Phase Transition Properties of 1,2- and 1,3-Diacylphosphatidylethanolamines with Modified Head Groups†

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ABSTRACT: The phase transition properties of dilute aqueous suspensions of "nonhydrated" (i.e., lipid suspensions which had not been heated above room temperature or above the main phase transition temperature of the fully hydrated lipid, whichever was lower) and hydrated 1,2(α)- and 1,3(β)-dipalmitoylphosphatidylethanolamines with modified head groups have been determined by high-sensitivity differential scanning calorimetry at a scan rate of 0.1 K min^{-1}. In both the 1,2 and 1,3 series, the head-group modifications of the phosphoethanolamine moiety included *N*-methyl, *N*,*N*-dimethyl, and *N*,*N*,*N*-trimethyl (phosphocholine). In the 1,2 series, additional modifications were dinitrophenyl, trinitrophenyl, *N*-(dinitrophenyl)aminocaproyl, *N*-(trinitrophenyl)aminocaproyl, and *N*-4-nitro-2,1,3-benzoxadiazole. Also included in this study were 1,2-dihexadecylphosphatidylethanolamine and the corresponding *N*-methyl-substituted lipid. In general, increasing bulkiness of the head-group substituent caused increasing lowering of the transition temperature, the most extreme cases among the hydrated lipids being the 45 °C lowering produced by the *N*-(dinitrophenyl)aminocaproyl substitution and its trinitrophenyl analogue in the 1,2 series. No simple trend is evident in the changes produced in the calorimetric enthalpy of transitions.

The physical characteristics, including thermodynamic properties, of the phase transitions of saturated, unsaturated, and branched-chain synthetic 1,2-diacylphospholipids as well as naturally occurring phospholipids with different head groups have been extensively examined (Mabrey & Sturtevant, 1978; Boggs, 1980; McElhaney, 1982) by using a variety of physical and chemical techniques (Levine, 1972; Andersen, 1978; Fein et al., 1984). This attention is due, in part, to the importance of lipid molecules in the structural and functional properties of cell membranes (Quinn, 1981) and enzymatic processess (Bonsen et al., 1972; Dennis, 1983). In this context, diacylphosphatidylethanolamines (diacyl-PE's)[1] are of considerable interest because of their ubiquitous presence in both procaryotic and eucaryotic organisms, not to mention their complex physical properties (Cullis & De Kruijff, 1978). However, 1,3- and 1,2-diacyl-PE's and their derivatives have been less extensively examined than other classes of phospholipids such as 1,2-diacyl-PC's. Even though 1,3-diacyl-PE's do not, to our knowledge, occur naturally in biomembranes, comparative physical investigations of such compounds and 1,2-diacyl-PE's with modified head groups are of interest for the insight they may give into the physical behavior and biological significance of both synthetic and naturally occurring phospholipids (Figure 1).

Experimental Procedures

Chemicals. All lipids were purchased from Calbiochem-Behring (La Jolla, CA) except for 1,2-DNPDPPE, 1,2-TNPDPPE, 1,2-DNPACDPPE, 1,2-TNPACDPPE, and 1,2-NBDDPPE which were supplied by Avanti Polar Lipids (Birmingham, AL). Spectroscopic-grade "Photrex" organic reagents were employed, and doubly deionized water was used throughout. Phospholipids were tested for purity by analytical TLC (Uniplate silica gel HL, 10 × 20 cm and 250 μm thick; Analtech, Newark, NJ) using as eluents (a) $CHCl_3$/MeOH/HOAc/H_2O (85:15:10:3 v/v), (b) $CHCl_3$/MeOH/H_2O (130:50:10), (c) $CHCl_3$/MeOH/7 N NH_3 (230:90:15), (d) $CHCl_3$/MeOH/H_2O (70:30:5), and (e) $CHCl_3$/CH_3OH/NH_4OH (10:5:1). Eluent b gave the best chromatographic resolution of 1,2 and 1,3 homologous series of PE's. 1,2-DNPDPPE and related lipids gave a single spot in solvent

Table I: R_f Values of Phospholipids before Purification

phospholipid	R_f values
1,2-NMeDPPE[b]	0.25, 0.48,[a] 0.6, 0.72
1,3-NMeDPPE	0.06, 0.23, 0.48, 0.6,[a] 0.7, 0.72
1,2-N,NMe_2DPPE	0.1, 0.2, 0.4,[a] 0.55
1,3-N,NMe_2DPPE	0.06, 0.15, 0.22, 0.26, 0.34, 0.36, 0.46,[a] 0.6, 0.76
1,2-NMeDHPE ether	0.2, 0.5,[a] 0.6, 0.65
1,2-DHPE ether	0.15, 0.44,[a] 0.55
1,2-NBDDPPE	0.15, 0.28, 0.55,[a] 0.85, 0.9
1,2-DNPDPPE[c]	0.53[a]
1,2-TNPDPPE	0.5[a]
1,2-DNPACDPPE	0.62[a]
1,2-TNPACDPPE	0.59[a]

[a] R_f values of lipid; other R_f values are those of impurities. [b] TLC using eluent b. [c] TLC using eluent d.

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[1] Abbreviations: DSC, differential scanning calorimetry; T_m, temperature of maximal excess apparent specific heat (C_{max}); $\Delta T_{1/2}$, transition width in degrees centigrade at $^1/_2C_{max}$; ΔH_{cal}, calorimetric enthalpy; ΔH_{vH}, van't Hoff enthalpy; ΔH_{cal}^T, total calorimetric enthalpy evaluated by integration of the curve of C vs. T (excess apparent specific heat vs. temperature); N, number of molecules in a cooperative unit ($N = \Delta H_{vH}/\Delta H_{cal}$); TLC, thin-layer chromatography; T_{NH}, temperature at C_{max} of "nonhydrated" lipid; PE, phosphatidylethanolamine; PC, phosphatidylcholine; 1,3-NMeDPPE, 1,3-dipalmitoyl-*sn*-glycero-2-phospho-*N*-methylethanolamine. Other head groups indicated as follows: N,NMe_2, *N*,*N*-dimethyl; DNP, dinitrophenyl; TNP, trinitrophenyl; DNPAC, *N*-(dinitrophenyl)aminocaproyl; TNPAC, *N*-(trinitrophenyl)aminocaproyl; NBD, *N*-4-nitro-2,1,3-benzoxadiazole; 1,2-DHPE, 1,2-dihexadecyl-*sn*-glycero-3-phosphoethanolamine.

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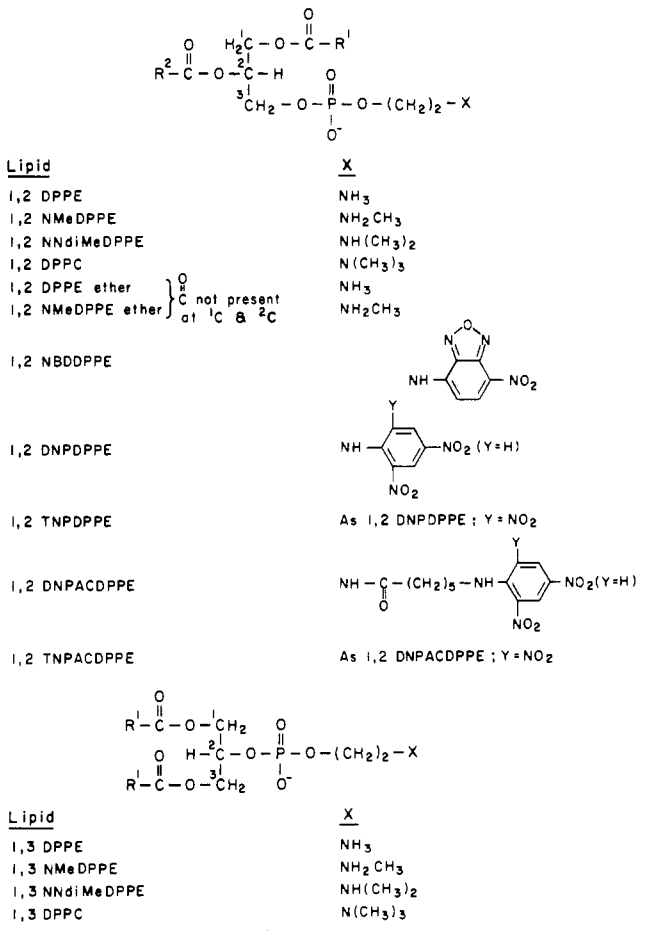

FIGURE 1: Formulas of the phospholipids studied. In all cases, $R_1 = R_2 = -(CH_2)_{14}CH_3$.

d, and their calorimetric properties did not change, within experimental error, before and after preparative TLC. 1,2-DNPDPPE and related lipids were also found to be of high purity by spectrophotometric assay (Six et al., 1973). All other lipids were found to contain impurities (Table I) and were purified by preparative TLC (20 × 20 cm plates; 500 μm thick) using eluent b except for 1,2-NBDDPPE which was purified with eluent c. Phospholipid bands were visualized by briefly placing TLC plates, which had been covered with saran wrap (except for 2 cm on both sides of the plates), in iodine vapor. The part of the desired lipid band not stained with iodine was collected, and 2.0 mL of 1.0% NaCl, 10 mL of MeOH, and 10 mL of $CHCl_3$ were added in consecutive steps; the sample was gently shaken for 2 min, warmed to 50 °C, and centrifuged, and the supernatant was collected. This elution process was repeated with half quantities of NaCl, MeOH, and $CHCl_3$. The combined supernatants were added to a separatory funnel containing 12.0 mL^{-1} of 0.1% NaCl, shaken for 1 min, and left to stand at room temperature for 4 h, and the nonaqueous (lower) fraction was collected. This fraction was reduced in volume to 1 mL^{-1} under a stream of nitrogen and further purified several times by recrystallization using chloroform/acetone (1:40 v/v). Excess solvents were removed by drying under a stream of nitrogen and then in vacuo for 1 h at 50 °C. TLC plates that had been used to purify lipids were placed in iodine vapor after the required lipid band had been scraped off to ensure that no other phospholipid material had been collected. After purification, the lipids were again checked for impurities by analytical TLC using unpurified material as control at loading concentrations of 800 μg cm^{-1}. No impurities were detected in the purified samples. 1,2-NBDDPPE was purified in the same manner except that TLC plates were not exposed to iodine (the inherent yellow color of the compound being sufficient for detection) and excess solvents were removed by drying under a stream of nitrogen and then in vacuo for 3 h at room temperature.

Preparation of Lipid Samples. Except for 1,2-DNPACDPPE and 1,2-TNPACDPPE, "nonhydrated" lipids, that is, aqueous suspensions which had not been heated above room temperature (~22 °C), were prepared for calorimetry by the addition of the appropriate volume of H_2O to crystalline lipid to give lipid concentrations in the range 1.0–5.0 mg mL^{-1}. Samples were vortexed for 1 min, placed in an acetone/dry ice mixture until frozen, thawed, and vortexed, for 5 min. This procedure was repeated twice. To ensure high homogeneity, the samples were passed several times through a 24-gauge hypodermic needle (sample preparation method 1). Nonhydrated suspensions of 1,2-DNPACDPPE and 1,2-TNPACDPPE were prepared by incubating the crystalline lipids under nitrogen in a sealed vial at 0 °C for 12 h followed by addition of the required amount of H_2O, at 0 °C, and vortexing intermittently to give a total vortexing time of 5 min. Care was taken to ensure that the temperature of the lipid suspensions remained below 2 °C and to protect the samples from light and oxygen (these latter precautions were also taken with 1,2-DNPDPPE, 1,2-TNPDPPE, and 1,2-NBDDPPE). All operations prior to loading the calorimeter were conducted in a cold room, and the samples were kept in ice between preparative operations. The time taken for preparation of nonhydrated suspensions did not exceed 15 min, after which the samples were immediately examined by calorimetry. Fully hydrated lipid samples were prepared either by following sample preparation method 1 and then heating and cooling the nonhydrated samples 3 times in the calorimeter, the heating being to a temperature 10 °C above the temperature of the single transition observed with nonhydrated samples (sample preparation method 2), or by performing the heating and cooling outside the calorimeter with vortexing for 20 s after each heating (sample preparation method 3).

Differential Scanning Calorimetry. Experiments were performed with a DASM-1M (Mashpriborintorg, 121200 Moscow, U.S.S.R.) differential scanning microcalorimeter (Privalov, 1980) using scan rates of 0.02–1.0 K min^{-1}. Unless otherwise stated, the scan rate in any particular experiment was 0.1 K min^{-1}. Instrumental base lines obtained by scanning doubly deionized water in both sample and reference cells were horizontal in the range 1.5–80 °C. Initial and final base lines for all curves of excess apparent specific heat vs. temperature (C vs. T) for purified phospholipids were also horizontal. The noise level of the instrument, in the experiments reported here, was in the range 0.015–0.03 cal K^{-1} g^{-1}. Nonhydrated lipid suspensions were always loaded into the calorimeter when the temperature of the calorimetric cells and of the lipid suspensions was 0.5 °C. All samples were rescanned at least twice to check on reproducibility and reversibility of transitions, as well as possible phospholipid degradation. After calorimetric examination, lipid suspensions were extracted (Bligh & Dyer, 1959) and examined by analytical TLC. There was no evidence of lipid degradation.

DSC Data Analysis. The calorimetric parameters T_m, $\Delta T_{1/2}$, C_{max}, ΔH_{cal}, ΔH_{vH}, ΔH^T_{cal}, and N were evaluated as previously described (Velicelebi & Sturtevant, 1979; Chen et al., 1980).

Results and Discussion

The calorimetric properties of all transitions of both nonhydrated and hydrated lipids were independent of lipid con-

Table II: Phase Transition Parameters for Synthetic Phospholipids at a Scan Rate of 0.1 K min^{-1}

lipid	T_{NH} (°C) (±0.02)	$\Delta T_{1/2}$ (°C) (±0.02)	ΔH_{cal} (kcal mol^{-1}) (±0.2–0.4)	C_{max} (cal K^{-1} g^{-1}) (±0.5–2.0)
Nonhydrated Lipids				
1,3-DPPE[a]	79.50	0.45	11.4	23.0
1,3-NMeDPPE	64.65	1.40	8.8	7.4
1,3-N,NMe$_2$DPPE	42.65	0.90	26.8	38.5
1,3-DPPC	24.20			4.0
	28.50		7.17[d]	1.0
	36.9	0.15	8.4	57.0
1,2-DPPE[b]	67.30	0.44	12.7	32.0
1,2-NMeDPPE	57.75	0.50	23.2	41.7
1,2-N,NMe$_2$DPPE	47.95	0.45	17.9	38.8
1,2-DPPC	34.30			3.9
	35.10		2.41[d]	1.1
	41.54	0.13	7.9	62.0
1,2-DHPE ether	68.30	0.70	17.6	23.0
1,2-NMeDHPE ether	62.65	0.75	18.3	23.0
1,2-DNPDPPE	35.07	0.14	13.7	61.4
1,2-TNPDPPE	33.96	0.14	13.4	59.8
1,2-DNPACDPPE[c]	19.50	0.35	16.0	4.2
1,2-TNPACDPPE[c]	19.80	0.34	15.5	3.9
1,2-NBDDPPE	35.12	0.63	12.8	18.8

lipid	T_m (°C) (±0.02)	$\Delta T_{1/2}$ (°C) (±0.02)	ΔH_{cal} (kcal mol^{-1}) (±0.2–0.4)	C_{max} (cal K^{-1} g^{-1}) (±0.5–2.0)	ΔH_{vH} (kcal mol^{-1}) (±45)	N
Hydrated Lipids						
1,3-DPPE[a]	42.80	0.90	4.50	6.1	765	170
	53.10	0.24	9.30	33.2	3060	330
1,3-NMeDPPE	42.60	0.90	4.90	8.5	764	160
	49.80	0.19	7.90	52.0	3790	480
1,3-N,NMe$_2$DPPE	42.55	0.48	25.30	49.5	1430	57
1,3-DPPC	25.00	1.8	4.53	2.3	613	130
	36.80	0.12	7.90	65.0	5520	700
1,2-DPPE[b]	63.40	0.31	3.12	11.1	2600	830
	63.66	0.22	1.76	10.4	3600	2040
	63.80	0.13	5.20	50.8	6100	1170
1,2-NMeDPPE	57.65	0.30	10.90	35.3	2520	230
1,2-N,NMe$_2$DPPE	47.90	0.26	11.10	32.8	2730	250
1,2-DPPC	34.80	0.90	1.35	1.9	727	540
	41.50	0.11	7.60	74.0	6210	820
1,2-DHPE ether	68.05	0.30	11.80	36.1	2680	230
1,2-NMeDHPE ether	61.65	0.35	15.28	37.4	2210	150
1,2-DNPDPPE	35.17	0.13	12.90	47.5	5050	390
1,2-TNPDPPE	33.99	0.13	13.30	48.2	5010	380
1,2-DNPACDPPE[c]	5.85	0.38	13.60	22.4	1410	100
	18.90	0.75	1.10	1.3	785	710
1,2-TNPACDPPE[c]	2.57	0.37	13.90	21.0	1420	100
	18.90	0.76	1.20	1.1	774	640
1,2-NBDDPPE	34.80	0.15	7.11	29.0	4360	610

[a] Chowdhry et al. (1984b). [b] Chowdhry et al. (1984c). [c] Samples scanned at 0.5 K min^{-1}. [d] These values of ΔH_{cal} represent enthalpy values for transitions with fine structure (Chowdhry et al., 1984a).

centration in the range 1–5 mg mL^{-1}.

Nonhydrated Lipids. All nonhydrated lipid suspensions (except 1,2-DNPACDPPE, 1,2-TNPACDPPE, 1,2-DPPC, and 1,3-DPPC) prepared by method 1 show a single transition in the temperature range 1.5–90 °C (Table II). In all these cases, the transition is asymmetrically broadened toward lower temperatures. 1,3-NMeDPPE differs from the other lipids in exhibiting a nonhydrated transition which is less asymmetric and has a lower value of C_{max} and a higher value of $\Delta T_{1/2}$ (Figure 2ii, Table II). Except for 1,2- and 1,3-DPPC phospholipid suspensions prepared by method 1 and scanned to 2 °C below the nonhydrated transition, cooled, and rescanned did not show any phase transitions between 0 °C and T_{NH}. In addition, suspensions prepared by method A when heated and cooled between temperatures 10 °C above and below T_{NH} either one time or several times did not exhibit the T_{NH} transition except for 1,2-DNPACDPPE and 1,2-TNPACDPPE. The T_{NH} transition was also not observed after incubation of fully hydrated suspensions for 12–96 h at temperatures between 0 and 4 °C or at 22 °C. This is in contrast to the metastable behavior of 1,2-diacyl-PE's for which complex transition behavior is observed after incubation of fully hydrated samples at different temperatures below the gel to liquid-crystalline transition for varying periods of time depending on the chain length of the 1,2-diacyl-PE (Mantsch et al., 1983; B. Z. Chowdhry et al., unpublished observations). Results from Fourier-transform infrared spectroscopy have been used as evidence that for suspensions of 1,2-diacyl-PE's (Mantsch et al., 1983) and 1,2-NMeDPPE as well as 1,2-N,NMe$_2$DPPE NMe$_2$DPPE (Casal & Mantsch, 1983) prepared by method 1 the T_{NH} transition represents concomitant hydration and acyl chain melting of the poorly hydrated polycrystalline lipids. Although, by analogy, this is probably the case for the T_{NH} transitions of all the lipids examined in this report, no unequivocal conclusion can be drawn on the basis of DSC studies alone.

In the homologous series 1,2-DPPE, 1,2-NMeDPPE, 1,2-N,NMe$_2$DPPE, and 1,2-DPPC as well as the analogous 1,3 series, the parameter $T_{NH} - T_m$ decreases with increasing methylation (Figure 3A; Table II), the degree of change being more pronounced for the 1,3 series than for the 1,2 series. In the case of 1,3-DPPE and 1,3-N,NMe$_2$DPPE and the DPPC's

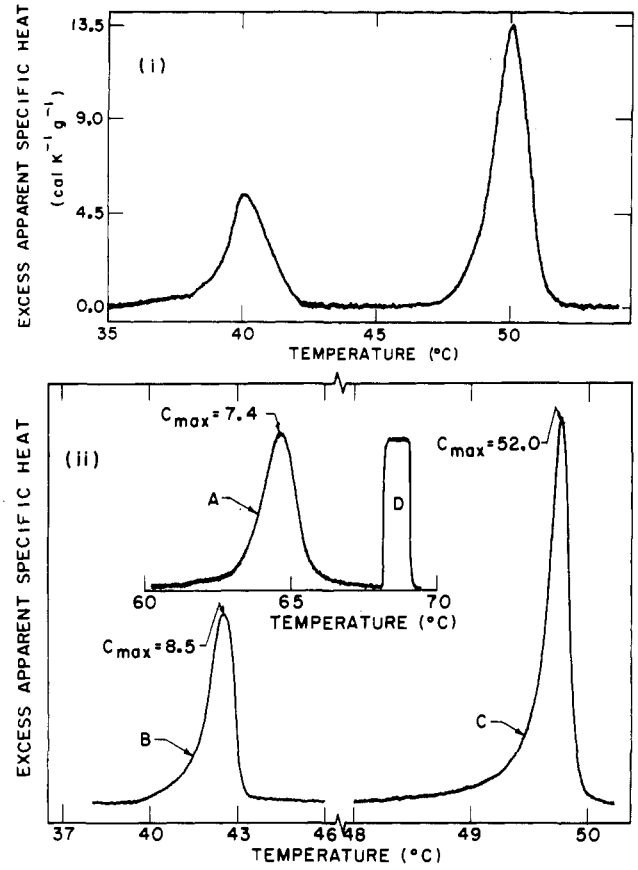

FIGURE 2: Calorimetric scans for 1,3-NMeDPPE. (i) Impure phospholipid prepared by method 3; (iiA) pure phospholipid prepared by method 1 (nonhydrated); (iiB and iiC) lower and higher temperature transitions, respectively, of fully hydrated samples of pure phospholipid prepared by methods 2 or 3; (iiD) an electrical calibration mark. In this figure (and all subsequent figures), the lipid concentration was 1 mg mL^{-1}, and unless otherwise stated, a scan rate of 0.1 K min^{-1} was used. In (ii), the units of C_{max} are calories per degree kelvin per gram. In this and all subsequent figures, the scans have been copied directly from the recorder tracings.

of both series, however, the nonhydrated transition occurs very close to the transition of the fully hydrated sample. This, when viewed in light of results obtained on the first scan of nonhydrated 1,2- and 1,3-DPPC, indicates that the parameter $T_{NH} - T_m$ is a function not only of the position of the head group along the glycerol backbone but also of the nature and number of substituents on the head group.

Nonhydrated samples of 1,2-DNPDPPE (Figure 4), 1,2-TNPDPPE, 1,2-DNPACDPPE (Figure 5), 1,2-TNPACDPPE, and 1,2-NBDDPPE do not show any transitions below T_{NH} on the first scan. The T_{NH} transitions of 1,2-DNPDPPE, 1,2-TNPDPPE, and 1,2-NBDDPPE are again asymmetric, being broader on the low-temperature side. The T_{NH} transition for 1,2-TNPDPPE occurs at a temperature 1.21 °C lower than that of 1,2-DNPDPPE. In both these cases, the T_{NH} transitions occur at a temperature similar to that of their respective gel to liquid-crystalline transition temperatures (Table II) in fully hydrated samples. Nonhydrated samples of 1,2-DNPACDPPE and 1,2-TNPACDPPE show more complex behavior than that of the other phospholipids examined although the behavior of the two lipids is very similar. Nonhydrated samples of these two lipids do not show any transitions at temperatures below 10 °C on the first scan although T_{NH} transitions are observed above this temperature. The T_{NH} transitions of these lipids at a scan rate of 0.5 K min^{-1} are not simple asymmetric curves, there being a shoulder on the higher temperature side, and the values of $\Delta T_{1/2}$ and C_{max} on the first scan of nonhydrated suspensions are higher and lower, respectively, than those for the comparable scans of 1,2-DNPDPPE and 1,2-TNPDPPE. With each subsequent scan, the main T_{NH} transition temperature decreases, as does $\Delta T_{1/2}$, while C_{max} increases; the shoulder on the high-temperature side (Figure 5i) becomes less prominent, and its temperature relative to the "main" T_{NH} transition changes until the third rescan after which it is not observed. At the same time, the transitions at 5.85 °C for 1,2-DNPACDPPE and at 2.57 °C for 1,2-TNPACDPPE, representing the transitions of fully hydrated lipids, become more prominent on each rescan. This behavior is very different from that of the other lipids examined in this study in which, after scanning to 10 °C above T_{NH} and rescanning, no transition is observed at temperatures higher than the transition of the fully hydrated suspensions.

Hydrated Lipids. Lipid preparation methods 2 and 3 gave the same C vs. T curve for each of the lipids examined in this study. The T_m of both the 1,2 and 1,3 series of methyl-substituted PE's decreases with increasing methylation (Figure 3B). The difference in T_m between equivalent members of the two homologous series decreases with increasing methylation (Figure 3C). Furthermore, the difference in T_m between successive members of each homologous series also decreases with increasing methylation (Figure 3D). For fully hydrated samples of both homologous series of lipids, calorimetric scans were fully reproducible after scanning to 1 °C above the gel to liquid-crystalline transition, cooling to 1.5 °C, and rescanning. No fine structure was observed in any of the transitions of the pure hydrated lipids except that of 1,2-DPPE (Chowdhry et al., 1984a,b) at scan rates of 0.02–0.1 K min^{-1}. Apparently, even a single methyl substituent on the head group is sufficient to prevent the formation of the structure(s) presumably responsible for the complex transition seen with 1,2-DPPE.

Mulukutla & Shipley (1984) interpreted their data for the T_m of the gel to liquid-crystalline transition as a function of the number of *N*-methyl groups on the head groups for the 1,2-dimyristoyl and 1,2-dipalmitoyl series in terms of a linear relationship. Taking into account the high purity, as reflected in the calorimetric data, of the samples examined in this report, it appears that for both the 1,2- and 1,3-dipalmitoyl series there are small but significant deviations from a linear dependence of T_m on the number of *N*-methyl groups (Figure 3B). Mulukutla & Shipley (1984) do not give full calorimetric data for each lipid so that direct comparisons between their results and those presented here cannot be made. However, it is to be noted that these workers considered their samples pure by TLC criteria. $\Delta T_{1/2}$ values for the gel to liquid-crystalline transitions obtained by Mulukutla & Shipley (1984) are of the order of 1–2 °C or more, and although in many cases the gel to liquid-crystalline transitions are asymmetric, they are, in contrast to the results presented here, broader on the high-temperature side. Vaughan & Keough (1974) also obtained a linear relationship between T_m and the number of *N*-methyl groups; a similar criticism applies in this case, especially in view of the high $\Delta T_{1/2}$ values and scan rates (10 K min^{-1}) used. Recently, the phase transition properties of 1,2-NMeDPPE and 1,2-N,NMe_2DPPE have been studied by DSC at scan rates of 5 K min^{-1} (Mio et al., 1984). These workers obtained T_m values of 59.1 and 48.6 °C, respectively, for these two lipids. Values for $\Delta T_{1/2}$ of about 1.4 °C and for C_{max} of 6 cal K^{-1} g^{-1} have been estimated for 1,2-NMeDPPE from the data given by Mio et al. (1984). These

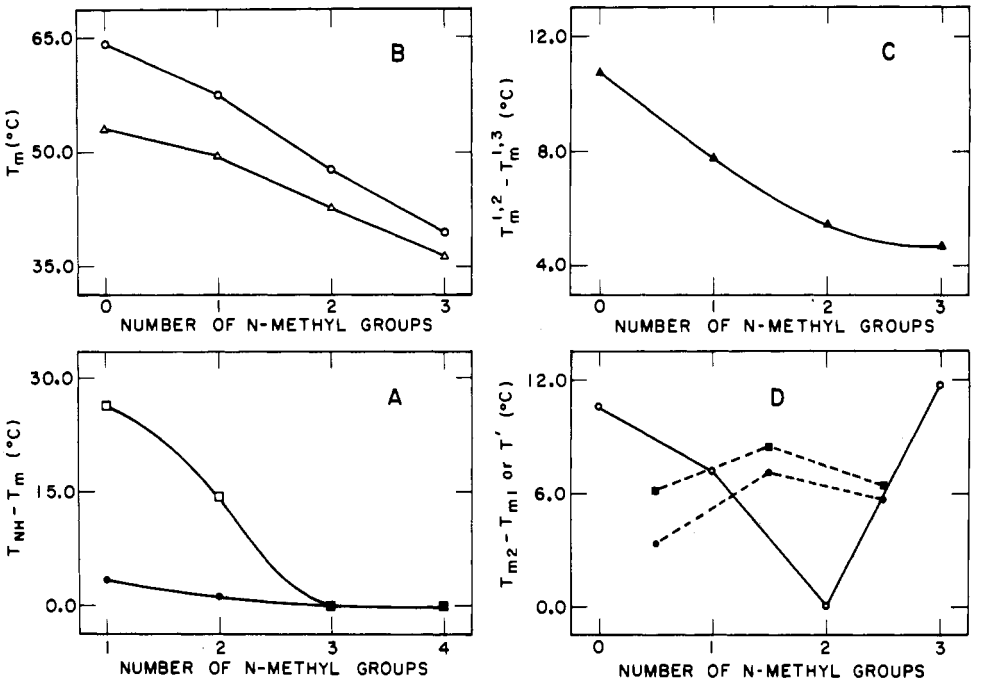

FIGURE 3: Plots of (A) $T_{NH} - T_m$ vs. number of N-methyl groups [(□) 1,3 series; (●) 1,2 series], (B) T_m vs. number of N-methyl groups [(○) 1,3 series; (△) 1,2 series], (C) $T_m^{1,2} - T_m^{1,3}$ of the gel to liquid-crystalline transition vs. number of N-methyl groups, and (D) $T_{m_2} - T_{m_1}$ vs. number of N-methyl groups for the 1,3 series (○) where T_{m_2} is the T_m of the gel to liquid-crystalline transition and T_{m_1} is the T_m of the lower transition ($T_{m_2} - T_{m_1}$ for 1,3-N,NMe$_2$DPPE is assumed to be zero). Also in (D) is the plot of T' (difference in T_m of the gel to liquid-crystalline transition between subsequent members of each homologous series) vs. number of N-methyl groups for the 1,3 series (●) and the 1,2 series (■).

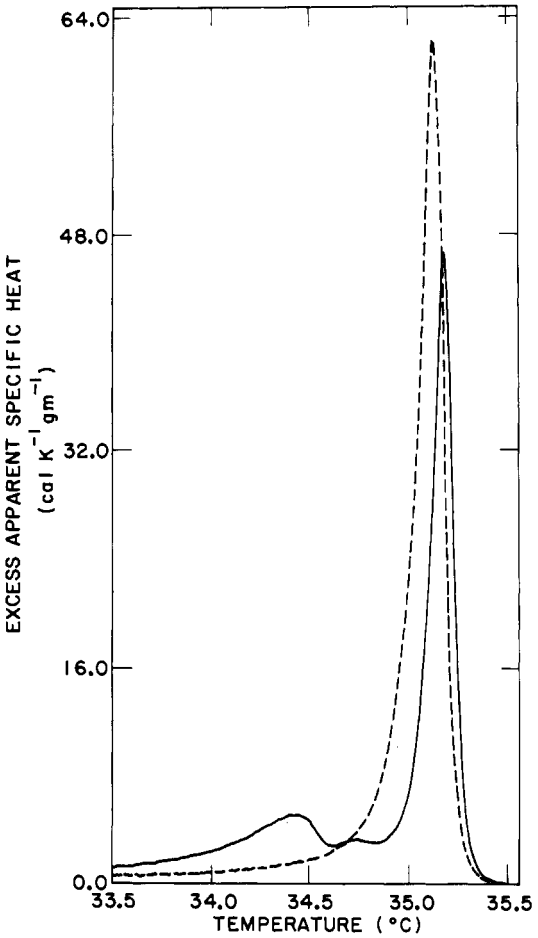

FIGURE 4: Calorimetric scans of 1,2-DNPDPPE: (---) scan of nonhydrated lipid prepared by method 1; (—) scan of fully hydrated lipid prepared by method 2 or 3. 1,2-TNPDPPE gives similar transition profiles but in a slightly lower temperature range (see Table II).

values for $\Delta T_{1/2}$ and C_{max} are much higher and lower, respectively, than those obtained in the present study (Table II). Moreover, some of their conclusions concerning the effect of 1,2-NMeDPPE on the fluidity of 1,2-DPPE bilayers were based upon the differences in T_m between 1,2-NMeDPPE and 1,2-DPPE. This differences amounted to 3.9 °C whereas the value obtained in the present study is 5.75 °C. It should be emphasized that it cannot be claimed that the values for $\Delta T_{1/2}$ and C_{max} obtained in this report are due merely to the low scan rates employed. The values of these parameters are dependent on the purity of the lipid samples and instrumental characteristics as well as on the scan rates employed.

The phase transition parameters of impure and purified lipids are very different (see, for example, Figures 2i,ii, 6, and 8i,ii). The change in purity is reflected in T_m, $\Delta T_{1/2}$, C_{max}, ΔH_{cal}, and the difference in excess apparent specific heat between the gel phase and the liquid-crystalline phase (the chemical base line of the gel to liquid-crystalline transitions). The reason for the lower $\Delta T_{1/2}$ of impure 1,2-N,NMe$_2$DPPE at a scan rate of 1.0 K min^{-1} compared to that at 0.1 K min^{-1} (Figure 8i) is not understood, as it would be expected that the reverse situation would hold.

1,3-NMeDPPE shows two phase transitions (Figure 2ii) with C_{max} at 42.6 and 49.8 °C. The 42.6 °C transition is attributed, by analogy to that found in 1,3-DPPE (Chowdhry et al., 1984c), to a "crystalline" phase, probably of low hydration and slow motion in the head-group region. On the basis of its calorimetric properties compared to that of other phospholipids, the 49.8 °C transition is attributed to a gel to liquid-crystalline transition. 1,3-N, NMe$_2$DPPE, on the other hand, shows a single transition in the temperature range 0–90 °C with an unusually large enthalpy, 25.30 kcal mol^{-1}. Comparison with the T_m values for the two transitions exhibited by the other members of this series suggests that this transition of 1,3-N, NMe$_2$DPPE is actually the sum of two transitions which cannot be resolved under the present ex-

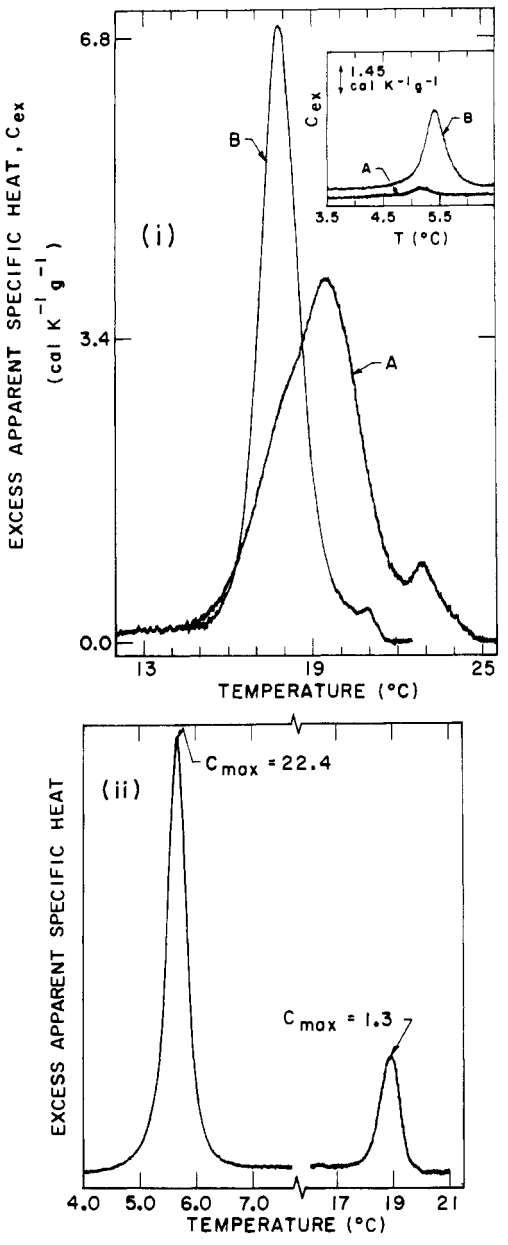

FIGURE 5: Excess apparent specific heat as a function of temperature for (i) nonhydrated and (ii) hydrated samples of 1,2-DNPACDPPE at a scan rate of 0.5 K min^{-1}. In (i), (A) is the first scan of "unhydrated" suspensions, and (B) is the rescan of the same sample after heating to 25 °C and cooling to 1.5 °C. The inset shows the calorimetric profiles obtained in the temperature range 3.5–6.5 °C for scans A and B. Scans of 1,2-TNPACDPPE were similar (see text). In (ii), the units of C_{max} are calories per degree kelvin per gram.

perimental conditions, but even so, the enthalpy is inexplicably large. It may be noted that the single transition of the isomeric 1,2-N, NMe_2DPPE has an enthalpy less than half as large. If it is assumed that $T_{m_2} - T_{m_1}$, the differenct in T_m between the upper and lower transitions of each lipid, is zero in the case of 1,3-NNMe_2DPPE, then the variation of $T_{m_2} - T_{m_1}$ with the number of methyl groups takes the form shown in Figure 3D.

The phase transition properties of 1,3-DPPC are different from those of 1,3-N,NMe_2DPPE in exhibiting two transitions separated by a significant temperature interval (Stümpel et al., 1983; Serralach et al., 1983). It appears that the changes in transition properties of the lipids in going from 1,3-DPPE to 1,3-NMeDPPE are much smaller than those which occur upon addition of subsequent methyl groups.

1,2-NMeDPPE and 1,2-N,NMe_2DPPE both exhibit single asymmetric transitions (Figures 7 and 8)in the temperature

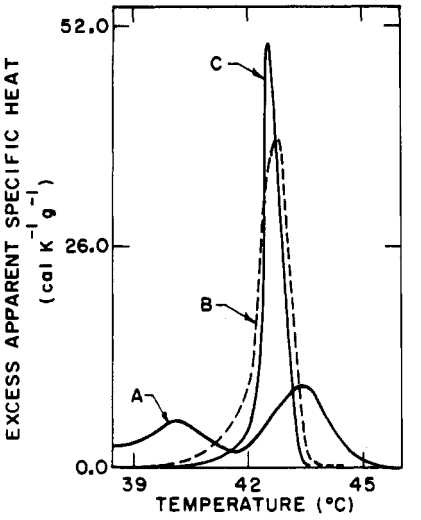

FIGURE 6: Calorimetric scans of (A) impure fully hydrated 1,3-N,NMe_2DPPE, (B) purified samples of nonhydrated lipid, and (C) purified samples of fully hydrated lipid obtained by method 2 or 3.

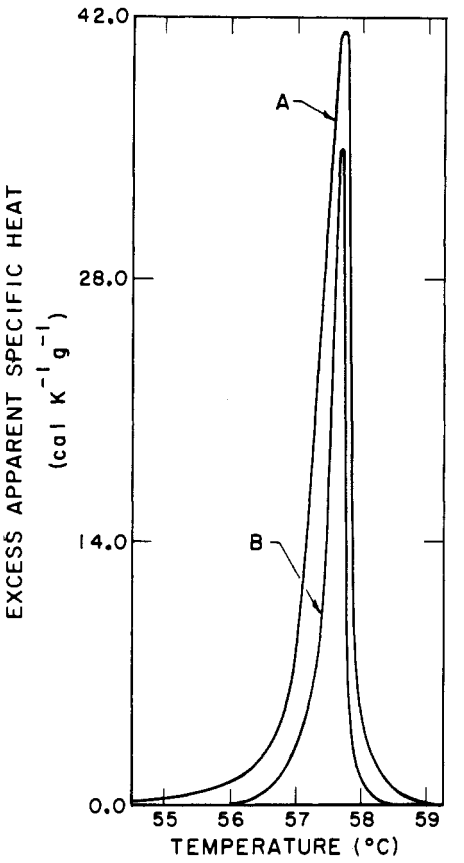

FIGURE 7: Excess apparent specific heat as a function of temperature for 1,2-NMeDPPE. (A) is the scan of nonhydrated and (B) the scan for fully hydrated samples.

range 1–90 °C which are attributed to gel to liquid-crystalline transitions (Casal & Mantsch, 1983). These transitions do not, under the experimental conditions used, show any fine structure or give any indication of metastability in the phases involved. This is in contrast to the first and last members of the series, 1,2-DPPE and 1,2-DPPC. The first of these lipids has thermotropic behavior showing both fine structure and phase metastability (Chowdhry et al., 1984a), and 1,2-DPPC shows additional transitions, apart from the gel to liquid-crystalline transition, one of which, the subtransition, appears only after prolonged incubation at temperatures below 6 °C

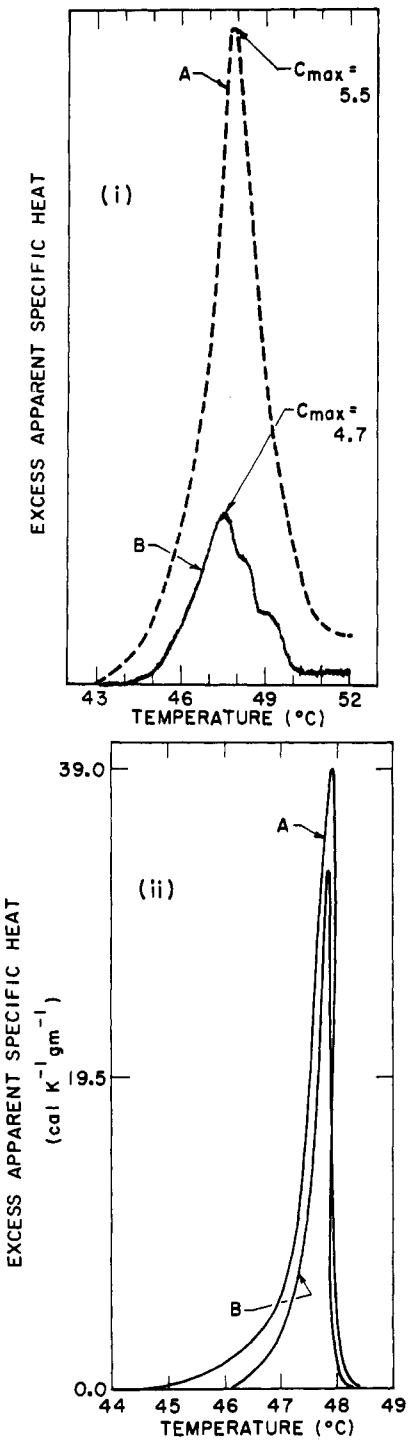

FIGURE 8: Calorimetric scans of 1,2-N,NMe$_2$DPPE. In (i), (A) is a scan of a fully hydrated suspension of impure lipid scanned at 1.0 K min^{-1}, and (B) is the rescan of this material at 0.1 K min^{-1}. The transition profile in (B) was identical with that obtained by using freshly prepared samples and scanning at 0.1 K min^{-1}. (ii) Transition profiles of nonhydrated and hydrated samples of purified lipid.

(Chen et al., 1980) and involves an obviously metastable phase. The fully hydrated forms of the compounds in each homologous series show no changes in phase transition behavior when incubated at temperatures between 6 and 22 °C for periods up to 96 h. Recently, Mulukutla & Shipley (1984), using DSC and X-ray diffraction techniques, reported that following prolonged incubation at −4 °C (7–53 days) the bilayer gel phase of 1,2-NMeDPPE and 1,2-N,NMe$_2$DPPE and of the corresponding dimyristoyl compounds is converted to a low-temperature crystalline phase.

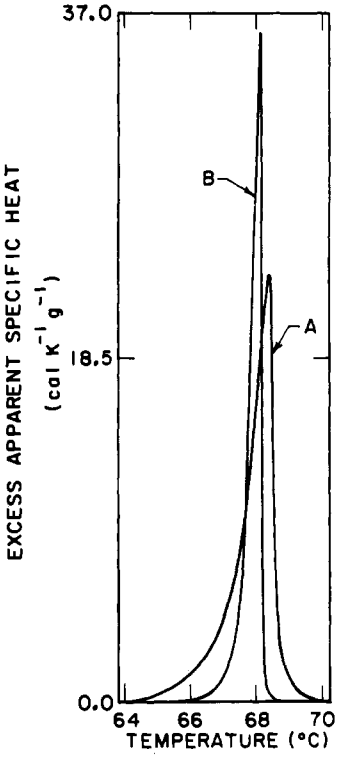

FIGURE 9: Excess apparent specific heat as a function of temperature for 1,2-DPPE ether. (A) Nonhydrated lipid; (B) hydrated lipid.

Vaughan & Keough (1974) found that 1,2-N,NMe$_2$DPPE had a slightly higher ΔH_{cal} than other members of the 1,2 series, while the data of Casal & Mantsch (1983) indicated that all four members of the 1,2 series gave, within experimental error, similar values for ΔH_{cal}. Our results agree with those of Mulukutla & Shipley (1984) in assigning higher enthalpies to the transitions of the partially methylated members of the 1,2 series than to either 1,2-DPPE or 1,2-DPPC.

Recent Fourier-transform infrared (FT-IR) studies (Casal & Mantsch, 1983) indicate that the properties of 1,2-NMeDPPE and 1,2-N,NMe$_2$DPPE are closer to those of 1,2-DPPC than to those of 1,2-DPPE. This is also reflected in the calorimetric data in terms of stability of lipid suspensions and differences in T_m between hydrated and nonhydrated suspensions. However, the opposite appears to be the case for 1,2-NMeDPPE and 1,3-N,NMe$_2$DPPE, the properties of which appear to be closer to those of 1,3-DPPE than 1,3-DPPC.

1,2-DHPE ether (Figure 9) and 1,2-NMeDHPE ether (Figure 10) also show only single asymmetric transitions, and no signs of metastability can be detected upon storage at room temperature or at 4 °C for 1–12 h. The parameter $T_{NN} - T_m$ is similar for both these phospholipids (0.1 and 0.25 °C) but less than that for 1,2-DPPE (3.5 °C). The difference in T_m between 1,2-DHPE ether and 1,2-NMeDHPE ether (6.4 °C) is very similar to the difference between the two ester analogues (6.25 °C), and as expected, the T_m of the monomethylated lipid is lower than that of the nonmethylated lipid. The difference in T_m between 1,2-DHPE ether and 1,2-DPPE is greater than that between 1,2-DHPC ether and 1,2-DPPC by 2.25 °C.

1,2-DNPDPPE and 1,2-TNPDPPE show complex phase transition properties similar to those shown by 1,2-diacyl-PE's under the same experimental conditions (Chowdhry et al., 1984b). In both instances, the transition profiles are similar, differing only in temperature and showing three maxima of

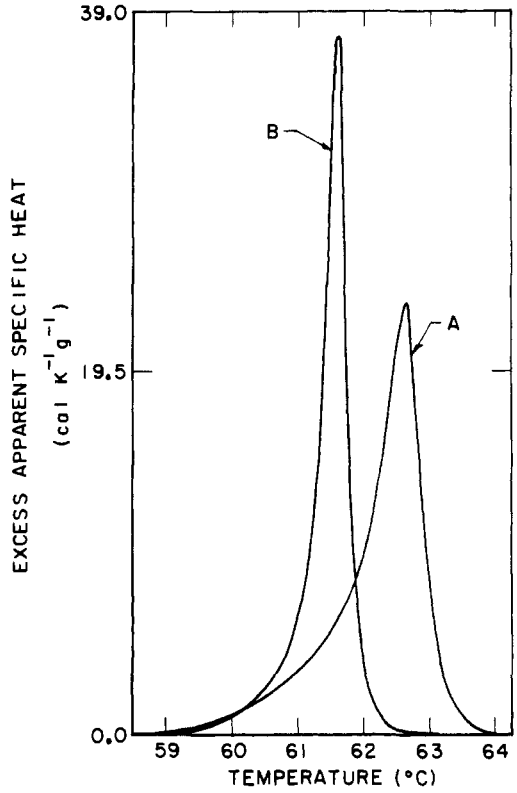

FIGURE 10: Excess apparent specific heat as a function of temperature for 1,2-NMeDPPE ether. (A) Nonhydrated lipid; (B) hydrated lipid.

excess apparent specific heat (Figure 4, Table II). No attempt has been made to resolve the transition profiles into two-state component curves since the fact that the low-temperature sides of the transitions of both 1,2-DNPDPPE and 1,2-TNPDPPE are clearly asymmetric makes it evident that more than three-component transitions would be required. Comparison of the calorimetric properties of the transitions at the highest temperature of these two lipids with those of other 1,2-diacylphospholipids makes it seem likely that they are gel to liquid-crystalline transitions. The fine structure of the transitions is observed at both 0.1 and 0.5 K min^{-1}.

The phase transition behavior of 1,2-DNPACDPPE and 1,2-TNPACDPPE is more complex than that of 1,2-DNPDPPE and 1,2-TNPDPPE in that a small transition is seen with fully hydrated samples at a temperature 13–16 °C above what is presumably the gel to liquid-crystalline transition (Figure 5, Table II). As in the case of the latter lipids, the trinitro compound exhibits a transition at a lower temperature than the dinitro compound, but the difference in T_m's for these lipids is greater than that for 1,2-DNPDPPE and 1,2-TNPDPPE. In contrast to the situation with 1,2-DNPDPPE and 1,2-TNPDPPE, the transitions of 1,2-DNPACDPPE and 1,2-TNPACDPPE do not show any fine structure at a scan rate of 0.5 K min^{-1} (instrumental limitationsprevent scanning at 0.1 K min^{-1} in the interval 0–10 °C), and the symmetry of their transitions suggests that no fine structure would be seen at much lower scan rates.

The transitions of 1,2-DNPACDPPE and 1,2-TNPACDPPE at 18.9 °C are not seen if the samples are cooled from 25 to 15 °C and rescanned either one time or several times. If the samples are cooled to 0.5 °C, both the lower temperature transition and the 18.9 °C transition are again observed with no change in calorimetric properties.

Fully hydrated 1,2-NBDDPPE shows a single asymmetric transition at 34.8 °C (Figure 11, Table II). Since this temperature is close to the T_m for the nonhydrated lipid, to ensure

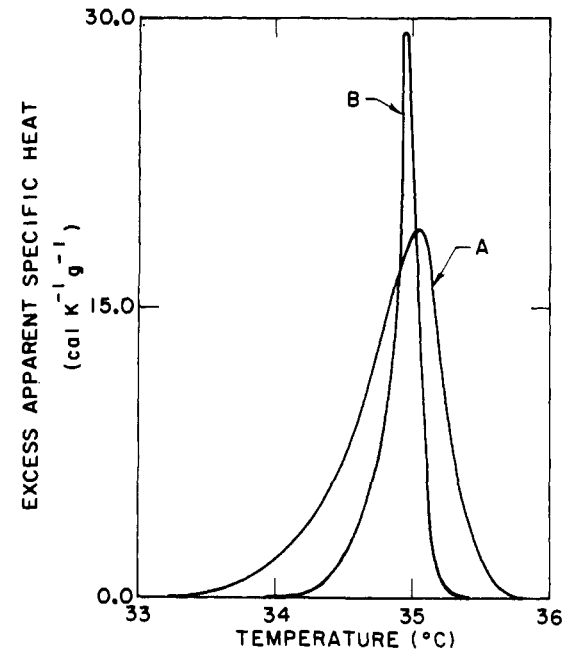

FIGURE 11: Calorimetric scans of purified samples of 1,2-NBDDPPE. (A) Nonhydrated lipid; (B) hydrated lipid.

full hydration the lipid should be heated to at least 45 °C. It is interesting that the T_m of this lipid is close to that of 1,2-DNPDPPE, suggesting that the head groups of the two lipids have approximately equal effective volumes.

1,2-Diacyl-PC's are known to be hygroscopic while the corresponding 1,2-diacyl-PE's are difficult to hydrate (Mantsch et al., 1983). All the lipids examined in this study (except for 1,2-DPPE) were found to be readily hydrated; the lipids containing nitro groups gave yellow (reddish yellow in the case of 1,2-NBDDPPE) suspensions with no visible turbidity, and all the others gave fine-grained opalescent suspensions typical of well-dispersed samples.

No hexagonal phase transition was detected for any of the lipids examined. Such a phase (or a micellar one) cannot be excluded for those lipids such as 1,2-DNPACDPPE which exhibit no visible turbidity and also in view of the phase transitions exhibited by 1,2-DNPACDPPE and 1,2-TNPACDPPE at 18.9 °C. Some members of both the 1,3 and 1,2 series, e.g., 1,3-DPPE and 1,3- and 1,2-NMeDPPE, may also exhibit a hexagonal phase at temperatures above 90 °C since it it is known that under certain conditions 1,2-diacyl-PE's exhibit such transitions at temperatures above 100 °C (Seddon et al., 1983). The instrumental capabilities of the DASM-1M calorimeter employed in the present studies do not allow scanning at temperatures above 100 °C.

In conclusion, we propose that all calorimetric studies should give full calorimetric parameters (C_{max}, T_m, $\Delta T_{1/2}$, and ΔH_{cal}) and be conducted, in the case of pure lipids, at scan rates of 0.1–0.25 K min^{-1} so that strict interlaboratory comparisons can be made.

Registry No. 1,3-NMeDPPE, 96760-47-3; 1,3-N,NMe_2DPPE, 96760-48-4; 1,2-NMeDPPE, 3930-13-0; 1,2-N,NMe_2DPPE, 3922-61-0; 1,2-DHPE ether, 61423-61-8; 1,2-NMeDHPE ether, 96760-49-5; 1,2-DNPDPPE, 67151-59-1; 1,2-TNPDPPE, 87706-96-5; 1,2-DNPACDPPE, 87706-97-6; 1,2-TNPACDPPE, 84804-96-6; 1,2-NBDDPPE, 92605-64-6.

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Dissociation of the Receptor for Immunoglobulin E in Mild Detergents†

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ABSTRACT: We previously showed that, in the absence of phospholipids, exposure of the tetrameric receptor for immunoglobulin E to mild detergents dissociates the intact β chain and two γ chains from the α chains. Having developed a practical method for assaying the dissociation, we have now explored a variety of different detergents, detergent concentrations, temperatures, times, salts, pHs, and other factors that influence the detergent-induced dissociation. Our findings should be useful for optimizing the stability of the receptor and for future studies on recombination of the subunits. The data suggest the following: (1) The critical perturbant is micellar detergent. (2) Unlike solubilization of membranes, where a molar ratio of micellar detergent:lipid of 2 is adequate, dissociation of the receptor is incomplete even at molar ratios of micellar detergent:receptor of $>10^5$ and may be limited by a reversible component. (3) Detergents that are best for solubilizing membranes are also best for dissociating the receptors. (4) The latter observation and other data implicate bound lipid as stabilizing the receptor. Our findings may be applicable to the study of interactions between membrane proteins in general.

Mast cells and related cells have plasma membrane receptors that bind immunoglobulin E (IgE)[1] and which when aggregated initiate degranulation of the cells. The IgE interacts directly with a 45-kD glycoprotein (α) exposed on the surface of the cells (Conrad & Froese, 1976; Kulczycki et al., 1976; Kumar & Metzger, 1982), but a variety of data indicate that the unit receptor contains in addition a 33-kDa β chain and two 10-kDa, disulfide-linked, γ chains (Perez-Montfort et al., 1983; Metzger et al., 1984).

An unusual property of the $\alpha\beta\gamma_2$ complex is that intact β and γ_2 dissociate from α in the mild detergents generally used to solubilize cells (Rivnay et al., 1982). In this paper we describe studies on rat basophilic leukemia cells in which we examined the conditions that promote the dissociation of β and of γ_2 from α.

Materials and Methods

Proteins and Cells. Monoclonal mouse anti-dinitrophenyl-IgE from hybridoma H1-DNP-e-26.82 (Liu et al, 1980) and human myeloma IgE from patient PS were prepared as described previously (Holowka & Metzger, 1982; Kulcyzcki & Metzger, 1974). Mouse IgE was iodinated with

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[1] Abbreviations: IgE, immunoglobulin E; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; cmc, critical micelle concentration; HLB, hydrophilic/lipophilic balance; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.