Differential Scanning Calorimetric Study of Bilayer Membrane Phase Transitions

A Biophysical Chemistry Experiment

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We present a series of calorimetric experiments developed to probe the gel to liquid-crystalline phase transition in model phospholipid bilayer systems. Previous calorimetric studies of these systems led to a greater understanding of the energetics governing lipid interactions and how these energetics are affected by chemical composition and molecular geometry (J). By conducting the experiments detailed here, students gain an understanding of the importance of weak interactions on phase transitions for model bilayer systems. This knowledge is used to further their understanding of the importance of chemical and physical properties of lipids influencing physiological biomembrane processes.

The need for this experiment arose with the development of a new course in our curriculum, “Physical Chemistry for the Life Sciences”, a requirement for our biological chemistry majors. When planning the material for this course and its associated laboratory, we sought to develop biologically relevant physical chemistry laboratory experiments. This article is aimed at instructors who may be in similar situations, primarily physical chemists with little background in biology. Biochemists may also find these experiments useful, but will have to bear with our detailed description of biomembranes.

Our primary goal was to enable students to see connections between traditional, relatively dry, thermodynamics topics (including calorimetry, phase transition temperatures, enthalpies, and entropies) and the biological membrane structure. All of the interrelated experiments presented use a single approach and require a high-sensitivity differential scanning calorimeter (DSC), an important tool in both academic and industrial laboratories.

Students study the following compositional changes on the gel to liquid-crystalline bilayer phase transition: (i) phospholipid chain length, (ii) phospholipid headgroup composition, (iii) cholesterol incorporation into the phospholipid bilayer, and (iv) mixed-chain-length bilayers. Results from the first two reveal much about lipid bilayer phase transitions in general, whereas the others provide a more realistic picture of a complicated membrane system. Finally, the students relate the results from all experiments to the structure of biomembranes and the thermodynamics of phase transitions. The instructor or student may modify and use some or all of the experiments in a one-week or multi-week laboratory.

Background

Biological membranes are complicated systems containing a variety of molecular components, including lipids, proteins, and carbohydrates. While each of these components is vital to cellular function, the structural foundation of the membrane is formed by a phospholipid bilayer. The bilayer is analogous to a solvent in which other “functional” components are dissolved. To understand even the simplest biomembrane processes, one must examine the phospholipid bilayer in a controlled manner, and these experiments are designed to do so.

The predominant phospholipids in most membranes are phosphoacylglycerols, phosphates esters of glycerol. All phospholipids contain three main functional groups: (i) two long acyl chains, usually with an even number of carbon atoms, (ii) the glycerol component, and (iii) the phosphate headgroup. Figure 1 shows the structure of two classes of phospholipids studied in these experiments, phosphatidylcholines and phosphatidylethanolamines. Because phospholipids are amphipathic molecules, they spontaneously assemble into multilayer vesicles (MLVs) in an aqueous solution. The hydrophilic headgroups are dissolved in the surrounding water (facing outward) while the hydrophobic acyl chains are ordered and pointed toward the center of the bilayer. The formation of MLVs is entropy driven as the individual phospholipids shed the surrounding water. The MLVs prepared as we outline in this experiment resemble an onion of concentric spheres of phospholipid bilayers with diameters between 1000 and 50,000 Å (J) and serve as the model membrane throughout these experiments.

Lipid bilayers have properties distinct from either pure liquids or crystals, many of which arise from their two-dimensional structure. For example, thermally induced phase changes occur in aqueous suspensions of bilayers, but are not observed in the high-melting (mp ~200 °C) anhydrous lipids. Such behavior has also been noted in both prokaryotes and eukaryotes, which exhibit a “adaptive response” when exposed to different growth conditions. This response allows the cell membrane to maintain its fluidity over a wide range of temperatures and is vital to the survival of a cell in different thermal environments. This cellular response frequently occurs near the temperature of the gel to liquid-crystalline phase transition of the model phospholipid bilayers.

In the MLVs, in the lower temperature gel state, the phospholipids are tightly packed by strong van der Waals interactions on phase transitions for model bilayer systems. This knowledge is used to further their understanding of the importance of chemical and physical properties of lipids influencing physiological biomembrane processes.

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forces. At the onset of the phase transition, the phospholipids “cooperatively melt” (2). In the resulting liquid-crystalline state, the phospholipids are more loosely associated, owing to weakened van der Waals forces between the acyl chains, weakened polar interactions of the phospholipid headgroups, and a lateral expansion of the acyl chains. Because these weak van der Waals interactions dictate the structure of the membrane, the acyl chain length and the identity of the phospholipid headgroup clearly are the major contributors to changes in the nature of the gel to liquid crystalline phase transition (3, 4).

Since the 1970s, freeze-fracture electron microscope images of MLVs at different temperatures have given researchers pictures of the bilayer structure (5). Below the main phase transition, large domains of the gel phase were identified by their characteristic striated appearance and highly ordered structure, and small regions of the lower order (liquid crystalline) assembly were also observed. These images contributed to the creation of the fluid mosaic model of bilayer membranes, which allows for lateral diffusion of the lipids within the bilayer structure (6). Because of diffusion and phase separation, molecular cooperativity is thought to play a large role in the gel to liquid-crystalline phase transition. Molecular cooperativity is an omnipresent concept in biology, as it is found in many biological systems, including the denaturation of proteins. In proteins, subunits may act cooperatively or independently during denaturation and the difference between these two types of behavior can be determined in DSC experiments (7). Several researchers have determined the degree of molecular cooperativity in gel to liquid-crystalline phase transition of phospholipid bilayers by fitting the DSC data to a “non-two-state” model (2, 10, 11). This analysis can contribute to the usefulness of the DSC experiments in the study of bilayer phase transitions and will be discussed in detail. In summary, the experiments consist of preparing well-characterized MLVs, observing the phase transition by DSC, and then analyzing the results for transition temperature, enthalpy, entropy, and size of the cooperative unit.

Materials and Equipment

All lipids can be obtained in high purity from either Sigma or Avanti Polar Lipids (Alabaster, AL)

- α-phosphatidylethanolamine, dipalmitoyl (DPPE, C16:0 PE) (CAS: 5681-36-7)
- α-phosphatidylethanolamine, dimyristoyl (DMPE, C14:0 PE) (CAS: 998-07-2)
- α-phosphatidylethanolamine, dilauroyl (DLPE, C12:0 PE) (CAS: 59752-57-7)
- α-phosphatidylethanolamine, dipalmitoyl (DPPE, C16:0 PE) (CAS: 5681-36-7)
- α-phosphatidylethanolamine, dimyristoyl (DMPE, C14:0 PE) (CAS: 998-07-2)
- α-phosphatidylethanolamine, dipalmitoyl (DPPE, C16:0 PE) (CAS: 5681-36-7)
- differential scanning calorimeter (MicroCal VP-DSC or other high-sensitivity DSC, repeatability 5 µcal/min [0.35 µW, 1.0-mL sample volume])
- glass beads (3 mm in diameter)
- rotary evaporator or vacuum line
- 10-mL pear-shaped flasks

Hazards

There are no major hazards associated with this experiment. Chloroform is possibly carcinogenic to humans. Avoid skin contact, ingestion, and inhalation. Perform transfers in a ventilated hood.

Experiments

In our course, all students perform one DSC run from experiment 1 below and then choose one of the four experiments as an independent project. Class data are pooled and discussed after every group has completed the experiment.

Experiment 1. Effect of Chain Length on Phase Transition Temperatures

This experiment is straightforward and can be accomplished in one afternoon. Bilayers are prepared from phosphatidylcholines (PCs) of different chain lengths and the results are analyzed as a function of chain length. In a 3- to 4-hour session, 3 or 4 samples may be prepared and a series of three phospholipids analyzed by DSC. More information about length of time for each experiment is available in the instructor’s notes.

Preparation of PC Bilayers. Stock solutions (2.0 mg/mL) of each of the carefully weighed PCs are prepared in chloroform. A small volume (2.5 mL) of the stock phospholipid solution is transferred by pipet to a 10-mL pear-shaped flask. The flask is placed on a rotary evaporator in a hot water bath (∼70 °C) until the chloroform has completely evaporated and the remaining lipid is uniformly distributed on the walls of the flask. Then 1.0 mL of degassed distilled water and two small glass beads are added to the flask. The flask is swirled in the same hot water bath until a cloudy dispersion forms and the walls of the flask appear clean.

Experiment 2. Effect of Headgroup on Phase Transition Temperature

Phosphatidylethanolamine (PE) Bilayers. Because these lipids do not dissolve well in chloroform, mixtures of chloroform and methanol are used to make the stock solutions. Sample preparation is similar to the procedure in experiment 1. More details are available in the instructor’s notes.

Experiment 3. Effect of the Addition of Cholesterol to a DPPC Bilayer

In this experiment, cholesterol is incorporated into the DPPC bilayer in varying proportions. Bilayer mixtures of DPPC with cholesterol are prepared by mixing stock cholesterol solutions (1.0 mg/mL in chloroform) with 2.5 mL of stock DPPC solution (2.0 mg/mL in chloroform) to obtain cholesterol concentrations ranging from 10 to 50 mol %. After mixing, the procedure in experiment 1 is followed for vesicle formation.

Experiment 4. Effect of Mismatching Chain-Length in PC Bilayers

Mixtures of phospholipids (1:1 v/v) are prepared by mixing 1.5 mL of each of two PC stock solutions (e.g. DPPC with DSPC). All of this mixture is added to a 10-mL pear-shaped flask, and the procedure in experiment 1 is followed to form the dispersion of “mixed” bilayers.
Instrumental Details. Degassed distilled water is used to fill the reference cell. Samples are slowly injected into the sample cells by syringe, taking care to expel all air bubbles that may be trapped in the cell. The experimental parameters for each scan are set using DSC VPViewer software. A scan rate of 60 °C/h, a pre-scan thermostat time of 10 min, and a filter period of 16 s were used for all scans. The temperature range is modified for each scan for each phospholipid, usually at least ±20 °C on either side of the expected phase transition. We have found it unnecessary to collect background scans using our instrument for this experiment. Differential scanning calorimeters heat the reference (water) and sample cells simultaneously. The power required to maintain both cells at the same temperature is measured and converted to give an output of heat capacity (sometimes referred to as excess heat capacity) versus temperature.

Results and Analysis

Figures 2–5 show the DSC results from all experiments outlined above, the numerical results of which are summarized in Tables 1–4. For first-order phase transitions such as the bilayer gel to liquid-crystalline transition, the transition temperature, $T_m$, is where the heat capacity, $C_p$, reaches its maximum value. The value of the calorimetric enthalpy ($\Delta H_{cal}$) for the phase transition is determined by integrating the area under the peak.

$$\Delta H_{cal} = \int C_p dT$$

From these values, the entropy of the phase transition is determined:

$$\Delta S = \frac{\Delta H_{cal}}{T_m}$$

Comparison of $\Delta H_{cal}$, $\Delta S$, and $T_m$ shows the effect of a structural modification (e.g. chain length) on the thermodynamics of the phase transition. However, unlike a simple organic compound’s crystal-to-liquid melting transition, the phase transition in bilayers involves more than just the initial and final states. In fact, intermediate “states” are formed during the transition, and a “non-two-state” model is necessary to fit these data. These intermediate states result from the formation of domains (e.g. liquid crystalline within the gel) before the phase transition temperature, and are due to lateral movement of the phospholipids within the bilayer. The asymmetric shape of the DSC peak reflects the fact that a non-two-state transition is occurring.

Table 1. Pretransition and Main-Phase Transition Data for Phosphatidylcholines, Experiment 1

<table>
<thead>
<tr>
<th>PC</th>
<th>$T_f$/°C</th>
<th>$T_m$/°C</th>
<th>$\Delta T_{1/2}$/°C</th>
<th>$\Delta H_{cal}$/kcal/mol</th>
<th>$\Delta H_{vH}$/kcal</th>
<th>$\Delta S$/cal/mol/K</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Expfl</td>
<td>Lit</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMPC (C14:0)</td>
<td>9.50</td>
<td>23.97 ± 0.01</td>
<td>23 (12]</td>
<td>23.9 (16]</td>
<td>1.8</td>
<td>5.03 ± 0.08</td>
</tr>
<tr>
<td>DPPC (C16:0)</td>
<td>34.8</td>
<td>41.51 ± 0.01</td>
<td>41 (12]</td>
<td>41.4 (16]</td>
<td>1.1</td>
<td>5.80 ± 0.07</td>
</tr>
<tr>
<td>DSPC (C18:0)</td>
<td>49.6</td>
<td>54.97 ± 0.01</td>
<td>58 (12]</td>
<td>58.9 (16]</td>
<td>1.6</td>
<td>7.60 ± 0.08</td>
</tr>
</tbody>
</table>

To understand this model, we refer to a two-state model and note where modifications are required for our system. For any phase transition that occurs between two phases, A and B, an equilibrium constant characterizes this process:

$$K = \frac{a_B}{a_A}$$

where $a_B$ and $a_A$ represent the activity of each phase. The equilibrium constant is related to enthalpy by the van’t Hoff equation (eq 4):

$$\left(\frac{\partial \ln K}{\partial T}\right)_p = \frac{\Delta H_{vH}}{RT^2}$$

The van’t Hoff enthalpy, $\Delta H_{vH}$, has units of energy and is equal to the amount of heat required for each cooperative unit to undergo the phase transition. For a two-state transition, the van’t Hoff enthalpy is equal to the calorimetric enthalpy ($\Delta H_{cal}$). After subtracting a baseline from our data, which negates any temperature dependence of $\Delta H_{cal}$, we use eq 4 to obtain an expression to fit our data (9):
\[ C_p(T) = \frac{K(T)\Delta H_{vth} \Delta H_{cal}}{(1 + K(T))^2RT^2} \]  

(5)

where \( K(T) \) is just our equilibrium constant (eq 3) and has the value

\[ K(T) = \exp \left[ \frac{\Delta H_{vth}}{RT} \left( 1 - \frac{T}{T_m} \right) \right] \]  

(6)

The software provided by Microcal, Origin, completes this fit and provides the values of \( \Delta H_{cal}, \Delta H_{vth} \) and \( T_m \) (9); however, these data may be fit by using the expression above and a Simplex minimization routine. For a more physical picture of the van’t Hoff enthalpy, \( \Delta H_{vth} \) can be calculated from the calorimetric data (7):

\[ \Delta H_{vth} = 4RT^2 \frac{dx}{dT} \]  

(7)

where \( dx/dT \) is the midpoint slope of a curve of enthalpy, \( \Delta H_{vth} \) versus \( T \) (the integrated plot of the original \( C_p \) versus \( T \) graph). A narrower transition results in a larger value of \( \Delta H_{vth} \). However, these data may be fit by using the expression above and is defined as the cooperative unit, C.U. Sturtevant and others have used the value of C.U. to define the number of molecules cooperating to undergo the specified transition (2, 10, 11). For this phase transition in pure phospholipids, values between 50 and 1000 are observed (2). The values of \( \Delta H_{vth} \) are very sensitive to scan speed of the DSC; thus a slower scan speed will result in increased thermal equilibration during the transition, and \( \Delta H_{vth} \) will be greater. Therefore, the C.U. should only be taken as a relative value within a series of experiments. In the case of phospholipids, the C.U. is an indication of the number of subunits within the protein cooperating in a phase transition, providing a widely accepted method for using calorimetric data to determine molecular cooperativity in proteins. This method of analysis is more controversial for phospholipid bilayers and has not been universally accepted (7). Therefore, although C.U. can be calculated, our analysis concentrates on the differences between values of \( T_m, \Delta T_{1/2} \), and \( \Delta H_{cal} \) within a series of phospholipids. The values of \( T_m \) and \( \Delta H_{cal} \) provide an indication of how strongly bound the phospholipids are in the gel phase, and \( \Delta T_{1/2} \) indicates how cooperative they are when undergoing the transition.

Experiment 1. Effect of Chain Length

Figure 2 shows the DSC scans resulting from this experiment, and Table 1 summarizes the values of \( T_m, \Delta T_{1/2}, \Delta H_{cal} \) and \( \Delta H_{vth} \) from these data. The main endothermic phase transition temperature increases with increasing chain length, as one might expect from an increased number of van der Waals interactions between acyl chains. The main transitions are fairly narrow, \( \Delta T_{1/2} \) values being on the order of 1.1–1.8 °C for each phospholipid. A small peak in \( C_p \), the “pre-transition,” occurs before the main transition and is associated with a polar headgroup reorganization accompanying a change in the angle of the lipid chains from a tilted to a vertical conformation prior to melting (12, 13). The values of \( T_m, \Delta T_{1/2}, \) and \( \Delta H_{cal} \) can be compared within this series, and the trends are what one would expect for van der Waals interactions between long acyl chains. One can also use the values for \( \Delta H_{vth} \) and \( \Delta H_{cal} \) to obtain a value for the cooperative unit. We have found the value not to be reproducible, but on the order of 70–100 for this PC series. As noted above, the values of \( \Delta H_{vth} \) and C.U. are sensitive to scan speed. These data were acquired at 60 °C/h. Conducting the experiments at slower scan speeds does not alter \( T_m \) or \( \Delta H_{cal} \) but does make the experiment prohibitively slow.

Experiment 2. Phosphatidylethanolamines

Figure 3 shows the DSC data from this experiment, and the results are summarized in Table 2. The change in the phase transition with headgroup composition is evident from comparing Figure 3 for the PE series with the data in Figure 2 for the PC series. Both systems show the same increase in \( T_m \) with chain length; however, the PE series has higher melting temperatures / °C

<table>
<thead>
<tr>
<th>Relative ( C_p ) (kcal deg mol⁻¹)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature / °C</td>
<td>20</td>
<td>40</td>
<td>60</td>
<td>80</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3. Results for the PE series in experiment 2, showing the gel to liquid-crystalline phase transition. Numerical values are given in Table 2.

### Table 2. Gel to Liquid-Crystalline Phase Transition Data for Phosphatidylethanolamines, Experiment 2

<table>
<thead>
<tr>
<th>PE</th>
<th>( T_m )/°C</th>
<th>( \Delta T_{1/2} )/°C</th>
<th>( \Delta H_{cal} )/(kcal/mol)</th>
<th>( \Delta H_{vth} )/(kcal/mol)</th>
<th>( \Delta S_{cal} )/(cal K⁻¹ mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLPE (C12:0)</td>
<td>30.25 ± 0.01</td>
<td>1.1</td>
<td>0.95 ± 0.01</td>
<td>464 ± 7</td>
<td>3.1 ± 0.03</td>
</tr>
<tr>
<td>DMPE (C14:0)</td>
<td>49.54 ± 0.01</td>
<td>1.2</td>
<td>2.3 ± 0.01</td>
<td>559 ± 8</td>
<td>7.0 ± 0.06</td>
</tr>
<tr>
<td>DPPE (C16:0)</td>
<td>63.83 ± 0.01</td>
<td>1.2</td>
<td>1.9 ± 0.02</td>
<td>640 ± 10</td>
<td>5.8 ± 0.06</td>
</tr>
</tbody>
</table>

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points for the same chain length as the PC series. From Table 2, one notes that we did not observe the expected trend in \( \Delta H_{\text{ad}} \) for the PE series. Because of the lower solubility of the PEs in organic solvents, we do not know the molarity of our vesicle suspensions. The values we calculated assume complete mixing. The values of \( T_m \) and \( \Delta T_{1/2} \) may still be compared with those obtained for the PCs. Because the PE headgroup is smaller and very polar, these phospholipids can pack more tightly in the bilayer, resulting in higher transition temperatures.

**Experiment 3. Cholesterol Incorporation**

Cholesterol, a component in many biological membranes, intercalates into the bilayer membrane and alters its structure. At low concentrations it is thought to control the fluidity of the hydrocarbon chains by disrupting the “crystalline” lattice in the gel phase and by preventing chain flex in the liquid crystalline phase (14). When no cholesterol is present in the DPPC bilayer, the hydrocarbon chains assume a tilted conformation relative to the membrane surface. With the addition of a small amount (ca. 7.5% mol) of cholesterol, this changes to perpendicular strengthening the bilayer (14). As the concentration of cholesterol increases, a reduction in the van der Waals forces between the hydrocarbon chains in the phospholipid occurs, causing a slight reduction in \( T_m \), a reduction in \( \Delta H_{\text{ad}} \), and an increase in the water layer between the bilayers in the MLVs. As the percentage of cholesterol increases, a broadening of the phase transition is noticeable (Fig. 4). The measured widths of these transitions are given in Table 3. Students were asked to look at space-filling and computer-generated models of cholesterol and the phospholipids so they could gain a better understanding of how cholesterol intercalates into the bilayer structure (4).

It is clear that the peak is broadened as the proportion of cholesterol in the bilayer increases. This peak may be viewed either as one broad peak or as a single sharp peak with an overlapping broader peak. Using Origin (9) it is possible to fit these data as one or two peaks. The observation of two overlapping peaks has been explained by lateral phase separation in which islands of pure DPPC form and undergo a phase transition at the normal phase transition temperature (the sharp peak), whereas impure DPPC undergoes a phase transition over a wider temperature range. A figure of both single- and double-fit peaks for the 30% cholesterol data is contained in the instructor’s notes.

![Figure 4. Results from experiment 3. Cholesterol is added to a DPPC bilayer. Numerical results are tabulated in Table 3.](image)

### Table 3. Data for DPPC Bilayers with Intercalated Cholesterol, Experiment 3

<table>
<thead>
<tr>
<th>Cholesterol (mol %)</th>
<th>( T_m /^\circ C )</th>
<th>( \Delta T_{1/2} /^\circ C )</th>
<th>( \Delta H_{\text{ad}} / \text{kcal/mol} )</th>
<th>( \Delta S / \text{cal/K/mol} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>40.54 ± 0.03</td>
<td>1.9</td>
<td>2.76 ± 0.07</td>
<td>304 ± 10</td>
</tr>
<tr>
<td>20</td>
<td>40.99 ± 0.05</td>
<td>3.9</td>
<td>2.06 ± 0.05</td>
<td>158 ± 5</td>
</tr>
<tr>
<td>30</td>
<td>42.3 ± 0.1</td>
<td>5.8</td>
<td>1.82 ± 0.05</td>
<td>87 ± 3</td>
</tr>
<tr>
<td>40</td>
<td>42.5 ± 0.1</td>
<td>8.2</td>
<td>1.16 ± 0.02</td>
<td>72 ± 2</td>
</tr>
<tr>
<td>50</td>
<td>40 [very broad]</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

![Figure 5. Results from experiment 4. Mixtures of phosphatidylincholines of two different chain length form “mismatched” bilayers. Numerical results are found in Table 4.](image)

### Table 4. Data for Mixed Chain-Length Phosphatidylincholine Bilayers, Experiment 4

<table>
<thead>
<tr>
<th>Mixture of</th>
<th>( T_m /^\circ C )</th>
<th>( \Delta T_{1/2} /^\circ C )</th>
<th>( \Delta H_{\text{ad}} / \text{kcal/mol} )</th>
<th>( \Delta S / \text{cal/K/mol} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMPC (14.0)</td>
<td>31.34 ± 0.03</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>DPPC (16.0)</td>
<td>33.42 ± 0.03</td>
<td>1.44 ± 0.08</td>
<td>321 ± 7</td>
<td>4.7 ± 0.3</td>
</tr>
<tr>
<td>DMPC (14.0)</td>
<td>30.19 ± 0.03</td>
<td>1.59 ± 0.09</td>
<td>117 ± 8</td>
<td>5.2 ± 0.3</td>
</tr>
<tr>
<td>DSPC (18.0)</td>
<td>44.04 ± 0.03</td>
<td>2.7 ± 0.1</td>
<td>90 ± 5</td>
<td>8.5 ± 0.3</td>
</tr>
<tr>
<td>DPPC (16.0)</td>
<td>46.20 ± 0.03</td>
<td>3.2 ± 0.1</td>
<td>417 ± 6</td>
<td>6.7 ± 0.3</td>
</tr>
<tr>
<td>DSPC (18.0)</td>
<td>47.64 ± 0.03</td>
<td>4.1 ± 0.1</td>
<td>316 ± 7</td>
<td>12.8 ± 0.3</td>
</tr>
<tr>
<td>total</td>
<td>80.0 ± 0.0</td>
<td>4.7 ± 0.2</td>
<td>738 ± 10</td>
<td>19.2 ± 0.3</td>
</tr>
</tbody>
</table>
Experiment 4. Mixed Chain Length Bilayers

The results of this experiment are given in Figure 5 and Table 4. These mixtures provide structurally simple models to study the effect of different chain lengths on the packing properties of lipid chains in the bilayer. To understand these results, one must consider the energetic principles dictating the packing of the phospholipid chains in the gel phase. Ideally the lipids attempt to maximize the van der Waals contacts between the chains and pack the chains without void. But in the mixed phospholipid case, a mismatch of chain lengths on neighboring phospholipids will weaken the total van der Waals interactions. Therefore, the change in the phase transition temperature(s) and structure is due entirely to the weaker van der Waals contacts in the interdigitated region of the bilayer. A mismatch as short as four carbons (e.g. Fig. 5, DMPC and DSPC) has a striking effect on the nature of the phase transition. As for the DPPC and cholesterol, lateral phase separation is reflected in these data. Particularly in the case of DMPC with DSPC, two phase transitions are observed. These may be attributed to a two-phase region, impure DMPC and DSPC domains. In fact, this mixing has been thoroughly examined and phase diagrams for these mixtures have been developed by McConnell et al. (6).

Conclusions

The experiments outlined in this article give students a clear understanding of the importance of weak interactions on phase transitions. As in the fluid mosaic model of cell membranes (15), we view the bilayer as a solvent. The gel to liquid-crystalline phase transition is not only an unusual consequence of the two dimensional model bilayer but is also found in lipids obtained from cellular membranes (7) and in the adaptive response of cell membranes. When the structure of the phospholipid is altered (by changing either the headgroup or the acyl chain length), we can clearly see the effect of the changing van der Waals forces binding the bilayer on the phase transition. As we add a foreign substance (cholesterol) to the bilayer, the $\Delta T_{1/2}$ increases owing to decreasing purity of the phospholipid bilayer. Lateral phase separation is observed in the mixed samples and can be discussed in terms of two component phase diagrams, in which mixing is nonideal (6). Again, we find space-filling or computer-generated models helpful in understanding how structure and phase transitions are related. As a secondary benefit of the experiment, students gain an appreciation of calorimetry as a useful experimental technique, particularly in examining biological systems. Because there are four interconnected experiments, students will work independently, but may share their results with others. Therefore, each student can get a complete picture of how bilayer structure affects the thermodynamics of phase transitions. Because the experiments are presented in order of increasing bilayer complexity, students may relate what they understand from their physical chemistry lecture (simple phase transitions) to a complex biological system. To extend these experiments, one may perform this DSC experiment on egg lecithin, a natural bilayer membrane containing a mixture of phosphatidylcholines. The measured phase transition is extremely broad. Asking students to connect their egg lecithin experiment with previous results encourages them to connect the model bilayer experiment with more complex biomembranes.

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"Supplemental Material"

Detailed notes for the instructor are available in this issue of JCE Online.

Literature Cited

13. For a sketch of this behavior, see Jain, M. K.; Wagner, R. C. Introduction to Biological Membranes; Wiley: New York, 1980, p 94.