ratio and a decrease of unsaturated:saturated acid ratio (24). The phospholipid components of biological membranes are amphipathic and mainly made of phosphatidylcholine and phosphatidylethanolamine. Human erythrocytes (25) and microsomal membranes (23) have a higher percentage of phosphatidylcholine than phosphatidylethanolamine. Other lipids are present as minor components in biological membranes, including cholesterol, triacylglycerol, phosphatidylserine, and phosphatidylinositol.

The Gel-Liquid Crystalline Transition Studies on Pure Phospholipids:

The thermal analysis of lipids was initiated by Champan in the 1960's (15). When a pure phospholipid is dispersed in water by mechanical agitation, it forms a suspension which usually exhibits a thermal phase transition. This phase transition can be detected by DSC. The phosphatidylcholines, containing two identical saturated fatty acids, have been the most studied by DSC and other physical techniques (26,10).

Typical DSC scans of dipalmitoylphosphatidylcholine have shown two peaks. First, the main transition temperature peak which is characterized by a sharp, symmetrical, and endothermic transition. A second peak that is relatively smaller, has a broader endothermic transition, and is located several degrees below the gel to liquid-crystalline transition, is known as the pretransition.

The pretransitions of a series of simple disaturated phosphatidylcholines are characterized by ΔH values of 1.0-1.8 kcal/mol and there is a slight change in ΔH with chain length. The pretransition does not exist if the saturated fatty acid chains of the disaturated phosphatidylcholine is less than 14 carbons. Also the pretransition disappears on addition of small amounts of cholesterol (10), fatty acids (27), or other compounds. It was thought (28) that the appearance of the pretransition of disaturatedphospholipids was due to a conformational change of polar head group. However, X-ray diffraction data (4) indicate that the hydrocarbon chains of fully hydrated diacylphosphatidylcholine are aligned and tilted at an angle of about 30° to the normal of the bilayer plane in the gel state. As the temperature increases, the chains become normal to the plane in the liquid-crystalline phase. It has been suggested that the change in tilt angle is an integral part of the pretransition in phosphatidylcholines (29).

The main transition or phase transition which appears after the pretransition as the temperature increases is characterized by enthalpy of transition (ΔH) values for each molecular species of phospholipids. This phase transition involves an important change in the structure of phospholipid bilayer. These structural changes at the phase transition involve: first, an enthalpic change from an ordered and condensed state in

the hydrated gel phase to a fluid and expanded state in the liquid-crystalline phase (8). Second is a configurational change of the carbon-carbon bonds of the hydrocarbon tails from antiperiplaner or all trans structure to a synclinal or gauche structure (30). Third, are conformational changes of the glycerol backbone of the phospholipid molecule which allow two fatty acids to stack at at distance of 0.48 nm in the gel phase and as temperature increases, the interchain distance is increased to 0.6 nm (31). Fourth, is an increase in surface area. Fifth is an increase of total volume occupied by phospholipid molecules. Sixth is a decrease in the thickness of the phospholipid bilayer (8). Seventh is an increase of the coefficient of lateral diffusion (32). All the structural changes which occur at the phase transition are summarized in (Table 2).

The thermodynamic parameters associated with the main transition of simple saturated symmetric-chains of phosphatidylcholines have been investigated by a number of laboratories (11,33). They reported that ΔH values increase progressively with chain length from about 1.7 kcal/mol for dilauroylphosphatidylcholine to about 10.6 kcal/mol for distearoylphosphatidylcholine. Also, ΔS values increase progressively with increasing chain length. The transition temperature which increases with increasing the chain length (11) has a linear relationship with ΔH for the series of even-chained phosphatidylcholines from 12 to 22 carbons (10). The transition temperature is chain-length dependent for diacylphosphatidylethanolamines also (33).

The thermotropic phase behavior of isobranched phosphatidylcholines have been investigated by Silvius and McEthaney (13). They reported that

phospholipid Bilayer

Trans. temp.

Hydrated Gel ⟶ liquid-crystalline

ΔH

ordered and condensed → a fluid and expanded

Configuration of	all antiperiplaner	⟶	all synclinal
fatty acid	or all trans	⟶	or gauche
interchain distance	0.48 nm	⟶	0.6 nm
of phospholipid molecule			
surface area		⟶	
total volume		⟶	
coefficient of	$<10^{-10}$ cm^2/sec	⟶	$\sim 1 \times 10^{-8}$ cm^2/sec
lateral diffusion		decrease	
Bilayer thickness		⟶	

Table 2. Structural changes at phase transition as temperature increases.

the transition temperature, which increases with the increase of chain length for the series of odd and even isobranched fatty acid of 12 to 18 charbons, is 20°C lower than the corresponding straight-chains of diacylphosphatidylcholines. Furthermore, the transition temperature of diacylphosphatidylcholines containing two identical n-saturated fatty acids is decreased when cis-double bonds or cis-cyclopropyl groups are introduced into the diacylphosphatidylcholines (14).

The temperatures at which the transitions occur are affected by the degree of unsaturation and the type of double bonds of fatty acids present in the diacylphosphatidylcholines (14). An increase of two carbons in chain length of fatty acylphosphatidylcholine for cis or trans-unsaturated fatty acids causes the transition temperature to increase by about 20°. The transition temperature values of 16 carbon members is decreased in this order: trans-dipalmitoleoylphosphatidylcholine (-4.0°C) > cis-dipalmitoleoylphosphatidylcholine (-35.5°C).

A comparison can be made of the various changes in fatty acyl residues of phosphatidylcholine which affect the transition temperature (13,14). The transition temperature of the 18 carbon members of phosphatidylcholines decreased in the following order: n-saturated (54.8°C) > methylisobranched (36.5°C) > trans-cyclopropane (16.3°C) > trans-mono-unsaturated (12.9°C) > cis-cyclopropane (-0.5°C) > cis-mono-unsaturated (-15.8°C). Furthermore, the pretransition, which is observed with di-n-saturated phosphatidylcholines does not appear for the branched chain, unsaturated or cyclopropane fatty acid-containing phosphatidylcholines.

Mixed-acid saturated phosphatidylcholines which contain myristic acid, palmitic acid or stearic acid, have been investigated by high-sensitivity differential scanning calorimetry (34) and DSC (35). An interesting

observation was made that both the transition temperature and Enthalpy of transition were not the same for each isomeric pair of phosphatidylcholines. The isomer that has the longer fatty acyl residue at position two of sn-glycerol has the higher T_t and ΔH. Also, when the fatty acyl residue is constant at position two, an increase of acyl chain length at position one will raise T_t and will not affect ΔH of the main transition. But if the fatty acid is constant at position one, an increase of acyl chain length at position two will increase both T_t and ΔH. The addition of each carbon to the fatty acid in the second situation will increase ΔH by 0.5 kcal/mol. Also, all mixed-acid saturated phosphatidylcholines were observed to have pretransitions with ΔH's at lower temperature than those observed when the two fatty acids were identical.

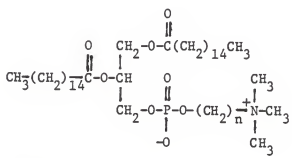
The thermotropic behavior of mixed-acid phosphatidylcholines containing a saturated and an unsaturated chain have been investigated by Davis, et al. using DSC (36). They reported that the transition temperature of the gel to liquid-crystalline phase transition of aqueous dispersions of the same mixed-acid saturated-unsaturated phosphatidylcholines which occur at a wide range of temperature, depends on the procedure being used for the synthesis of these compounds, and on the content of the reversed isomer present. The transition temperature value for 1-palmitoyl-2-oleoylphosphatidylcholine, which contains 8% of the reversed isomer, is about -2.6°C and the enthalpy of transition is about 5.4 kcal/mol. For the same compound, but using a different procedure for synthesis, the transition temperature value is 5°C and ΔH is of 8.1 kcal/mol (37). The transition temperature values for the reversed isomer 1-oleoyl-2-palmitoylphosphatidylcholine range from -11°C to -9.3°C and ΔH values range from 6.7 to 4.6 kcal/mol. All the results support the

observation that the transition temperature and ΔH values of each isomer pair of phosphatidylcholines are different, with the isomer having the saturated chain at position 1 of sn-glycerol exhibiting higher T_t and ΔH values. When the unsaturated fatty acid at position 2 is constant, increasing the chain length of the saturated fatty acid at position 1 will increase the transition temperature T_t and vice versa is true (36,37).

An important factor in the thermotropic behavior of phospholipids is the nature of the polar headgroup. This has been examined by differential scanning calorimetry (38,39,40). The data from calorimetric studies of a number of phosphatidylethanolamines indicate that the transition temperature values, are always higher than for the corresponding phosphatidylcholines. All the diacylphosphatidylethanolamines which show a single asymmetric transition (main transition), do not exhibit a pretransition and the transition temperature T_t and ΔH increase progressively with chain length (40). Disaturated phosphatidylethanolamines are more stable in the gel state than the corresponding disaturated phosphatidylcholines. This increased stability is due to both the smaller size of the polar headgroup of phosphatidylethanolamine (27) and the ability of the amino group of phosphatidylethanolamine to form hydrogen bonds between the protonated amino group and surrounding water molecules or the amino group of adjacent phosphatidylethanolamine molecules (38). Eibl, (41) observed that increasing the pH to 12 will deprotonate the amino group of dipalmitoylysophosphatidylethanolamine and decrease the transition temperature from 63°C to 41°C which is the transition temperature for diaplmitoylphosphatidylcholine (DPPC). This observation supports the latter view.

Bach et al (42) studied the thermotropic behavior of dipalmitoylphos-

phatidylcholine analogues which contain a different number of hydrocarbon chains between the phosphate group and the nitrogen group by DSC (see Fig. 3).



n = 2 to 11 and 16

(Fig. 2) The Chemical Structure of Dipalmiloylphosphatidylcholine analogues.

They reported that the transition temperature is not affected by increasing the hydrocarbon chain length in the polar headgroup of dipalmitoylphosphatidylcholine. They concluded that the stability of the layer structure does not depend on the interaction between the positively charged nitrogen atom and the negatively charged phosphate group.

Studies of Phospholipid Mixtures:

Since biological membranes normally contain several different phospholipids and different kinds of fatty acid, the studies of the thermotropic phase behavior of single component multilamellar dispersions of phospholipids are not realistic models. Although these studies are necessary and valuable to gain more information about realistic models for biological membranes, the DSC studies of various phospholipids mixtures are a first step toward understanding the interaction of both the polar and

nonpolar portions of different lipids present in a mixture. From the observed calorimetric phase transition of these mixtures, phase diagrams can be constructed (25).

Almost ideal behavior is exhibited by disaturated phosphatidylcholines differing by only two carbons in chain length in binary phospholipid-water dispersions. A difference of four carbons in the hydrocarbon chain results in behavior far from ideal. With these simple diacyl phosphosphatidylcholines, in general, the smaller the difference in their transition temperature the more nearly ideal is their mixing behavior (43,44).

The thermotropic behavior of binary mixtures of disaturated phosphatidylcholine and di-*cis*-monounsaturated phosphatidylcholines have been examined by differential scanning calorimetry by Phillips et al (45). Their calorimetric data indicate that these two compounds are largely immiscible in the gel state, whatever the chain length of disaturated phosphatidylcholine. On the other hand, the mixing behavior of disaturated phosphatidylcholine with di-*trans*-monounsaturated phosphatidylcholine approaches perfect miscibility in all proportions, but the mixing becomes less ideal with decreasing chain length of the disaturated phosphatidylcholine (46).

Increasingly non-ideal behavior was achieved by mixing simple phosphatidylglycerols and phosphatidylcholines with differences in unsaturation or increasing differences in hydrocarbon chain length. This non-ideal behavior was no more than what was observed with the corresponding binary mixtures of phosphatidylcholines. This indicates a high amount of miscibility of the phosphatidylglycerol and phosphatidylcholine headgroups (68). Also the non-ideal behavior was exhibited by

mixing phosphatidylcholine with phosphatidylethanolamine having identical fatty acyl groups (46).

Microscopic mixing may contribute significantly to non-ideality in liquid-crystalline and gel phases although no microscopic lateral phase separations occur (47). Because changes in ionic strength and pH can cause significant changes in the transition temperature, isothermal lateral phase separation in liquid-crystalline mixtures of acidic lipids such as phosphatidylglycerol or phosphatidylserine and Zwitterionic lipid such as phosphatidylcholine can be induced by changes in Ca^{2+} concentration or pH (48). Mg^{+2} is not as effective at inducing lateral phase separation in binary mixtures (49).

Effect of Cholesterol on the Phase Transition of Phospholipids

Cholesterol and related sterols are found in substantial concentrations in the membrane of eukaryotic cells (10). Consequently, the effect of cholesterol on the thermotropic behavior of phospholipids has been investigated by using DSC and other physical techniques (10,50,51). These studies have established that cholesterol plays an important role in determining the physical properties of phospholipid bilayers and the fluidity of biological membranes. Cholesterol interacts with phospholipid molecules forming an intermediate state in which the hydrocarbon chains are intermediate between what they would be in the gel state and liquid crystalline state. In this condition there is no phase transition and the structure of the mixture does not depend on the temperature. Some generalization can be made from these studies concerning the structural basis for the cholesterol-phospholipid interactions. The interaction requires a hydroxyl group in the β configuration at cholesterol carbon atom number 3, a planar cholesterol ring system, and a hydrophobic chain at C_{17}

of cholesterol containing not less than 8 carbon atoms.

The thermotropic behavior of mixtures of phosphatidylcholines cholesterol have been examined by DSC (10,37,52). All studies concerning the cholesterol-phosphatidylcholine complex have a general agreement that an increase in the cholesterol concentration will make the gel to liquid-crystalline phase transition of phosphatidylcholine wider and broader and will reduce its enthalpy of transition. However, these are disagreements about the concentration of cholesterol which is necessary to remove the phase transition completely and the direction of the shift in the transition temperature. Studies using high sensitivity differential scanning calorimetry (53,54) of dipalmitoylphosphatidylcholine-cholesterol system have provided additional information about the nature of the cholesterol-phospholipid interaction and may have resolved this argument. High sensitivity DSC scans of the phase transition of DPPC-cholesterol mixtures containing between 0-20 mol percent cholesterol, can be resolved into two components, one sharp and the other one broad. The sharp component which is located at the T_t of pure DPPC shifted to a lower temperature as the result of cholesterol addition. An inverse relationship between the cholesterol concentration and the decreased enthalpy of transition was observed in which ΔH falls from an initial value of 8.0-8.5 kcal/mol to zero at 20 mol percent cholesterol. The broad component, which is located at a temperature slightly above the T_t of pure DPPC, shifted to higher temperatures as the result of cholesterol addition. The enthalpy of transition ΔH of this component increases first and then decreases with increasing cholesterol concentration and then goes to zero at 50 mol percent cholesterol. It has been reported that 33 mol percent is the amount of cholesterol necessary to completely abolish the phase transition

of diacylphosphatidylethanolamine (55). However, findings similar to phosphatidylcholine-cholesterol complexes have been obtained using high sensitivity differential scanning calorimetry (56) for phosphatidylethanolamine-cholesterol complexes, except that the T_t of the broad component shifts to lower rather than to higher temperature.

A number of calorimetric studies of the effect of cholesterol on lysophosphatidylcholine have been carried out (57,58). In general, the addition of cholesterol causes the phase transition peak to broaden and decrease in area until a 1:1 molar ratio is reached, at which no phase transition can be observed. The T_t will shift to higher temperatures as the result of the increase in cholesterol concentration and ΔH will decrease with increased cholesterol content.

It appears that cholesterol associates preferentially with different classes of lipids in binary mixtures. Cholesterol preferentially interacted with the lower melting lipid in mixtures of phosphatidylcholine-phosphatidylglycerol, or phosphatidylserine-phosphatidylethanolamine (53,54,59). In other binary systems cholesterol did not show a preference for the lower melting phospholipid. Cholesterol order of preference has been established as PS PG > PC >> PE.

Effects of Metal Ions and Small Molecules on the Thermotropic Behavior of Phospholipids

Besides temperature, water content, ligands such as lipid-soluble small molecules and metal ions produce effects on biological membranes. Many investigators, utilizing DSC and other physical techniques (27,60,61,62), have established that gel to liquid-crystalline phase transitions of aqueous dispersions of phospholipids can be affected by

addition of small molecules or metal ions. Salts may alter the structure of water which may result in changes in interactions among the hydrocarbon chains (63), or may partition into the hydrocarbon chains and destroy the ordered interaction of phospholipid bilayers. Also, electrolytes may interact with the polar region of phospholipid affecting the packing of hydrocarbon chains in the bilayers (64).

The effect of metal ions on the thermotropic behavior of phosphatidylcholine have been investigated by DSC (60,62). There was general agreement that the presence of divalent cations, such as Ca^{2+}, will broaden the gel to liquid-crystalline phase transition, increase its ΔH, and shift the phase transition temperature to a higher value. Chowdhry et al (61) have reported similar results with the trivalent cation, La^{3+}, while Simon et al. (62) reported that La^{3+} does not affect the transition temperature.

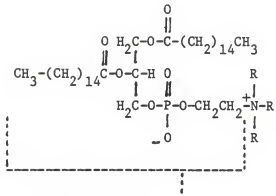
Simon et al. (62), reported that the thermal properties of dipalmitoylphosphatidylcholine were not affected by the presence of several monovalent cations (H^+, Na^+, Li^+) and divalent cations (Ba^{2+}, Mg^{2+}, and Sr^{2+}). However, the presence of the divalent cations, Ca^{2+} and Cd^{2+}, or the trivalent cation, Fe^{3+}, shifted the phase transition temperature to higher values.

The presence of free fatty acids as minor components in many biological membranes can alter the permeability, change the activity of certain enzymes and allow the fusion of phospholipid vesicles and cells. The effects of free fatty acids on the gel to liquid-crystalline phase transition have been studied by DSC and other physical techniques (27,65,66). In general, both the transition temperature T_t and enthalpy of transition ΔH increase as a result of the addition of small amounts of saturated free fatty acids of 12-18 carbons to dipalmitoylphosphatidyl-

choline dispersions. The pretransition is also completely eliminated. The lipid fluidity in the liquid-crystalline state did not change with the addition of saturated and unsaturated free fatty acids (67). At a mole ratio of 2:1 of palmitic acid to DPPC a T_t of 61.5°C resulted, which is very similar to the transition temperature of dipalmitoylphosphatidylethanolamine (27).

The effect of the addition of 1-palmitoyllysophosphatidylcholine on the thermotropic phase behavior of dipalmitoylphosphatidylcholine has been studied by DSC (57). A non linear decrease in the T_t of DPPC is a result of the addition of PLPC. PLPC alone exhibits a T_t at 3.4°C. An initial slight increase in the enthalpy of transition ΔH of DPPC is followed by a gradual decrease as the result of increasing concentrations of PLPC.

Diacyl and Monoacyl Compounds



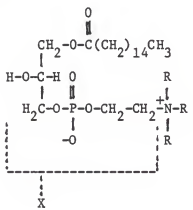
R = H or CH_3

Dipalmitoylphosphatidylethanolamine (DPPE)

Dipalmitoylphosphatidyl-N-methylethanolamine (DPP-N-methyl E)

Dipalmitoylphosphatidyl-N-N-dimethylethanolamine (DPP-N-N-dimethyl E)

Dipalmitoylphosphatidylcholine (DPPC)



$R = CH_3$ or H

Palmitoyllysophosphatidylethanolamine (PLPE)

Palmitoyllysophosphatidyl-N-methylethanolamine (PLP-N-methyl E)

Palmitoyllysophosphatidyl-N-N-dimethylethanolamine (PLP-N-N-dimethyl E)

Palmitoyllysophosphatidylcholine (PLPC)

MATERIALS AND METHODS

The dipalmitoylphosphatidylcholine, dipalmitoylphosphatidylethanolamine, palmitoylLysophosphatidylcholine, palmitoyllysophosphatidylethanolamine, cholesterol, and phospholipase A_2 (Crotalus adamanteus) enzyme were purchased from Serdary Research Laboratories, London, Ontario, Canada. The dipalmitoylphosphatidyl-N-methylethanolamine and dipalmitoylphosphatidyl-N-N-dimethylethanolamine were obtained through Tridon Chemical Inc., New York, New York, U.S.A.

Purities of each of these compounds were checked by thin layer chromatography before use.

Thin Layer Chromatography:

Silica gel 60 PF-254 with fluorescent indicator, was obtained from E. Merck Laboratories, Inc., Elmsford, N.Y. Silica gel was mixed with distilled water at a ratio of 2 ml of water/g of silica gel. The slurry was spread rapidly with a layer thickness of 0.75 mm onto cleaned (grease-free) glass plates. The plates were allowed to air dry and then activated in a 110°C oven for one hour. The plates were cooled and washed once in the 65:25:4 chloroform/methanol/water solvent system. This removed any contamination present in the plates and allowed visualization of free fatty acids at the solvent front.

Preparation of PLP-N-methyl E and PLP-N-N-dimethyl E

The lysophospholipids were prepared by phospholipase A_2 hydrolysis of the diacyl compounds (68). 200 mg sample of dipalmitoylphosphatidyl-N-methyl-ethanolamine was dissolved in 400 ml of anhydrous diethyl ether.

Five mg of phospholipase A_2 were dissolved in 20 ml of 0.1 M borate buffer which contains 2.5 mM calcium chloride (pH 7.0). The two phases were combined and the reaction was allowed to proceed at room temperature with shaking for 36 hours. The rate of hydrolysis of DPP-N-methyl E to free fatty acid and PLP-N-methyl E with phospholipase A_2 was slower than stated in the original procedure possibly because of the small amounts of enzyme employed. Thin layer chromatography was used to monitor the extent of the hydrolysis. This was accomplished as follows. One plate was spotted with the ether layer of the reaction mixture and separated in the 65:25:4 chloroform/methanol/water solvent system. DPP-N-methyl E and palmitic acid were used as standards. Another plate was spotted with aqueous layer of the reaction mixture and the standards. The spots were visualized by iodine vapors (solid iodine crystals in the bottom of TLC tank). On the plate on which the ether layer was spotted only one spot was visualized, corresponding to palmitic acid. On the plate spotted with the aqueous layer 3 spots were visualized, corresponding to palmitic acid, PLP-N-methyl E, and the enzyme at the origin. There was no spot corresponding to the DPP-N-methyl E which indicates 100% conversion to PLP-N-methyl E.

The reaction mixture was separated by a separatory funnel into an aqueous layer and ether layer. The ether layer, which contained a major portion of palmitic acid was disposed of. The aqueous layer, which contained PLP-N-methyl E, salts of the buffer and the enzyme, was washed with anhydrous diethyl ether to remove any remaining palmitic acid. The samples were lyophilized in a Vir Tis Super Freeze-Mobile for 8 hours. The salts of the buffer were removed by dissolving the precipitate in 30 ml of chloroform and filtering through filter paper. The chloroform layer, which contained the PLP-N-methyl E and the enzyme, was concentrated to

approximately 3 ml by a stream of nitrogen gas. PLP-N-methyl E was separated with the $CHCl_3/CH_3OH/H_2O$ solvent system on 20x20 cm thin layer plates, which were activated in a 110°C oven for one hour. A concentrated sample was applied to one plate in a narrow series of spots at the origin. After the solvent had migrated to the top of the plates, the plates were removed and air dried. A clean glass plate with the same dimensions was gently placed on the top of the dried silica gel plate and taped, leaving one edge exposed to the I_2 vapor. This spot is used as a reference to identify a band of spots across the plate. To assist in visualizing the spot, the plate was put in a UV light box (Chromato-vue, Model C-3; manufactured by Ultra-Violet Products, Inc., San Gabriel, CA) after developing with I_2 vapors. The bands which contained the PLP-N-methyl E in the silica gel were marked and scraped from the plates.

A number of different solvents were used in an attempt to extract the sample from the silica gel. The solvents isopropanol, dichloromethane, chloroform, acetone and 1:1 chloroform/methanol each dissolved significant amounts of silica gel. Methanol was chosen because it maximized dissolvability of sample, yet minimized the amounts of silica gel dissolved.

Each collected band of silica gel was extracted with 15 ml of methanol, vortexed for 2-3 min and transferred to centrifuge tubes (30 ml Corex). The mixture was divided into four equal portions by weight and centrifuged at 9000 g for 30 minutes at 20°C. The extraction was repeated with 5 ml of methanol for five times until no spot was observed on thin layer plates. The supernatant was collected each time and taken to dryness. The precipitate was redissolved in 10 ml of 9:1 (v/v) chloroform/methanol. The same was done using dipalmitoylphosphatidyl-N-N-

dimethylethanolamine to generate 1-palmitoyllysophosphatidyl-N-N-dimethyl-ethanolamine.

Sample Preparation for Differential Scanning Calorimetry

One hundred mg of each phospholipid was dissolved in 10 ml of 1:1 (v/v) chloroform/methanol. One ml of the stock solution which contained the appropriate amounts of phospholipid (total 10 mg) was evaporated under a stream of nitrogen gas. For the cholesterol studies, an appropriate amount (10,20,30,40 and 50 mole %) of a stock solution of cholesterol was added before evaporation with nitrogen gas. The samples were stored in an evacuated dessicator overnight. Aqueous dispersions of phospholipids were formed by heating and vortexing with 50 µl of buffer which contained 100 mM NaCl, 10mM Tris-HCl, and 0.2 mM EDTA at pH 7.0 to final concentration to 100 mM (69). For palmitoyllysophosphatidylcholine, it was necessary to include 10% ethylene glycol in the same buffer to obtain a sharp peak. Five, 10 and 15% ethylene glycol were tested. The best results were with 10% ethylene glycol. About 15 µl of the dispersed phospholipid, was added to a Perkin-Elmer aluminum sample pan and was sealed by a vise. Differential scanning calorimetry experiments were performed on a Perkin-Elmer DSC-1B. The samples were heated at scan rates of 5°C/min or 10°C/min and sensitivity ranges of 2 or 4. At least three curves were recorded for each sample.

Phosphorus Determination

The amount of phospholipid present in the sample pan can be measured by phosphorus determination according to Fiske and Subbarow (70). Phospholipid was released from the pan which was ruptured in a small test

tube. One ml of 1:1 chloroform/methanol was added with shaking to dissolve the phospholipid. Four test tubes containing 0.1 ml of the sample solution were evaporated in the oven at 130°C for half an hour. When all the tubes were cooled, 0.25 ml of 70% perchloric acid was added. At the same time a set of standards were prepared by taking 0,2,4,7,10,15,20 ug of phosphorus equivalent (monobasic potassium phosphate) and adding 0.25 ml of 70% perchloric acid. Each tube was covered with a marble, and was inserted in a heating block at 220°C to digest overnight. After all the tubes were cooled, 4 ml of 2.5% (w/v) ammonium molybdate in 5N sulfuric acid and 2 ml of 15% (w/v) sodium metabisulfite were added. All tubes were stirred with a thin glass stirring rod. After proper mixing all tubes were incubated in a boiling water bath for 30 minutes. The samples were cooled and absorbance measured at 830 nm on a Beckman DU-2 double beam spectrophotometer (Beckman Instruments Inc., Fullerton, CA).

A standard curve was prepared by least squares analyses of absorbance at 830 nm vs micrograms of phosphorus for each sample. Unknowns were calculated using the point-slope method.

Gas-Liquid Chromatography

Fatty acid methyl esters of lysophosphatidyl-N-methylethanolamine were analyzed qualitatively for purity and separated on a programmable Hewlett-Packard 5880A. A coiled glass column 2.5 meter long and 2 mm inside diameter was packed with 7.5% diethylene glycol succinate on Chromosorb W 100/120 mesh. Column temperature was maintained at 165°C, injector temperature at 220°C and detector temperature at 230°C. Nitrogen gas was used as the carrier at a flow rate of 15 ml/min. The methyl esters were detected by a hydrogen flame ionization detector. Methyl esters of

lauric, myristic, palmitic and stearic acids were used as standards.

Methyl esters of lysophosphatidyl-N-methylethanolamine were prepared by adding 1 ml of 10% (w/v) boron trichloride in anhydrous methanol and heated at 70°C for 30 minutes. The esters were recovered by neutralization and extraction with hexanes and distilled water. The extraction was repeated three times. The hexane solutions were combined, washed with distilled water and dried with anhydrous sodium sulfate. The dried hexane solution was concentrated by a stream of nitrogen gas and injected into the column.

CALCULATIONS

When the differential scanning calorimetry curves were recorded, a number of important parameters could be determined. These parameters are necessary to determine the thermotropic phase transition temperatures and enthalpies of lysophosphatidylethanolamine derivatives differing in the degree of methylation of the ethanolamine.

The Phase Transition Temperature

This parameter is usually denoted T_t and can be directly determined from the DSC trace. T_t is taken as the intercept of the predominate slope of the baseline and the first appearing arm of the transition peak. Since we recorded three curves for each sample pan, the temperature in these results is an average value.

Known sample weights of deionized water were scanned and used not only as a standard for the transition temperature, but also the peak area of these scans were used to calibrate the instrument. This was done for each set of samples. To obtain the T_t in °C of the sample, it was necessary to subtract the T_t of water from the average T_t. For pure PLP-N-N-dimethyl E, the T_t of the first, second and third scans were 385.2, 384.4 and 384.8 respectively. The average T_t for this compound was 384.4 and the T_t for water was 370.

$$T_t = 384.4 - 370 = 14.8 \pm 0.35°C.$$

Differential Scanning Calorimetry Peak Areas

Three heating scans for each sample pan were recorded and two sample pans were filled for each phospholipid. After scanning, the DSC traces

were photocopied. The area under the peaks, which corresponded to the gel to liquid-crystalline phase transitions, were calculated by cutting and weighing the peaks after the baselines were drawn. The baselines were drawn from the transition temperature to the point the transition returned to a consistent slope. The area was multiplied by the range setting of the instrument. This brings the areas of all peaks to the same scale. For pure PLP-N-N-dimethyl E, the average weight of the area under the peak were 0.04436 g and the range setting of the instrument was 2.

Total area = 0.04436 g x 2 = 8.872×10^{-2} g

Differential Scanning Calorimetry Calibration factor

A preweighed sample of deionized water was scanned on the DSC and the DSC trace was photocopied. The total area per gram was obtained by multiplying the weight of the area under the water peak with the range setting of the instrument. The heat of fusion of ice at 0°C has been calculated as 79.7 cal/g (71). Using this information the calibration factor was obtained.

$$\text{Calibration Factor} = \frac{\text{Heat of fusion of ice in cal/g x weight of water in g}}{\text{weight of the area under the water peak in g x range}}$$

$$= \frac{79.7 \text{ cal/g} \times 0.0112 \text{ g}}{0.129468 \text{ g} \times 32} = 0.216 \text{ cal/g}$$

Heat of Transition

The heat of transition was obtained by multiplying the total area in grams for the sample peak by the calibration factor of the instrument. For pure PLP-N-N-dimethyl E, the total peak area in grams was 8.872×10^{-2} g.

Heat of transition = 8.872×10^{-2} g x 0.216 cal/g

= 1.916×10^{-2} cal

Moles of PLP-N-N-dimethyl E

After the sample pan was scanned by DSC, the pan was ruptured and the pan contents analyzed for phosphorus as described in Materials and Methods. The number of moles of phospholipid in the sample pan was calculated in the following way:

The average absorbance for the phosphorus determination on the PLP-N-N-dimethyl E pans at 830 nm was 1.2425. From the phosphorus standard curve, this absorbance corresponds to 9.225 mg phosphorus in 0.1 ml. Considering the dilution factors and molecular weight of phosphorus (31 g/mole):

$$\text{Moles of phosphorus} = 1\ \text{ml} \times \frac{\mu\text{g of P}}{0.1\ \text{ml}} \times \frac{1\text{g P}}{1\times10^{6}\ \mu\text{g P}} \times \frac{1\ \text{mole P}}{31\ \text{g P}}$$

Therefore, the number of moles of phosphorus in the pan was 2.976×10^{-6} for this compound.

Enthalpy of Transition

This parameter is usually denoted ΔH and can be obtained by dividing the heat of transition in cal by the number of moles of the phospholipid.

Mole Percent of Cholesterol

In the experiments in which the effects of cholesterol content on the phase transitions were observed, the phospholipid content was held constant and the cholesterol was varied to get the desired mole ratios. For the PLP-N-N-dimethyl E, 1.30×10^{-5} moles of the lipid were used. The stock

solution of cholesterol contained 2.59×10^{-3} moles in 50 ml. The volume of stock cholesterol solution needed to produce a 50 mole % mixture was calculated as follows.

$1.30 \times 10^{-5} = 2.59 \times 10^{-3}/50$ ml x ml cholesterol.

ml stock cholesterol = 0.251

RESULTS AND DISCUSSION

Before the phospholipids were scanned by differential scanning calorimetry, the purities were analyzed by thin layer chromatography. All these compounds showed one spot when developed in iodine vapors except DPP-N-methyl E. It showed two spots, a large spot corresponding to this compound and another small one for PLP-N-methyl E. Further investigation was made by gas liquid chromatography which showed only one peak corresponding to palmitic acid.

Because PLPC exhibited its phase transition near 0°C more studies (57,58), were necessary to determine its calorimetric behavior. This compound was dispersed in distilled water containing 50% (v/v) ethylene glycol to prevent freezing of the water. However the study by Van Echteld et al. (69) reported the thermotropic behavior of phospholipids, depending on the phospholipid species, can be affected in many ways by the presence of 50% (v/v) ethylene glycol. First, the pretransition of disaturated phosphatidylcholines is completely abolished. Secondly, for dispersions of unsaturated phosphatidylcholine, not only was an exothermic transition introduced but also a 50% increase in the transition enthalpy was observed. Finally, for dispersions of PLPC, an increase of about 25% in the transition enthalpy was also observed. Therefore, they suggested the PLPC be dispersed in a buffer containing 100 mM NaCL, 10 mM Tris-HCl, 0.2 mM EDTA at pH 7.0 to a final concentration to 100 mM. The sample was cooled in a calorimeter to -33°C, and scanned, as the sample was heated until the ice-water transition appeared. The scanning was halted, the ice allowed to melt, the temperature of the sample reduced to -8°C and a heating scan recorded.

When we followed their procedure for this compound, we found that the ice-water transition overlapped and obscured the gel to liquid-crystalline phase transition. In an attempt to separate the peaks, we cooled the sample to -33°C, scanned until the ice-water transition appeared and stopped scanning. The pen was allowed to return to the baseline and the sample was allowed to stand for 15, 30, or 60 minutes at this temperature. After standing, the heating scan was begun, but no peaks were observed.

By adding 10% (w/v) ethylene glycol to the same buffer, we were able to do a continuous, single scan beginning at -33°C. This separated the sample phase transition from the ice-water transition.

Using this method, we obtained T_t = 3.5°C ± 0.21 and a ΔH = 4.515 ± 0.18 kcal/mole. These results are in agreement with earlier literature reports (57,58,59).

The transition temperature and enthalpies of transition for all of the lipids studied as determined by DSC are summarized in Table 3. For the diacyl compounds the transition temperatures were found to decrease with increasing degree of methylation of the amino group. Addition of the first methyl reduced the transition temperature by 6.5°C below that for DPPE, while the second reduced the temperature by an additional 7.3°C, and the third reduced it another 7.3°C. Thus, the addition of each methyl group on the nitrogen atom of the polar headgroup of DPPE causes a decrease in the transition temperature of an average 7°C (see Figure 3). We found similar results with monoacylphosphatidylethanolamine derivatives. The transition temperature for PLPE (38.6°C) was reduced with the addition of one methyl group on the nitrogen atom of the polar headgroup of PLPE at a time by an average of 11.7°C (see Figure 3). There were slight increases in the transition enthalpies of the diacyl compounds with methylation while there

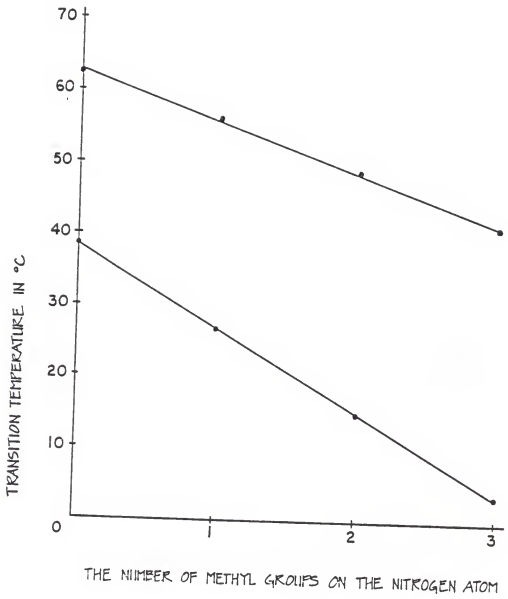
Table 3
The Transition Temperatures and Transition Enthalpies of Diacylphosphatidylethanolamine and Monoacyllysophosphatidylethanolamine Derivatives Differing in the Degree of Methylation of Ethanolamine Group.

	T_t (°C)		ΔH (Kcal/mol)	
No. of methyl groups on Nitrogen	Diacyl	Monoacyl	Diacyl	Monoacyl
0	62.5 ± 0.3	38.6 ± 0.25	8.01	7.77
1	56 ± 0.35	26.5 ± 0.4		6.90
2	48.7 ± 0.25	14.8 ± 0.35	8.37	6.05
3	41.4 ± 0.49	3.5 ± 0.21	8.53	4.75

Figure 3. Effects of Methylation of Phosphatidylethanolamine Derivatives on Transition Temperature.

■ - diacylphosphatidylethanolamine derivatives data

● - monoacyllysophosphatidylethanolamine derivatives data

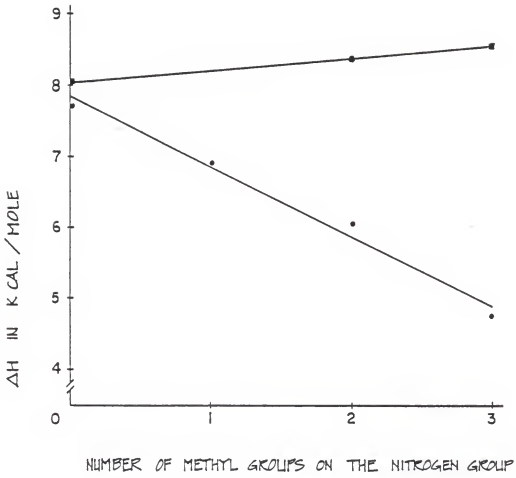

70
60
50
40
30
20
10
0
1
2
3
TRANSITION TEMPERATURE IN °C
THE NUMBER OF METHYL GROUPS ON THE NITROGEN ATOM

was a large linear decrease by about 1 kcal/mole in of monoacyl compounds with the addition of one methyl group on the nitrogen atom of the enthanolamine group (see Figure 4). [ΔH values were taken from the intercepts of ΔH in kcal/mol vs mole percent of cholesterol.] The ΔH value for DPP-N-methyl E was omitted because the DPP-N-methyl E contained some PLP-N-methyl E as a contaminant. Previously the T_t and ΔH for DPPC were reported to have a value of 41 to 41.5°C and 7.6 kcal/mol, respectively (11,38,61). Our results for the same compound were about the same. The T_t was 41.4°C while the ΔH was 8.71 kcal/mol [8.51 kcal/mol that was obtained from the intercept of ΔH vs the mole percent cholesterol (Figure 9B)]. The T_t and ΔH for DPPE were reported to have values of 62.2°C and 8.6 ± 0.6 kcal/mol (10) or 63.1°C and 8.8 kcal/mol (38), respectively. Our thermal data were 62.5°C and 8.76 kcal/mol (calculated) or 8.01 kcal/mol obtained from the intercept (Figure 8A). We obtained values for T_t of 3.5°C and for ΔH of 4.5 kcal/mol (calculated), 4.75 kcal/mol (intercept) while the reported T_t and ΔH values were from 3°C or 3.5°C and 4.0 or 4.5 kcal/mol (57,69). Nuzback (12) studied the PLPE and reported the T_t for PLPE to be 70.2°C and a ΔH of 8.5 kcal/mole. Our thermal data for the same compound were 38.6°C and 7.99 kcal/mol (calculated) or 7.77 kcal/mol (intercept) (Figure 11B). We also studied PLP-N-methyl E, PLP-N-N-dimethyl E and PLPC and when the T_t values for all of these compounds are plotted against the number of methyl groups on the nitrogen atom of the ethanolamine, a straight line is obtained. So our results are internally consistent and are based on more compounds than Nuzback's. As can be seen from the above, the values which we obtained for T_t and ΔH are consistent with published values for those compounds which are available. Until today, there have been no published data available on the mono- and diacylphosphatidyl-

Figure 4. Effects of methylation of phosphatidylethanolamine Derivatives on Transition Enthalpies.

■ - diacylphosphosphatidylethanolamine derivatives data

● - Monoacyllysophosphatidylethanolamine derivatives data


9
8
7
6
5
4
0
1
2
3
ΔH IN K.CAL / MOLE
NUMBER OF METHYL GROUPS ON THE NITROGEN GROUP

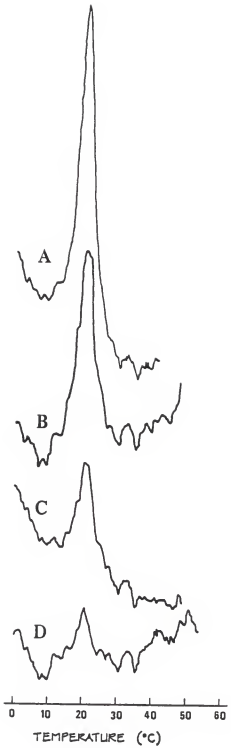
ethanolamine derivatives differing in degree of methylation. It has been reported that the transition temperature for DPPE is greater than the corresponding DPPC (38,4). Our data not only agree with the reports but we also found that the PLPE transition temperature is greater than that of PLPC. The effect of degree of methylation on the transition temperature of the monoacyl compounds is greater than that observed for the corresponding diacyl compounds. The increased stability of the disaturated phosphatidylethanolamine in the gel state appears to be due to the smaller size of the phosphatidylethanolamine polar headgroup compared to the phosphatidylcholine head group (27). This stability seems to be due to the ability of the protonated amino group of phosphatidylethanolamines to form hydrogen bonds between the surrounding water molecules or the amino groups of adjacent phosphosphatidylethanolamine molecules (38). Addition of each methyl group on the nitrogen atom of phosphatidylethanolamine reduces this stability because this decreases the ability to form hydrogen bonds. Our data supports this view and a corresponding, unpublished phenomena with the lysophosphatidylethanolamine.

The effects of cholesterol on the gel to liquid-crystalline phase transitions of dispersions of the phosphatidyl ethanolamine and lysophosphatidylethanolamine derivatives differing in the degree of methylation of ethanolamine were studied by differential scanning calorimetry. Heat scans of each individual sample were performed in the absence or presence of 10,20,30,40,50 mole percent cholesterol. A typical series of scans for PLP-N-N-dimethyl E is shown in Figure 5.

Trace A in Figure 5 represents a calorimetric scan of PLP-N-N-dimethyl E in the absence (0 mole %) of cholesterol. It reveals that this phospholipid undergoes an endothermic phase transition at about 14.8°C.

Figure 5. A series of Differential Scanning Calorimetry scans of PLP-N-N-dimethyl E.

A - 0 mole % cholesterol
B - 10 mole % cholesterol
C - 20 mole % cholesterol
D - 30 mole % cholesterol


A
B
C
D
0
10
20
30
40
50
60
TEMPERATURE (°C)

Trace B represents a calorimetric scan of the same compound in the presence of 10 mole % cholesterol. When the cholesterol concentration is 10%, the behavior of the differential scanning calorimetry curve changes by showing a decrease in the area of the phase transition peak. The peak also becomes broader. The transition temperature was affected by the presence of cholesterol and shifted to a higher temperature (about 15.7°C).

Increasing the concentration of cholesterol (20 and 30 mole percent) further broadened the gel to liquid-crystalline phase transition. The total area of the peak becomes smaller and smaller until a cholesterol concentration of 40% is reached, at which point the phase transition is completely abolished. Also, the transition temperatures were shifted to higher values with increasing cholesterol concentration.

The effect of cholesterol content on diacylphosphatidylethanolamine derivatives differing in the degree of methylation on the ethanolamine group have been analyzed by differential scanning calorimetry. The results for the transition temperatures and enthalpies of transition are summarized in Table 4. It can be seen that the presence of increasing concentrations of cholesterol with DPPE, DPP-N-methyl E, DPP-N-N-dimethyl E or DPPC caused the gel to liquid crystalline phase transition peaks to shift to lower temperatures. As the mole percent of cholesterol increased from 0 to 30, the average decrease in the transition temperature for DPPE, DPP-N-methyl E, DPP-N-N-dimethyl E and DPPC are respectively 2.7°C, 2.3°C, 1.2°C and 1.9°C.

A linear relationship between the transition temperature and the mole percent of cholesterol was obtained for each compound (see Figure 6). For DPP-N-methyl E at 30 mole % cholesterol, no phase transition was observed. A possible reason for this is that the original compound contained a very

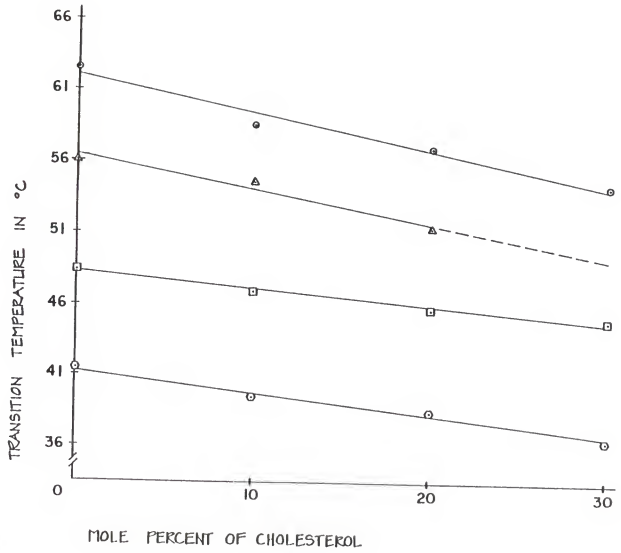
Table 4

Effects of Cholesterol Content on Transition Temperatures and Transition Enthalpies for Diacylphosphatidylethanolamine Derivatives.

	DPPE		DPP-N-methyl E		DPP-N-N-dimethyl E		DPPC	
% mol of cholesterol	T_t (°C)	ΔH (kcal/mole)	T_t (°C)	ΔH (kcal/mole)	T_t (°C)	ΔH (kcal/mole)	T_t (°C)	ΔH (kcal/mole)
0	62.5±0.3	8.76±0.13	56±0.35	7.32±0.43	48.7±0.25	8.28±0.75	41.4±0.49	8.71±0.16
10	58.5±0.4	4.65±0.86	54±0.15	3.66±0.06	46.9±0.3	5.97±0.04	39.5±0.59	5.81±1.29
20	57±0.4	2.72±0.08	51.4±0.25	1.08±0.04	45.7±0.25	2.95±0.06	38.5±0.3	3.57±0.32
30	54.3±0.4	1.25±0.38	-	-	45.1±0.24	0.53±0.18	36.6±0.35	1.26±0.37

Figure 6. Effects of Cholesterol Content on Transition Temperatures for Diacylphosphatidylethanolamine Derivatives.

⊙ - DPPE data
▲ - DPP-N-methyl E data
◫ - DPP-N-N-methyl E data
◔ - DPPC data


66
61
56
51
46
41
36
0
10
20
30
TRANSITION TEMPERATURE IN °C
MOLE PERCENT OF CHOLESTEROL

small amount of the lysocompound.

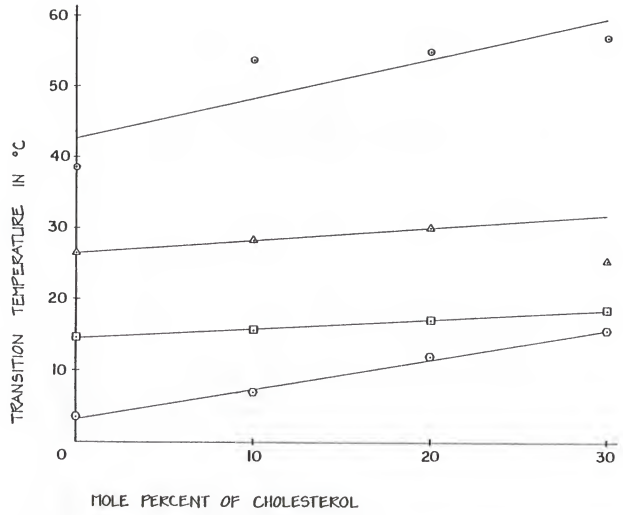
Mixtures of cholesterol and lysophosphatidylethanolamine derivatives differing in degree of ethanolamine group methylation were also studied by differential scanning calorimetry. These data are summarized in Table 5. The cholesterol increased the transition temperatures for PLPE, PLP-N-methyl E, PLP-N-N-dimethyl E and PLPC by average amounts of 6.17°C, 1.8°C, 1.23°C and 4.0°C, respectively for each 10 mole % increase of cholesterol.

The transition temperature for PLPE changed by an unusually high temperature shift of 15.2°C with 10% cholesterol. After this, the increase in temperature was slightly lower than the first shift by an average of 2.1°C. New samples of a PLPE-cholesterol mixture, which contained 10, 20, or 30 mole % of cholesterol, were made and scanned by DSC. The same results were obtained. The transition temperatures for PLP-N-methyl E were increased with 10% cholesterol. At this concentration a small peak similar to a pretransition appeared about 5°C before the main peak. This peak became larger when the cholesterol content was increased to 20 mole percent. We were however, still able to determine the transition temperature of the main peak. At 30 mole % of cholesterol, both peaks formed one very broad peak with transition temperature of 25.53°C. This temperature was lower than for those containing less cholesterol because at 30 mole % it was no longer possible to distinguish the early peak from the main peak. Therefore the transition temperature for the sample containing 30 mole % reflects the transition temperature of the early peak.

When the transition temperature for PLPE, PLP-N-methyl E, PLP-N-N-dimethyl E and PLPC were plotted against the mole percent of cholesterol, a linear plot was obtained (see Figure 7). Note that when the

Figure 7. Effects of Cholesterol Content on Transition Temperatures for Monoacyl-Phosphatidylethanolamine Derivatives.

● - PLPE data
▲ - PLP-N-methyl E data
■ - PLP-N-N-dimethyl E data
◎ - PLPC data


TRANSITION TEMPERATURE IN °C
60
50
40
30
20
10
0
10
20
30
MOLE PERCENT OF CHOLESTEROL

plot for PLP-N-methyl E was made, the transition temperature at 30% mole concentration of cholesterol was not included because the two peaks could not be resolved.

Our thermal data concerning the cholesterol effect on DPPE, DPPC, and PLPC are in agreement with other published reports. Although no specific values were indicated, the transition temperatures for DPPE and DPPC were shifted to lower temperatures (10,37,54) while that for PLPC shifted to a higher temperature (58, 70). Both lysophosphatidylethanolamine and diacylphosphatidylethanolamine derivatives were studied here. It can be concluded that the presence of increasing cholesterol concentration made the gel to liquid-crystalline phase transition broaden. The transition temperatures were shifted to lower temperatures for the diacylcompounds on addition of cholesterol and to higher temperatures for lysocompounds.

The enthalpies of transitions for the diacylcompounds were affected by cholesterol in a fashion similar to that observed with the transition temperatures. The enthalpy changes of DPPE, DPP-N-methyl E, DPP-N-N-dimethyl E and DPPC were decreased respectively by an average of 2.5, 3.12, 2.6 and 2.5 kcal/mol for each 10 mole % cholesterol as the mole percent of cholesterol is increased from 0 to 30 (see Table 4).

The lysocompounds showed decreases in ΔH's with increasing cholesterol while the T_t increases. The enthalpy changes of PLPE, PLP-N-methyl E, PLP-N-N-dimethyl E and PLPC values decreased an average of 2.3, 1.9, 2.03 and 1.21 kcal/mol as the mole percent of cholesterol is increased (see Table 5).

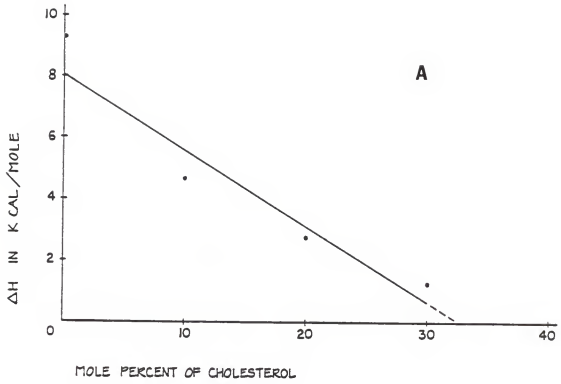
A linear relationship exists between the ΔH values for each diacyl compound and lyso compound and the mole percent of cholesterol. This can be seen in (Figures 8 and 9) for the diacylcompounds and (Figures 10 and

Table 5

Effects of Cholesterol Content on Transition Temperatures and Transition Enthalpies for Monoacyllysophosphatidylethanolamine Derivative.

	PLPE		PLP-N-methyl E		PLP-N-N-dimethyl E		PLPC	
% mol of cholesterol	T_t (°C)	ΔH (kcal/mole)	T_t (°C)	ΔH (kcal/mole)	T_t (°C)	ΔH (kcal/mole)	T_t (°C)	ΔH (kcal/mole)
0	38.6±0.2	7.99±0.36	26.5±0.4	7.06±0.0	14.8±0.35	6.57±0.18	3.5±0.21	4.515±0.18
10	53.8±0.38	5.43±0.08	28.4±0.15	4.86±0.2	15.7±0.21	3.47±0.07	7.0±0.4	3.86±0.13
20	55.1±0.45	2.53±0.25	30.03±0.38	2.83±0.06	17.3±0.3	1.41±0.04	12±0.31	2.20±0.02
30	57.1±0.21	1.23±0.08	25.53±0.31	1.35±0.06	18.5±0.2	0.48±0.04	15.6±0.25	0.89±0.09

Figure 8. Effects of Cholesterol Content on Transition Enthalpies for Diacylphosphatidylethanolamine Derivatives.
A - DPPE data
B - DPP-N-methyl E data


A
ΔH IN K CAL./MOLE
10
8
6
4
2
0
10
20
30
40
MOLE PERCENT OF CHOLESTEROL

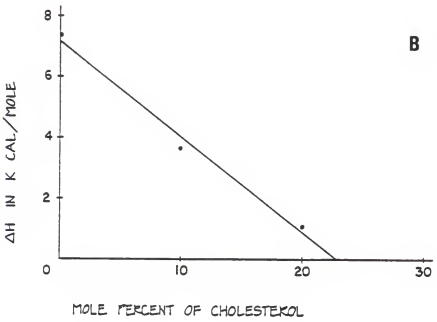
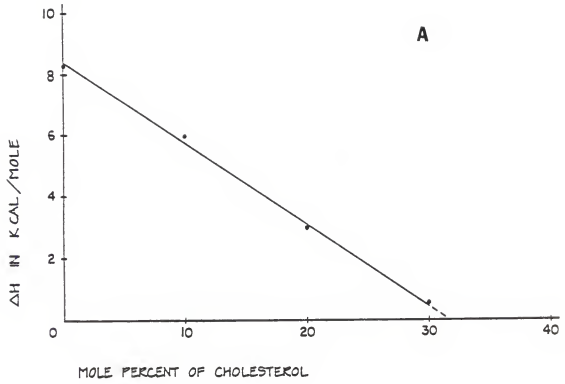

B
ΔH IN K CAL./MOLE
8
6
4
2
0
10
20
30
MOLE PERCENT OF CHOLESTEROL

Figure 9. Effects of Cholesterol Content on Transition Enthalpies for Diacylphosphatidylethanolamine and Diacylphosphosphatidyl-N-N-dimethyl-ethanolamine Derivatives

A - DPP-N-N-dimethyl E data
B - DPPC data


A
ΔH IN K CAL/MOLE
10
8
6
4
2
0
10
20
30
40
MOLE PERCENT OF CHOLESTEROL

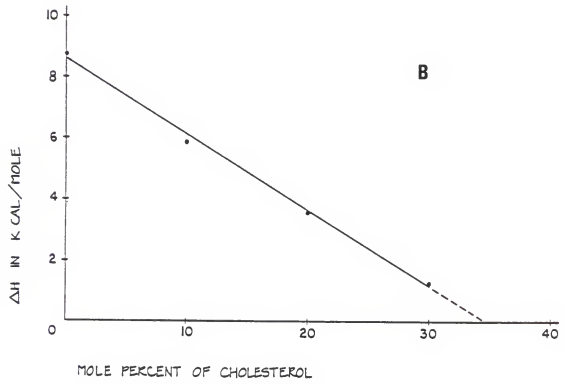
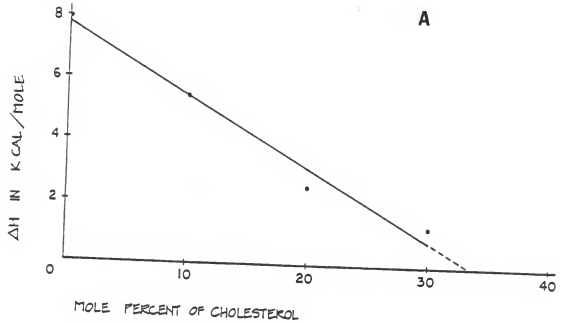

B
ΔH IN K CAL/MOLE
10
8
6
4
2
0
10
20
30
40
MOLE PERCENT OF CHOLESTEROL

Figure 10. Effects of Cholesterol Content on Transition Enthalpies for Monoacylphosphatidylethanolamine Derivatives

A - PLPE data

B - PLP-N-methyl E data

⊡ - Data point for main transition peak only


A
8
6
4
2
0
10
20
30
40
ΔH IN K CAL/MOLE
MOLE PERCENT OF CHOLESTEROL

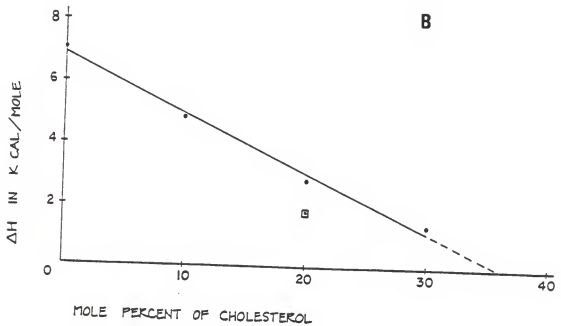
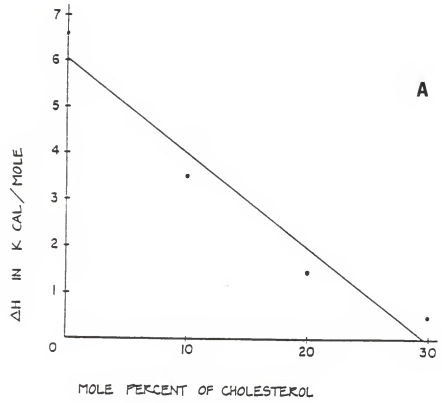
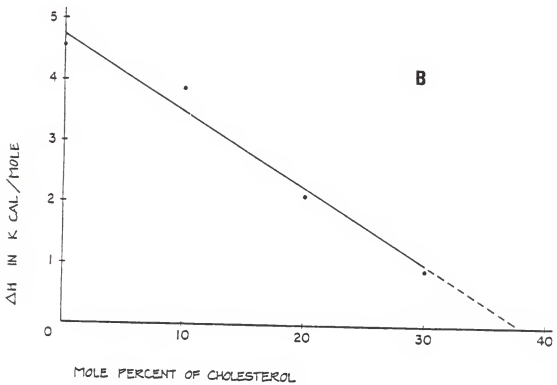

B
8
6
4
2
0
10
20
30
40
ΔH IN K CAL/MOLE
MOLE PERCENT OF CHOLESTEROL

Figure 11. Effects of Cholesterol Content on Transition Enthalpies for Monoacylphosphatidylcholine and Monoacylphosphatidyl-N-N-dimethylethanolamine Derivatives

A - PLP-N-N-dimethyl E data

B - PLPC data


A
ΔH IN K CAL./MOLE
0
1
2
3
4
5
6
7
10
20
30
MOLE PERCENT OF CHOLESTEROL


B
ΔH IN K CAL./MOLE
0
1
2
3
4
5
10
20
30
40
MOLE PERCENT OF CHOLESTEROL

11) for the lysocompounds. This relationship can also be seen on the DSC scans. As the cholesterol increases the peaks broaden, become shorter and the area under the peak becomes smaller. This shows that the area under the peak is proportional to ΔH.

At 20 mole % of cholesterol for PLP-N-methyl E two peaks were obtained. Calculation for ΔH using only the area under the main peak gave a value of about 1.8 kcal/mol whereas using the area under both peaks, ΔH calculation gave a value of 2.4 kcal/mol which falls within experimental error (see figure 10B).

The mole percentage of cholesterol necessary to completely abolish the phase transition for DPPC is either 33 mole % or 50 mole % (10,37,52). We obtained 34.8 mole percent of cholesterol necessary to abolish the phase transition peak of DPPC by extrapolating the slope of Figure 9B. For DPPE the reported value is 33% (55). Our studies obtained the same value (Figure 8A). No values for 50 mole % cholesterol have been reported for DPPE.

Klopfenstein et al (57) have reported that the cholesterol concentration necessary to completely abolish the phase transition of PLPC was 50 mole %. Nuzback (12) reported a value of 50 to 60 mole percent of cholesterol abolished the phase transition for the same compound. Our study determined a value of 37.6 mol % of cholesterol was necessary.

The mole percentages of cholesterol necessary for the elimination of the phase transition for both diacylphosphatidylethanolamine derivatives and monoacyllysophosphatidylethanolamine derivatives studied here, range between 30% to 40% with the exception of DPP N-methyl E. It is possible these differences are due to the purity of each sample or the rate of scanning in the DSC.

SUMMARY

Since the knowledge of the thermal properties of the lysophosphatidylethanolamine derivatives differing in the degree of methylation of the ethanolamine group is very limited, a comparison of the transition temperatures and enthalpies of transition of these compounds was completed. Also the results obtained for the lysocompounds were compare to the values for diacylphosphatidylethanolamine derivatives differing in the ethanolamine group methylation. The thermal properties for the lysocompounds and diacylcompounds were studied by differential scanning calorimetry. The transition temperature for DPPE was 62.5°C. The addition of one methyl group at a time on the nitrogen atom of the amino group reduced the transition temperature by an average of about 7.0°C for the diacylcompounds. The same result was obtained for the lysocompounds except that the transition temperatures were reduced with an average of about 11°C per methyl group. Transition enthalpy of DPPE was 8.01 kcal/mol. The addition of one methyl group at a time on the nitrogen atom of ethanolamine caused a slight increase in ΔH values (an average of 0.26 kcal/mol). The enthalpy of transition for the PLPE was 7.77 kcal/mol. Addition of each methyl group caused a decrease in ΔH values an average of 1 kcal/mole.

The effect of cholesterol content on both the diacyl and monoacyl compounds were also studied. The presence of increasing concentrations of cholesterol broadened the gel to liquid-crystalline phase transitions for both diacyl and monoacylphosphatidylethanolamine derivatives. The transition temperatures for the diacylphosphatidylethanolamine derivatives were shifted to lower temperatures as the mole percent of cholesterol increased from 0-30. However, increasing the cholesterol concentrations of

the monoacylphosphatidylethanolamine derivatives caused their transition temperature to shift to higher temperatures. A negative linear correlation was observed between the enthalpies of the transition and the mole percent of cholesterol in the mono and diacylphosphatidylethanolamine derivatives. Values between 30% to 40% cholesterol were necessary to completely abolish the phase transition peaks for mono and diacyl compounds.

These results appear to support Wilkison and Nagle (38). They reported the increased stability of diacylphosphatidylethanolamine in the gel state is due to the ability of the protonated amino group of phosphatidylethanolamine to form hydrogen bonds between the surrounding water molecules or the amino group of adjacent phosphatidylaethanolamine molecules. This stability is reduced by the addition of each methyl group on the nitrogen atom of ethanol-amine group for the diacyl compounds. We observed the same phenomena with the corresponding lysophosphatidylethanolamine derivatives.

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/PHYSICAL PROPERTIES OF PHOSPHATIDYLETHANOLAMINE AND LYSOPHOSPHATIDYLETHANOLAMINE DIFFERING IN THE DEGREE OF METHYLATION/

by

Khalid Omar Ąbulnaja

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AN ABSTRACT OF A MASTER'S THESIS

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requirements for the degree

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Graduate Biochemistry Group
Department of Biochemistry

Kansas State University
Manhattan, Kansas

1985

ABSTRACT

Palmitoyllysophosphatidyl-N-methylethanolamine and palmitoyllysophosphatidyl-N-N-dimethylethanolamine was prepared by enzyme hydrolysis of the corresponding diacyl compounds. These compounds and DPPE, DPP-N-methyl E, DPP-N-methyl E, DPPC, PLPE, and PLPC were studied by differential scanning calorimetry in the presence or absence of cholesterol. A comparison of the transition temperatures and transition enthalpies values for all these compounds was made. The diacylphosphatidylethanolamines have higher T_t's than the corresponding lysocompounds. The addition of one methyl group at a time on the nitrogen atom of the ethanolamine group of DPPE reduced the transition temperature by an average of about 7.0°C and transition enthalpy increased by an average of 0.26 kcal/mol for the diacylcompounds. For the corresponding lysophosphatidylethanolamine derivatives the transition temperature was reduced by an average of about 11.0°C and transition enthalpy was reduced by an average of 1 kcal/mol on addition of each methyl group. In the presence of increasing concentrations of cholesterol the T_t for each diacylcompound was shifted to a lower temperature while the T_t for each of monoacylcompound was shifted to higher temperature. Both the diacyl and monoacyl compound reduced the transition enthalpies ΔH as the mole % of cholesterol increased.