Titration Calorimetric and Differential Scanning Calorimetric Studies of the Interactions of *n*-Butanol with Several Phases of Dipalmitoylphosphatidylcholine†

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Received July 9, 1991; Revised Manuscript Received November 21, 1991

ABSTRACT: The interactions of *n*-butanol with dipalmitoylphosphatidylcholine (DPPC) were studied using titration calorimetry and differential scanning calorimetry (DSC). DSC results indicated that *n*-butanol induces the interdigitated phase in DPPC above 10 mg/mL butanol. A new application of titration calorimetry for measuring partition coefficients of nonsaturating solutes into lipids was developed. The partition coefficients and the heat of binding of *n*-butanol into DPPC were measured for the L_{β}', P_{β}', L_{α}, and $L_{\beta}I$ phases of DPPC. The partition coefficients were temperature dependent and ranged from 70 to 110 for the $L_{\beta}I$ phase, from 170 to 183 for the L_{α} phase, and similar to that for the $L_{\beta}I$ phase in the P_{β}' phase. The binding to the L_{β}' phase could not be detected, giving an upper limit for this partition coefficient of 23. The enthalpies for binding to the $L_{\beta}I$ and L_{α} phases were 1.0 and 1.5 kcal/mol, respectively. The van't Hoff enthalpy was in good agreement with the calorimetric enthalpy for the partitioning into the L_{α} phase; however, it was greater than the calorimetric enthalpy for the $L_{\beta}I$ phase, suggesting that the interaction of *n*-butanol with this phase is cooperative in some way.

The biological role of lipid phase behavior has not been clearly established; however, the well-established tight regulation of lipid compositions, combined with the increasing elucidation of the wide diversity of lipid physical properties, provides ever stronger circumstantial evidence for the importance of the unique physical properties of individual lipid species and classes in biological processes. The influence of solute molecules on the phase behavior of lipids is an obvious means by which the membranes can be controlled. There are many examples of isothermal changes in phase state which can occur as a function of solute ions, anesthetics, or metabolites [for a review, see Cevc (1991)].

We have been investigating the influence of alcohols in altering the phase behavior of phosphatidylcholines, and we have been particularly interested in the unique fully interdigitated phase which occurs at relatively high concentrations of alcohols. (Simon & McIntosh, 1984; Slater & Huang, 1989; Komatsu & Rowe, 1991; Nambi et al., 1988; Rowe, 1983, 1985a,b, 1987; Rowe & Nelson, 1990; Rowe et al., 1987; Rowe & Cutrera, 1990; Veiro et al., 1987). Most of our studies have focused on ethanol, but *n*-butanol was also studied by NMR (Herold et al., 1987; Rowe et al., 1987). Until the present, we have been focusing on the thermotropic behavior of PC's at various alcohol concentrations. This work showed that the alcohol concentration determines the phase behavior and therefore that the alcohols have differential interactions with each phase. In the present work, we have studied directly the partitioning of one alcohol, *n*-butanol, into each of the several phases of DPPC[1] in order to elucidate the role of alcohols in controlling this phase behavior.

It is clear that partitioning of solutes between the membrane and the aqueous phase is a very important aspect of the interaction of solutes with membranes. However, the determination of partition coefficients is still not a very common practice. Centrifugation has frequently been used to separate the membrane and aqueous phase (Katz & Diamond, 1974; Sarasua et al., 1989; Van Hoogevest & De Kruijff, 1978); however, it has low accuracy for solutes with low partition coefficients. This procedure can lead to errors due to the possibility of some membrane remaining in the supernatant, as well as solute cosedimentation without true partitioning (Van Hoogevest & De Kruijff, 1978). Some procedures have been presented for the determination of partition coefficients without requiring phase separation, based on fluorescence (Jain et al., 1985; Jones & Lee, 1985; Blatt & Sawyer, 1985) or ESR measurements (Lissi et al., 1990; Bianconi et al., 1988); however, the disadvantage of these methods is that foreign molecules such as fluorescent probes and spin labels are involved in these procedures.

Titration calorimetry has recently become available, and its methodology for studying the saturable high-affinity binding of ligands to proteins has been thoroughly described (Wiseman et al., 1989). In the present report, we have expanded this method to the measurement of nonsaturable partitioning of butanol into phospholipid. Using this method, we have measured the partition coefficients and the enthalpy of butanol–lipid interactions for each of several phases of DPPC. Differential scanning calorimetry (DSC) has been used to characterize the phase behavior and determine the thermodynamic characteristics of the transitions of DPPC in the presence of *n*-butanol.

Material and Methods

Chemicals. The dipalmitoylphosphatidylcholine (DPPC) was obtained from Avanti, Birmingham, AL. The *n*-butanol

† This work was supported by the Medical Research Service of the Veterans Administration and by a grant from the National Institute of Alcohol Abuse and Alcoholism (AA 05371).

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[1] Abbreviations: DPPC, dipalmitoyl-*sn*-glycero-3-phosphocholine; DSPC, 1,2-distearoyl-*sn*-glycero-3-phosphocholine; DHPC, 1,2-di-*O*-hexadecyl-*sn*-glycero-2-phosphocholine; L_{β}', tilted chain bilayer gel phase; P_{β}', rippled gel phase; L_{α}, liquid-crystalline bilayer phase; $L_{\beta}I$, interdigitated gel phase; DSC, differential scanning calorimetry; ITC, isothermal titration calorimetry.

was obtained from Aldrich Chemical Co. and was spectrophotometric grade.

Lipid Samples. Bangham's method was used to prepare the DPPC multilamellar vesicles (Bangham et al., 1967). Chloroform stock solutions of DPPC were dried under a steady stream of nitrogen gas and then kept overnight on a vacuum pump to remove all residual chloroform. The stock suspensions were hydrated with distilled water at 50 °C for at least 2 h with occasional vortexing. Aliquots were removed from the stock suspensions, and *n*-butanol was added accordingly. Extruded vesicles were prepared by extrusion of multilamellar vesicles through two stacked polycarbonate filters of 400-nm pore size at 50 °C, using the thermostated Extruder from Lipex (Vancouver, BC). The extrusion was repeated 10 times. The sizes of the extruded vesicles were determined by NICOMP model 370 submicron particle sizer (Pacific Scientific Co., Silver Spring, MD) to be 530 ± 270 nm. Lipid concentrations were determined by phosphorus assay by the method of Bartlett (1957).

High-Sensitivity Differential Scanning Calorimetry (DSC). DSC was performed using the MC2 scanning calorimeter from Microcal, Inc., Amherst, MA. The calorimeter is interfaced with an IBM PS/2 model 30 computer, and the software used is that provided by Microcal. A Keithley 150B microvolt ammeter is used as the preamplifier in the cell feedback circuit. A Haake FC3 refrigerated bath controlled by the computer is connected to the calorimeter for temperature control during cooling scans. For DSC experiments, the stock liposome sample was diluted with distilled water or *n*-butanol solutions at a temperature below the beginning temperature of heating scans, and the sample was held at this temperature until it was loaded into the calorimeter. The final lipid concentrations for DSC studies were between 0.25 and 0.50 mg/mL DPPC for studies of the main transition and from 3.0 to 4.0 mg/mL for studies of the pretransition or transition to the interdigitated $L_\beta I$ phase. Both heating and cooling scans were employed for the studies of the main transition, and the scan rates were 10 °C/h for both heating and cooling scans. For the studies of the pretransition or the transition to the interdigitated phase, a heating rate of 60 °C/h was used. The data were analyzed using ORIGIN software from Microcal, Inc., Amherst, MA.

Titration Calorimetry. Titration experiments were carried out using the Omega titration calorimeter from Microcal, Inc. (Northampton, MA). The calorimeter is interfaced with an IBM PS/2 model 30 286 computer, and the software used is ORIGIN, provided by Microcal. The 250-μL injection syringe was used. The Omega titration calorimeter is described in detail in the literature (Wiseman et al., 1989).

The interactions between DPPC and *n*-butanol were studied by placing the lipid suspension in the syringe and the butanol solutions in the sample cell. This was found to be most satisfactory because the butanol has a very large heat of dilution, whereas the heat of dilution of the lipid suspension is negligible. For titration experiments, the stock liposomes were combined with *n*-butanol at room temperature, and the sample was incubated at 50 °C for 1 h; they were stored at room temperature prior to use. For the studies of the interdigitated phase, the liposome samples were heated to 50 °C for 5 min immediately before being loaded into the injection syringe to make sure that they were in the interdigitated phase at all of the temperatures studied. (At 30 mg/mL *n*-butanol, the L_β' to $L_\beta I$ transition is for practical purposes irreversible at the temperatures studied here.) The concentration of lipid in the syringe was between 30 and 70 mg/mL.

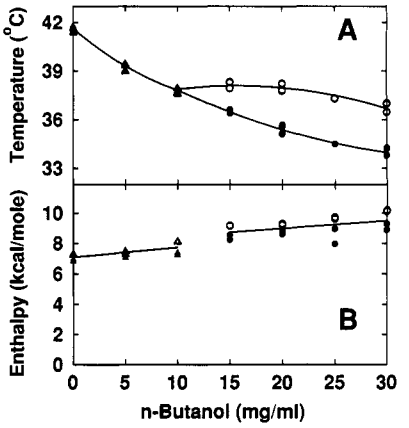

FIGURE 1: Main transition temperatures (A) and enthalpy (B) of DPPC as a function of *n*-butanol concentration measured by DSC. Triangles represent the P_β' to L_α transition; circles represent $L_\beta I$ to L_α transition. Open symbols represent heating scans; filled symbols represent cooling scans.

Titration experiments were performed in each phase using both multilamellar vesicles and unilamellar vesicles prepared by extrusion. The results obtained were identical within experimental error. Because the multilamellar vesicles are more convenient to prepare at the high lipid concentrations desired for these experiments, the multilamellar preparations were used in the present study.

RESULTS

Differential Scanning Calorimetry Studies. Differential scanning calorimetry was used to establish the phase behavior of DPPC as a function of *n*-butanol concentration, so that the partition coefficients and enthalpies of binding could be determined for each of the phases of DPPC. The DSC heating and cooling scans for DPPC in water for the main transition between the P_β' and L_α phases are very narrow; the width at half-height is 0.07 °C for the heating scan and 0.09 °C for the cooling scan. There is no significant difference between the transition temperatures for heating and cooling scans. Both heating and cooling transitions are broader in the presence of *n*-butanol; the width at half-height is 0.37 °C for the heating scan and 0.55 °C for the cooling scan in the presence of 30 mg/mL *n*-butanol, a concentration at which the lipid is interdigitated prior to melting, so that the observed transitions are between the $L_\beta I$ and L_α phases. There is a large hysteresis in this transition, as expected for transitions involving the $L_\beta I$ phase. The difference between the transition temperatures for heating and cooling was 3.0 °C at this butanol concentration.

The effect of *n*-butanol on the main transition temperature, enthalpy, and transition hysteresis of DPPC was studied by DSC. The main transition temperatures for heating and cooling for the P_β' to L_α and the $L_\beta I$ to L_α transition are plotted in Figure 1A as a function of *n*-butanol concentration. The results shown here are similar to our previously reported results on the interactions of *n*-butanol with DPPC using optical density measurements and ^{13}C NMR (Herold et al., 1987). This figure shows that the main transition temperature is decreased as a function of *n*-butanol concentration for the P_β' to L_α transition. Above the threshold concentration for interdigitation, the temperatures of the $L_\beta I$ to L_α transition for both heating and cooling scans also show a decrease with increasing *n*-butanol concentration; this is in contrast to the results with shorter chain alcohols, where the main transition temperature for heating scans increases with alcohol concentration above the threshold concentration (Rowe, 1983,

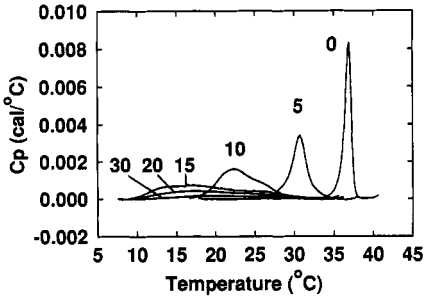

FIGURE 2: DSC heating scans of lower temperature transition as a function of *n*-butanol concentration. Numbers on the plot represent the concentration of *n*-butanol in mg/mL.

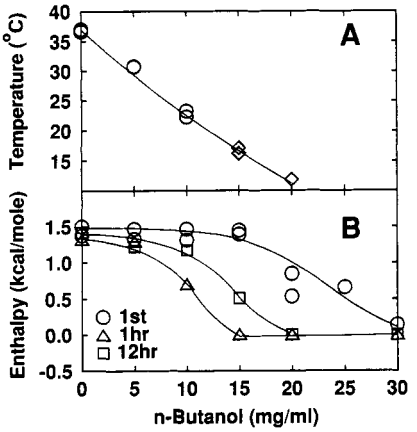

FIGURE 3: Transition temperatures (A) and enthalpy (B) for the lower temperature transitions of DPPC as a function of *n*-butanol concentration measured by DSC. (A) Transition temperatures for $L_{\beta'}$ to $P_{\beta'}$ (circles) and $L_{\beta'}$ to $L_{\beta}I$ (diamonds) transition in first heating scans. (B) Enthalpy of transition: Circles represent the first heating scans; the following heating scans were started after incubation at 4 °C. Triangles represent the scans with a 1-h incubation time; squares represent the scans with a 12-h incubation time.

1985a,b). At the threshold alcohol concentration for interdigitation, the onset of a large hysteresis between the heating and cooling scans is observed. This hysteresis is characteristic of the induction of the $L_{\beta}I$ phase in PC's by alcohols (Rowe, 1985a).

The enthalpy of the main transition was measured for both the $P_{\beta'}$ to L_{α} and the $L_{\beta}I$ to L_{α} transitions. Figure 1B shows the enthalpy of the main transitions as a function of *n*-butanol concentration. The data for the two transitions were separately fit to straight lines by least squares. Both transitions have a slight positive slope, but the two lines are separated by approximately 1 kcal/mol, showing that the $L_{\beta}I$ to L_{α} transition has a higher enthalpy than the $P_{\beta'}$ to L_{α} transition. This is consistent with the greater order of the $L_{\beta}I$ phase compared to the $P_{\beta'}$ phase, as determined by X-ray diffraction (Simon & McIntosh, 1984). The slightly positive slopes of these plots may be due to the contribution of the enthalpy of butanol binding to each phase.

Figure 2 shows the DSC heating scans of the lower temperature region at a series of *n*-butanol concentrations. The transition temperature decreases as the *n*-butanol concentration is increased. The shape of the transition changes abruptly between 5 and 10 mg/mL butanol, becoming increasingly broader with increasing butanol concentration. This observation is qualitatively similar to the effect of ethanol on the low-temperature transition of DSPC (Rowe & Cutrera, 1990) and suggests that the $L_{\beta'}$ to $P_{\beta'}$ transition is being replaced by the $L_{\beta'}$ to $L_{\beta}I$ transition as the *n*-butanol concentration is increased.

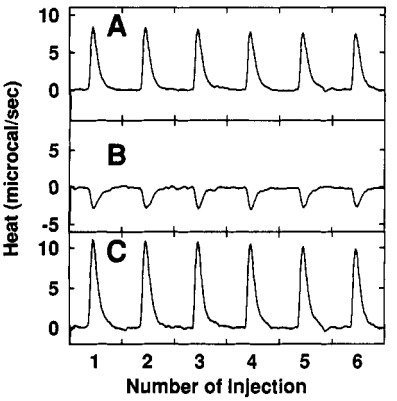

FIGURE 4: Raw data obtained for a sequence of injections, each of 15 μL of 16.6 mg/mL DPPC into 10 mg/mL *n*-butanol in the cell at 45 °C. (A) total heat; (B) blanks obtained for a sequence of injection of 15 μL of water into 10 mg/mL *n*-butanol in cell at 45 °C; (C) same as panel A after subtraction of the blank in panel B.

Figure 3A shows the temperature of the $L_{\beta'}$ to $P_{\beta'}$ and $L_{\beta'}$ to $L_{\beta}I$ transitions as a function of *n*-butanol concentration; these transitions are considerably more sensitive to the alcohol concentration than are the two main transitions shown in Figure 1A. Figure 3B shows a plot of the enthalpy of the low-temperature transition as a function of *n*-butanol concentration. In order to test the reversibility of the transition, the second and third heating scans were carried out with 1 and 12 h of incubation, respectively, at 4 °C after a previous heating scan. As seen in this figure, the transition is immediately reversible at *n*-butanol concentration up to 5 mg/mL, and the transition is not immediately reversible but can be recovered in a few hours at 10 mg/mL *n*-butanol. Above 20 mg/mL, the transitions are not reversible in more than 12 h. Our previous studies on DPPC in the presence of ethanol showed that the pretransition is immediately reversible, whereas the $L_{\beta'}$ to $L_{\beta}I$ transition is not (Nambi et al., 1988). The change in shape and reversibility of the low-temperature transition and the hysteresis of the main transition suggest that *n*-butanol induces the interdigitated phase in DPPC at *n*-butanol concentrations above 10 mg/mL. This is in agreement with our earlier report that the threshold concentration for butanol induction of interdigitation in DPPC is 8 mg/mL (Herold et al., 1987). Figure 3B also shows that the enthalpy for the pretransition is 1.4 ± 0.1 kcal/mol. The enthalpy of $L_{\beta'}$ to $L_{\beta}I$ transition at 10 and 15 mg/mL butanol is similar to that of the pretransition. Above this concentration, the apparent decrease in enthalpy is probably due to the low temperature of the transition such that, at the beginning temperature of the scan, the transition has already partially occurred.

Titration Calorimetry Studies. Titration calorimetry experiments were performed by adding aliquots of lipid suspension from the syringe into the reaction cell containing a solution of *n*-butanol. Figure 4 shows an example of a series of additions of 15-μL aliquots of DPPC suspension at 16.6 mg/mL lipid to a solution containing 10 mg/mL butanol at 45 °C. The area under the peak is integrated to give the heat released or taken up. Panel B shows the blank when water is added to the *n*-butanol solution; panel C shows the data corrected for the blank. Each experiment is performed at constant temperature; however, the temperature can be chosen over a wide range.

Figure 5 shows the temperature dependence of the heat taken up when DPPC is added to an *n*-butanol solution at a series of temperatures, expressed as heat per mole of lipid. At each temperature, 10 μL of DPPC suspension of 69.3 mg/mL

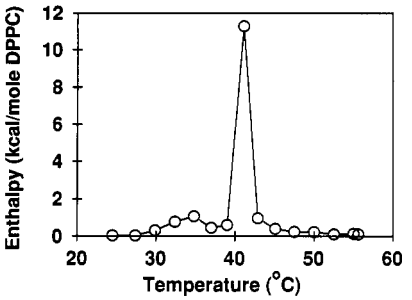

FIGURE 5: Temperature dependence of the enthalpy of *n*-butanol binding to DPPC in terms of kcal per mole of lipid. 10 μL of 69.3 mg/mL DPPC was injected into the reaction cell containing 5.4 mg/mL *n*-butanol.

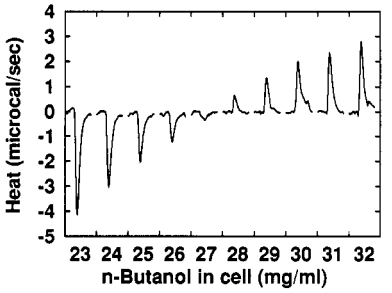

FIGURE 6: Raw data obtained for a sequence of injections of 15 μL of 52.0 mg/mL DPPC containing 30.0 mg/mL *n*-butanol into the reaction cell, which contained different concentrations of *n*-butanol solutions from 23 to 32 mg/mL, at 30 °C.

lipid concentration was injected into the reaction cell containing 5.4 mg/mL *n*-butanol. Blanks were measured by injecting 10 μL of DPPC suspension into water and by injecting 10 μL of water into 5.4 mg/mL *n*-butanol. In both cases, the blank was less than 0.02 kcal/mol. The apparent maxima in the enthalpy at 37 and 41 °C are due to the effect of butanol on the pretransition and the main transition of DPPC, respectively. Since butanol lowers these transition temperatures, the addition of butanol at constant temperature induces the transition to take place. The enthalpy is expressed in terms of kcal/mol of lipid, and the maximum at 41 °C corresponds well with the enthalpy for the main transition plus the heat of binding.

Titration calorimetry was used to measure the lipid:water partition coefficient for *n*-butanol into DPPC. Because the partitioning of butanol into lipid is not saturable, the titration calorimetry methods used for high-affinity protein–ligand interactions are not applicable. In our method, the free concentration of butanol in a butanol–lipid–water suspension is determined by a solvent null method. Aliquots from the syringe, containing a lipid–butanol–water suspension, are added to the reaction cell which contains various concentrations of butanol. Heat is absorbed if the free *n*-butanol concentration in the cell is higher than that in the suspension, and heat is released if the *n*-butanol concentration in the cell is lower than that in the suspension; when the concentration in the cell matches the free concentration in the syringe, no heat is generated upon mixing. The amount of *n*-butanol in the lipid is determined by subtraction of the free concentration from the total concentration, and the partition coefficient is then calculated from the formula $K_p = X_L/X_W$, where X_L is the mole fraction of *n*-butanol in the lipid phase and X_W is the mole fraction of *n*-butanol in the solvent.

Figure 6 shows the titration calorimetry results of a typical experiment in which 15 μL of DPPC suspension containing 30.0 mg/mL *n*-butanol was injected into the reaction cell, which contained different concentrations of *n*-butanol from

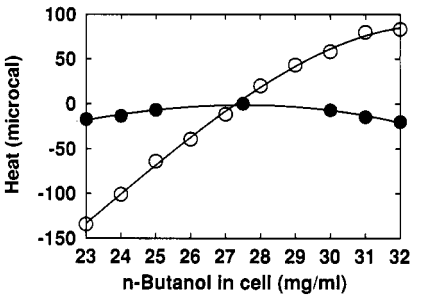

FIGURE 7: Heats of reaction from Figure 6 showing solvent blank contributions. (Open circles) Integrated heats from Figure 6. (Filled circles) Solvent blanks resulting from addition of 15 μL of 27.5 mg/mL *n*-butanol solution in the syringe into indicated *n*-butanol concentration in the cell.

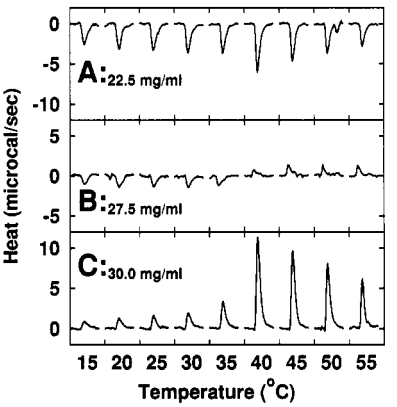

FIGURE 8: Raw data obtained from injection of 15 μL of DPPC containing 30 mg/mL *n*-butanol into the reaction cell containing *n*-butanol solution at a concentration of (A) 22.5 mg/mL; (B) 27.5 mg/mL; (C) 30 mg/mL. The lipid concentration in the syringe was 51.9 mg/mL. Experiments were carried out at nine different temperatures from 15 to 55 °C as indicated.

23 to 32 mg/mL, at a temperature of 30 °C. Heat is released at cell *n*-butanol concentrations of 23–27 mg/mL. The amount of heat decreases with increasing *n*-butanol concentration in the cell. At cell *n*-butanol concentrations of 28 mg/mL and above, heat is absorbed. The null concentration is therefore between 27 and 28 mg/mL.

Figure 7 shows a plot of the heat integrated from Figure 6 as a function of *n*-butanol concentration in the cell. The solvent blanks, also plotted in Figure 7, were determined by using a 27.5 mg/mL *n*-butanol solution in the syringe and different concentrations of *n*-butanol in the cell. In the blank experiments, heat was released in both cases, whether the *n*-butanol concentration in the syringe was higher or lower than that in the cell. The data from Figure 7 fit a straight line after correction with the solvent blank (not shown). All the titration results in this paper were corrected with the appropriate *n*-butanol solvent blanks. In this particular experiment, the free concentration was found to be 27.4 ± 0.2 mg/mL. The partition coefficient was then calculated to be 73 ± 8.

Using the partition coefficient, the measured heat can be expressed as heat per mole of bound butanol for each of the data points in Figure 7. The data show that the heat per mole bound was constant across the concentration range studied, whether the cell concentration was lower (i.e., unbinding) or higher (i.e., binding) than that in the syringe.

The partition coefficient of *n*-butanol between DPPC and water and the heat of *n*-butanol binding to DPPC were studied for the $L_{\beta'}$, $P_{\beta'}$, $L_{\beta}I$, and L_{α} phases of DPPC. At 30 mg/mL butanol, the lipid is in the $L_{\beta}I$ phase from 15 °C to its main transition temperature at about 40 °C. Figure 8 shows the

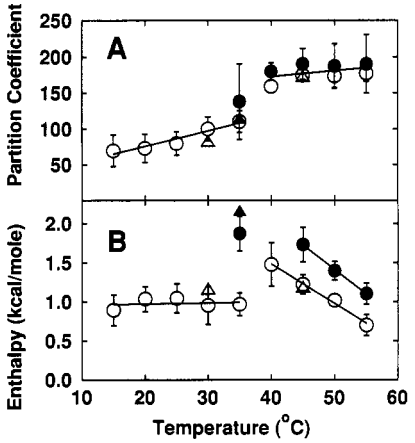

FIGURE 9: Partition coefficients of *n*-butanol between DPPC and water (A) and enthalpy of *n*-butanol binding to DPPC (B) as a function of temperature. The experiments were carried out with DPPC containing 30 mg/mL *n*-butanol (open symbols) and 5 mg/mL *n*-butanol (filled symbols). Circles represent multilamellar vesicles and triangles represent extruded unilamellar vesicles.

raw data obtained from the injection of 15 μL of DPPC containing 30 mg/mL *n*-butanol total concentration into the reaction cell containing *n*-butanol solution at concentrations of 22.5, 27.5, and 30 mg/mL, respectively, each at a series of temperatures. Each experiment for a particular *n*-butanol concentration in the cell was carried out by collecting data from 1 min before injection to 3 min after the injection at a constant temperature. Then the temperature was increased to the next value, and the calorimeter was allowed to equilibrate at the new temperature. Since the lipid initially contained 30 mg/mL butanol, the temperatures between 15 and 35 °C in Figure 8 correspond to DPPC in the interdigitated $L_\beta I$ phase. The temperatures from 40 to 55 °C correspond to DPPC in the liquid-crystalline phase. As seen in this figure, heat was released with 22.5 mg/mL *n*-butanol in the cell for DPPC in both the $L_\beta I$ phase and the L_α phase (panel A), indicating that the cell concentration was lower than the free concentration in the syringe. At an *n*-butanol concentration in the cell of 27.5 mg/mL (panel B), heat was still released for DPPC in the $L_\beta I$ phase. However, heat was absorbed for DPPC in the L_α phase, indicating that the free concentration in the syringe was lower than 27.5 mg/mL for the L_α phase. Heat was absorbed at 30.0 mg/mL *n*-butanol in the cell (panel C) for DPPC both in the $L_\beta I$ phase and the L_α phase, indicating that the free concentration in the syringe was lower than 30 mg/mL for both phases. These data illustrate the results and show that the free concentration is higher, i.e., the partition coefficient is lower, for the interdigitated $L_\beta I$ phase than for the L_α phase.

The partition coefficients measured by this method for *n*-butanol in DPPC for two initial concentrations of *n*-butanol are plotted against temperature in Figure 9A. With an initial concentration of 30 mg/mL *n*-butanol, as discussed above, the lipid is in the $L_\beta I$ phase below 40 °C and in the L_α phase above 40 °C. This graph shows that the partition coefficient is higher for the L_α phase than for the $L_\beta I$ phase. In the L_α phase, the partition coefficient is fairly constant with temperature.

In the presence of 5 mg/mL *n*-butanol, the lipid does not go into the $L_\beta I$ phase at any temperature. Our DSC results show that it exhibits a transition from L_β' to P_β' at 30 °C. Our attempts to measure the partition coefficient of *n*-butanol in the L_β' phase were unsuccessful because there was no detectable change in free concentration in the presence of 5 mg/mL *n*-butanol below 30 °C. This sets the upper limit of the partition coefficient for the L_β' phase at approximately 23, assuming the heat of binding was the same for L_β' as for $L_\beta I$. The readily measurable value of the partition coefficient for the P_β' phase, measured at 35 °C (filled circle and triangle at 35 °C), is within experimental error of the value obtained for the $L_\beta I$ phase at the same temperature. The agreement of the partition coefficients measured at 5 and 30 mg/mL *n*-butanol for the L_α phase is as expected for nonsaturable partitioning.

Table I: Thermodynamic Parameters for the Butanol–DPPC Interaction

phase	temp (°C)	K_p	ΔH (kcal/mol)	ΔG (kcal/mol)	ΔS [cal/(mol·K)]
$L_\beta I$	15	70	0.9	−2.43	11.5
	20	73	1.0	−2.50	12.1
	25	80	1.1	−2.59	12.2
	30	101	1.0	−2.78	12.3
	35	111	1.0	−2.88	12.5
P_β'	35	138	(1.9)[a]	−3.02	(15.9)[a]
L_α[b]	40	169		−3.19	
	45	182	1.5	−3.29	15.0
	50	180	1.2	−3.33	14.1
	55	184	0.9	−3.40	13.1

[a] The enthalpy and entropy for the P_β' phase has a large error due to the overlapping of the pre- and main transition as discussed in text. [b] The data for L_α phase were the average of the results obtained from DPPC containing 30 and 5 mg/mL *n*-butanol.

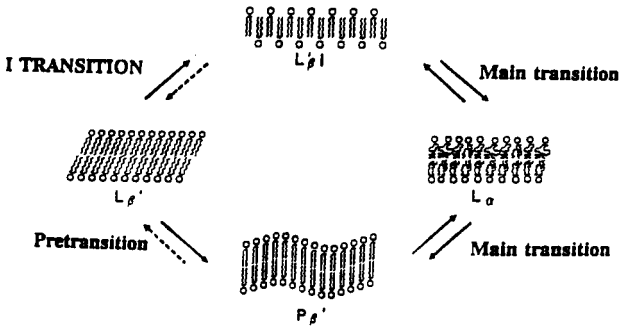

FIGURE 10: Illustration of the phase structures of DPPC, with temperature increasing from left to right. The upper pathway occurs in the presence of sufficient *n*-butanol; the lower pathway occurs in the absence of *n*-butanol or at low *n*-butanol concentration. The dashed arrows represent phase changes which are slow or which exhibit hysteresis on a macroscopic time scale.

The enthalpy measured for the partitioning of *n*-butanol into each of the lipid phases is given in Figure 9B. The enthalpy per mole of *n*-butanol bound in the $L_\beta I$ phase is approximately 1.0 kcal/mol. In the L_α phase, the enthalpy is considerably temperature dependent, with the heat capacity change ΔC_p about −58.0 cal/(mol·K); however, it is somewhat higher than that of the $L_\beta I$ phase. The enthalpy measured for the P_β' phase (filled circle and triangle at 35 °C) is higher than that for either of the other phases; however, this is probably not an accurate measurement of *n*-butanol binding alone. Because of the narrow region of existence of the P_β' phase, i.e., the overlap of the pretransition and main transitions, the measured enthalpy probably contains contributions from a shift of the phase equilibria in addition to the *n*-butanol binding.

The free energy of *n*-butanol bound to DPPC is given by the relation $\Delta G = -RT \ln (K_p)$, where K_p is the partition coefficient expressed in unitary (mole fraction) units. The entropy ΔS can be obtained from $\Delta G = \Delta H - T\Delta S$. The partition coefficients K_p, enthalpies ΔH, free energies ΔG, and entropies ΔS of *n*-butanol bound to different phases of DPPC at various temperature are listed in Table I.

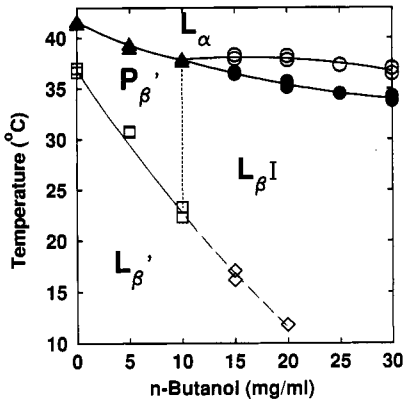

FIGURE 11: Transition temperatures of DPPC as a function of *n*-butanol concentration. Triangles represent the P_β' to L_α transition; circles represent the $L_\beta I$ to L_α transition; squares represent the L_β' to P_β' transition; diamonds represent the L_β' to $L_\beta I$ transition. Open symbols represent heating scans; filled symbols represent cooling scans.

DISCUSSION

Differential Scanning Calorimetry. Figure 10 shows a cartoon of the phase structures of DPPC as a function of temperature and *n*-butanol concentration. The upper pathway including the $L_\beta I$ phase occurs in the presence of alcohol above the threshold concentration, and the lower pathway occurs in the absence of alcohol as the temperature increases. Figure 11 shows the temperature and *n*-butanol concentration dependence of these phase distributions. The threshold concentration of *n*-butanol was found in this study to be approximately 10 mg/mL butanol at the lipid concentration of 5 mg/mL used in the DSC study. This was based on the shape and irreversibility of the low-temperature transition, as well as the onset of hysteresis in the main transition (Rowe, 1985a). This is in good agreement with our previous study of the DPPC–*n*-butanol system using optical density and ^{13}C nuclear magnetic resonance (Herold et al., 1987). Within the precision of our determination, the threshold concentration was approximately the same at both the main transition temperature and the temperature of the low-temperature transition. The dotted line in Figure 11 connecting the threshold points at these two temperatures approximates the boundary separating the P_β' and $L_\beta I$ phases; however, the precise location and shape of this line was not determined. The dashed line between the L_β' and $L_\beta I$ regions of the diagram represent the temperatures of the L_β' to $L_\beta I$ transition on heating scans; because the transition was not immediately reversible, the exact location of the true equilibrium line between these phases could not be determined.

The behavior of the temperature of the main transition as a function of *n*-butanol concentration in Figure 11 shows the onset of hysteresis between the heating and cooling scans which is characteristic of the induction of the $L_\beta I$ phase (Rowe, 1985a). However, the T_m for heating continues to decrease with *n*-butanol concentration above the threshold concentration, consistent with our observation that the partition coefficient for *n*-butanol is greater for the L_α phase than for the $L_\beta I$ phase. This is in contrast to the observations for the lower alcohols methanol, ethanol, and propanol (Rowe, 1985b), where the T_m for heating scans increased with concentration above the inflection, suggesting that, for the shorter chain alcohols, the partition coefficient is greater for the $L_\beta I$ phase than for the L_α phase.

The enthalpy of the $L_\beta I$ to L_α transition is approximately 1.0 kcal/mol greater than that of the P_β' to L_α transition. This is consistent with the X-ray structure of the two phases, which shows that the acyl chain region of the $L_\beta I$ phase is more compact than either the L_β' or P_β' phases (Simon & McIntosh, 1984). This is qualitatively similar to the results we obtained previously on the DSPC–ethanol system (Rowe & Cutrera, 1990).

The DSC results on the low-temperature transition are qualitatively similar to those reported in the DSPC–enthanol system (Rowe & Cutrera, 1990). At the threshold concentration of alcohol, as it changes to the L_β' to $L_\beta I$ transition, the low-temperature transition becomes broader and is irreversible on immediate rescanning. However, the enthalpy of the L_β' to P_β' transition is very similar to that for the L_β' to $L_\beta I$ transition for the DPPC–*n*-butanol system. This is in contrast to the results on the DSPC–ethanol system in which the enthalpy of the L_β' to $L_\beta I$ transition was approximately half of that for the L_β' to P_β' transition (Rowe & Cutrera, 1990). As discussed in the previous paper, the enthalpy change for the L_β' to $L_\beta I$ transition has contributions both from the acyl chain region of the bilayer, where the $L_\beta I$ phase has greater order than in the L_β' phase, and from the interfacial region, where the L_β' phase has greater order. The balance between these apparently opposite contributions to the enthalpy is probably different in the two lipids; it would be expected that the opposing negative enthalpy contribution from the greater packing in the acyl chain region would be less important in DPPC than in DSPC. The finding that the enthalpy of the L_β' to $L_\beta I$ transition is relatively larger in DPPC than in DSPC is consistent with this point of view. The difference in the alcohol used to induce interdigitation in the two studies may also contribute, particularly if there is a significant contribution to the enthalpy from the binding of the alcohol.

Titration Calorimetry. Titration calorimetry is a relatively new method which has recently become commercially available. The results presented here demonstrate a new application of this methodology to measure the partitioning of a solute between the aqueous phase and a phospholipid in suspension. The method relies upon a measurement of the free solute concentration in the lipid suspension by detecting the heat of dilution of the solute when the suspension is mixed with a solution of slightly different solute concentration. This method has the advantage over previously used methods that it does not require the separation of the two phases, it does not rely on any particular special properties of the ligand, and it does not involve any foreign probe molecules. It can be used for a variety of ligands with intermediate partition coefficients; the partition coefficient must be in a range in which there is a measurable change in the free concentration when it binds to the lipid under achievable conditions, and the ligand must have a significant heat of dilution. Using this method, we have measured the partition coefficients for *n*-butanol into DPPC for each of four lipid phases, L_β', P_β', $L_\beta I$, and L_α.

The partition coefficient values obtained from the titration calorimetry method for the liquid-crystalline L_α phase are in good general agreement with the literature values measured on lipids in their liquid-crystalline phases (Katz & Diamond, 1974; Roth & Seeman, 1972; Kamaya et al., 1981; Rowe et al., 1987). The values of the partition coefficient for the L_α phase obtained in this study are larger than that determined by T_m depression (Rowe et al., 1987). This is to be expected because the T_m depression calculation assumes that there is negligible binding in the phase below the transition. In the present study, we obtained a value of 138 for the P_β' phase and 180 for the L_α phase. This is consistent with our value obtained from the T_m depression of 63 (Rowe et al., 1987), because the T_m depression result represents the difference

between the partition coefficients for each of the two phases.

The partition coefficient for *n*-butanol in the interdigitated phase ranged from 70 to nearly 100, depending slightly on the temperature. It was lower than that for the L_α phase. This is consistent with the finding that the transition temperature for the $L_\beta I$ to L_α transition decreases with increasing *n*-butanol concentration. This is in contrast to the effect of ethanol on this transition temperature; for ethanol, the $L_\beta I$ to L_α transition temperature for heating scans increases with alcohol concentration (Rowe, 1985a; Nambi et al., 1988; Rowe & Cutrera, 1990), indicating preferential interactions of ethanol with the $L_\beta I$ phase relative to the L_α phase. This difference between ethanol and *n*-butanol is probably due to the greater partitioning of *n*-butanol into the L_α phase, due to its greater hydrophobicity. The published membrane:buffer partition coefficients of alcohols, all of which pertain to the liquid-crystalline phases of lipids, increase with the alcohol chain length (Seeman, 1972; Katz & Diamond, 1974; Kamaya et al., 1981). The larger alcohol chain length would not be expected to have as much effect on the alcohol interactions with the gel phases P_β' or $L_\beta I$ as it would on the interactions with the liquid-crystalline phase.

The partition coefficients for *n*-butanol into the P_β' and $L_\beta I$ phases were similar within the error of measurement. The precision of the measurement for the P_β' phase is considerably lower than that for the $L_\beta I$ phase because the P_β' phase only exists at low concentrations of *n*-butanol, thereby giving relatively small heat signals upon binding. Because the $L_\beta I$ phase apparently replaces the P_β' phase in the thermotropic phase sequence, it would be expected that *n*-butanol would interact more strongly with the $L_\beta I$ phase than with the P_β' phase. Our data suggest that such a difference must be relatively small.

The partitioning of *n*-butanol into the L_β' phase was not detectable in our measurements; there was no detectable change in the free concentration in the absence and presence of lipid. This is consistent with our previous carbon-13 measurements of *n*-butanol interactions with DPPC (Rowe et al., 1987). However, again this measurement is difficult because it must be carried out at *n*-butanol concentrations below the interdigitation threshold concentration. We have estimated the lower limits of a partition coefficient which could be measured for this phase of 23 if it has an enthalpy of binding similar to that for the $L_\beta I$ phase.

The thermodynamic parameters summarized in Table I indicate that the butanol–DPPC interactions are entropy-driven, which is characteristic of hydrophobic interactions (Tanford, 1980). From the relations $\Delta G = -RT \ln (K_p)$ and $\Delta G = \Delta H - T\Delta S$, we can calculate the van't Hoff enthalpy of *n*-butanol bound to DPPC from the slope of a plot of $\Delta G/T$ vs $1/T$. This calculation gave the van't Hoff enthalpy of 4.3 kcal/mol for *n*-butanol bound to the $L_\beta I$ phase of DPPC and 1.3 kcal/mol for *n*-butanol bound to the L_α phase of DPPC. The agreement of van't Hoff enthalpy and calorimetric enthalpy for DPPC in the L_α phase suggests that the butanol–DPPC interaction with lipid in the L_α phase is a simple binding interaction. However, the finding that the van't Hoff enthalpy is considerably greater than the calorimetric enthapy for DPPC in the $L_\beta I$ phase suggests that the butanol–DPPC interaction with lipid in the $L_\beta I$ phase has considerable cooperativity.

Registry No. DPPC, 63-89-8; *n*-butanol, 71-36-3.

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