Physicochemical Behavior of Cytotoxic Ether Lipids†

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ABSTRACT: The physicochemical properties of four biologically active alkyllysophosphatidylcholine analogs have been investigated using differential scanning calorimetry (DSC), ^{31}P NMR, and fluorescence spectroscopy. Each of the alkyl ether lipids, suspended in a buffer/ethylene glycol matrix, undergo a thermotropic structural rearrangement at temperatures near 0 °C. The axially asymmetric ^{31}P NMR spectra obtained for all four lipids, after prolonged incubation at −20 °C, are consistent with the notion that these amphiphiles are able to form extended lamellar structures at temperatures below the main phase transition temperature (T_m). We have attributed the sharp, isotropic ^{31}P resonance, observed for each derivative at temperatures above the T_m, to the presence of a micellar state. This has been confirmed via ^{31}P NMR experiments employing the lanthanide shift reagent $PrCl_3$. The critical micelle concentrations (cmc) of these lysolipids have been determined via the fluorescence titration of [6-palmitoyl-2-[[(2-trimethylammonium)ethyl]methyl]amino]naphthalene (PATMAN). The low values obtained for both the lamellar to micellar phase transition temperature (−7.88 ± 0.19 °C) and the cmc (0.15 ± 0.02 μM) for the 2-benzyloxylysolipid (**1**) indicate that this analog exhibits a marked preference for the micellar state relative to the 2-methoxy (**2**), 2-deoxy (**3**), and 2-hydroxy (**4**) substituted compounds.

Lysophosphatidylcholines (LPC)[1] are naturally occurring surface-active amphiphiles generated during normal phospholipid turnover in biological membranes (Poole et al., 1970). These lipids are known to be both fusogenic and cytolytic primarily owing to their ability to perturb the cell interface by inducing changes in membrane fluidity and permeability (Bierbaum et al., 1979; Gregson, 1989; Weltzien, 1979). Consequently, the physicochemical properties peculiar to LPC analogs have been the focus of some scrutiny within the last decade (Arvidson et al., 1985; Casal et al., 1984; Eriksson et al., 1987; Kramp et al., 1984; Nakagaki et al., 1986; Stafford et al., 1989). The thermotropic phase behavior of the C_{16} and C_{18} acyllysophosphatidylcholines in excess water has been elucidated using ^{31}P NMR (Van Echteld et al., 1981; Wu et al., 1982, 1983). The same technique has been employed, in conjunction with Raman spectroscopy, to demonstrate that 1-stearoyl-2-acetyl-*sn*-glycero-3-phosphorylcholine ("platelet activating factor") exhibits a highly ordered lamellar phase at low temperature (Huang et al., 1984).

Alkyllysophospholipids (ALP) are structural analogs of LPCs in which the ester functionality at the *sn*-1 position of the glycerol framework has been replaced with an alkyl ether moiety. Alkyl lysophosphatidylcholines have been demonstrated to be selective antitumor agents both in vitro and in vivo (Berdel et al., 1982; Westphal, 1987) and, more recently, have been reported to act as inhibitors of human immunodeficiency virus-type 1 (HIV-1) multiplication (Kucera et al., 1990). Although a few studies on the physicochemical properties of 1-alkylpropanediol-3-phosphorylcholines have been reported, the lyotropic phases assumed by these medicinally useful lipids have not, as yet, been systematically investigated. We have initiated such an investigation and report herein the physicochemical behavior of several hexadecyl (palmityl)-based ALPs. Differential scanning calorimetry (DSC) and ^{31}P NMR spectroscopy have been employed to examine phase transition behavior as well as to establish the presence of a micellar structural state for these lipids. We have also obtained highly reproducible cmc values for these lipids by employing a fluorescence-based protocol.

EXPERIMENTAL PROCEDURES

Materials. The hexadecyl ether lipids were synthesized (Arnold et al., 1967; Dick et al., 1992; Katti et al., 1969; Tsushima et al., 1982) and characterized (Dick et al., 1992) as previously described. Purification of compounds **1–4** was performed prior to each experiment via silica gel column chromatography using a $CHCl_3$–MeOH gradient. Lipid purity was assessed by ^1H and ^{31}P NMR in addition to thin layer chromatography employing a ternary solvent system $CHCl_3$:MeOH:H_2O (65:25:4) in conjunction with a molybdenum visualizing reagent. Large volume stainless steel calorimetric pans used for the preparation of DSC samples were purchased from Perkin-Elmer (Norwalk, CT). The lanthanide shift reagent $PrCl_3$ (99.9%) was obtained from Aldrich Chemical Co. (Milwaukee, WI) and deuterium oxide (99.8 atom % D) was purchased from MSD Isotopes (Rahway, NJ). PATMAN [[6-palmitoyl-2-[[(2-trimethylammonium)ethyl]methyl]amino]napthalene] used in the fluorescence cmc assay was acquired from Molecular Probes (Eugene, OR) and used as received. All reagents used for buffer preparation were purchased in the highest obtainable purity from Aldrich.

Differential Scanning Calorimetry. DSC samples were prepared by depositing a 75-μL aliquot of lipid (25–30 mM) dissolved in $CHCl_3$ onto a large volume, stainless steel calorimetry pan. The solvent was removed under a stream of N_2, and the resulting lipid film was further dried via vacuum dessication overnight. The dry lipid preparations were then hydrated with a 75-μL aliquot of a 1:1 mixture of 20 mM HEPES, 10 mM Na_2HPO_4 buffer (pH 7.5), and ethylene glycol. The pans were sealed and transferred to the sample chamber of a Du Pont model 910 differential scanning

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[1] Abbreviations: ALP, alkyl lysophospholipid; ANS, 8-anilino-1-naphthalenesulfonic acid; cmc, critical micelle concentration; DSC, differential scanning calorimetry; LPC, lysophosphatidylcholine; $\Delta\sigma_{CSA}$, chemical shielding anisotropy; PATMAN, [6-palmitoyl-2-[[(2-trimethylammonium)ethyl]methyl]amino]napthalene; SUV, small unilamellar vesicles; TNS, 2-*p*-toluidinylnaphthalene-6-sulfonic acid.

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calorimeter equipped with a Du Pont 2000 thermal analyzer. Calibration of the system was conducted using both indium and gallium standards. In addition to each lipid sample, a buffer–ethylene glycol (1:1) reference cell was equilibrated at −30 °C for 0.5 h prior to analysis. Thermograms were programmed in ascending temperature mode from −30 to +35 °C using a scan rate of 2–3 °C per min. Thermodynamic parameters for each lipid were obtained on at least three different sample preparations. Quantitation of lipid phosphorus was performed on small aliquots of lipid stock solutions using the method of Ames (1966).

^{31}P Nuclear Magnetic Resonance Spectroscopy. ^{31}P NMR spectra were recorded at 109 MHz on a JEOL FX270 Fourier-transform spectrometer using a fully phase cycled (32 pulse) Hahn spin echo sequence (Rance et al., 1983). A repetition rate of 1 s was employed to accumulate free induction decays from 500–2000 transients on 1-mL samples in 10-mm NMR tubes. Typical operating conditions include a 50-kHz sweep width, 10-μs (90°) pulse, and 2K data points. Temperature was controlled to ±2 °C using a Var-Temp thermostat apparatus, and prolonged heating of the sample was avoided by gating the ^{1}H decoupler on during acquisition and off for the remainder of the sequence. During signal enhancement, a line broadening of 100 Hz was generally applied to the broad asymmetric resonances indicative of bilayer-type structures, whereas a 25–50 Hz line broadening was introduced for the isotropic resonances obtained for micellar solutions. Lipids were suspended in 10 mM Tris-HCl, 50 mM KCl, 5 mM EDTA buffer (pH 7.2) containing 15% D_2O and 50% ethylene glycol to a final concentration of 100 mM. The clear micellar solutions at 25 °C were cooled to −20 °C to form a white opaque suspension, warmed to room temperature, and then recooled to −20 °C. After two additional heating–cooling cycles, samples were stored at −20 °C for 18 h prior to NMR measurements. For asymmetric spectra in which the low-field shoulder was poorly defined, estimation of the magnitude of the chemical shielding anisotropy ($\Delta\sigma_{CSA}$) was effected by treating the distance between the high-field peak and the emergent isotropic resonance as $^1/_3\ \Delta\sigma_{CSA}$ (Seelig, 1978). The structure of the lipid phase responsible for the isotropic ^{31}P NMR resonance was elucidated by employing a lanthanide shift reagent. ALP aggregates were prepared by vortexing 50 μmol of lipid in 1 mL of the ethylene glycol/buffer system described above until the solutions were completely homogeneous. A small aliquot (20 μL) of 150 mM $PrCl_3$ was subsequently added to 5-mm NMR tubes containing ALP aggregates.

Fluorescence Determination of Critical Micelle Concentration. Steady-state fluorescence spectra and intensities were obtained at 25 °C using a Perkin-Elmer LS-3 spectrofluorimeter modified to accommodate a thermostated cuvette chamber. Temperature was maintained at 25 ± 0.1 °C using a Lauda RL6 circulating water bath. The optimal excitation and emission wavelengths determined for PATMAN were 380 and 480 nm, respectively. Fluorescence measurements were made on individual samples containing a constant concentration of fluorophore (0.5 μM) and increasing aliquots of lipid stock solutions (50–200 μM) in 10 mM Tris-HCl and 50 mM KCl at pH 7.4. Analytical lipid concentrations were 10^{-7}–10^{-4} M and in no instance contributed to fluorescence emission throughout the concentration range studied. Since the optical density of 0.5 mM PATMAN at the excitation wavelength was less than 0.02 absorbance units, solutions were free of inner filter effects. Therefore, it was not necessary to apply a correction factor to the observed emission intensities. The units used to express relative fluorescence changes were kept consistent for each titration.

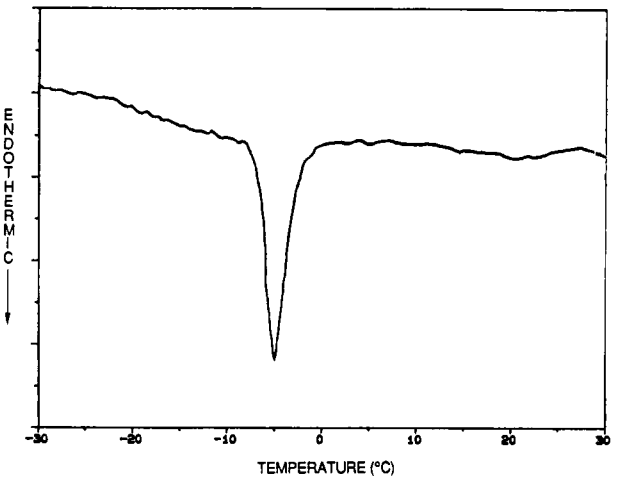
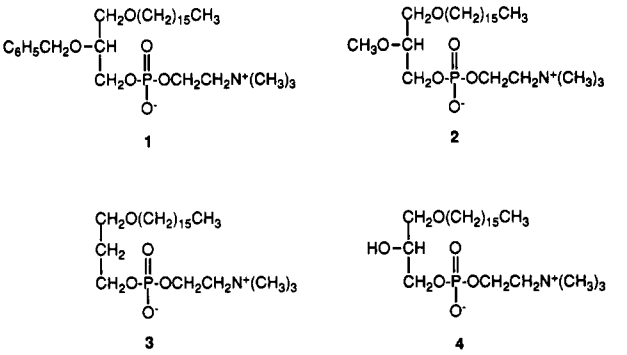


FIGURE 1: Calorimetric heating scan for the 2-methoxy substituted alkyllysophospholipid **2**.

Chart I



RESULTS

Differential Scanning Calorimetry. Figure 1 shows the DSC heating curve for 1-hexadecyl-2-methoxy-*rac*-glycero-3-phosphocholine **2**. Analogous experiments were performed on the corresponding racemic 2-benzyloxy (**1**), 2-deoxy (**3**), and 2-hydroxy (**4**) derivatives (Chart I). The results for lipids **1**–**4** are provided in Table I. Pretransitions were not observed in the DSC scans of any of these lipids. Ethylene glycol was employed in a 1:1 ratio with a 20 mM HEPES, 10 mM Na_2HPO_4 buffer (pH 7.5) to facilitate the acquisition of subfreezing transition temperatures. Previous DSC studies on the acyllysolipid 1-palmitoyl glycerophosphocholine, both in the presence and absence of ethylene glycol, indicate that the cryosuppressant does not significantly alter the experimentally derived thermodynamic parameters (Klopfenstein et al., 1974; Van Echteld et al., 1980).

The transition temperatures ranged from −7.9 °C for the 2-benzyloxy analog to 2.7 °C for the 2-hydroxy derivative. The marked tendency for the 2-benzyloxy-substituted lipid to undergo a structural transition is consistent with the results obtained in the ^{31}P NMR and cmc studies described below.

^{31}P Nuclear Magnetic Spectroscopy. At −20 °C or below, all of the hydrated ALP samples [100 mM lipid in 10 mM Tris-HCl, 50 mM KCl, 5 mM EDTA buffer (pH 7.2) containing 15% D_2O and 50% ethylene glycol] in this study were found to exhibit ^{31}P NMR spectra characterized by a broad, axially asymmetric resonance (powder pattern). The asymmetric resonance is comprised of two components: a high-field peak and a broad low-field shoulder. This peak

Table I: Physicochemical Properties of ALPs **1–4** As Determined by Differential Scanning Calorimetry (T_m, ΔH, $\Delta\sigma_{CSA}$), ^{31}P NMR Spectroscopy (ΔT_m), and Fluorescence Measurements (cmc)

lipid	R	T_m (°C)	ΔH (kcal/mol)	$\Delta\sigma_{CSA}$ (ppm)	ΔT_m (°C)	cmc (μM)
1	$-CH_2C_6H_5$	−7.88 ± 0.19	2.90 ± 0.22	25	−10 → −5	0.15 ± 0.02
2	$-OCH_3$	−5.23 ± 0.06	4.67 ± 0.10	25	−10 → −5	1.10 ± 0.17
3	−H	1.49 ± 0.09	5.84 ± 0.38	45	0 → 5	3.30 ± 0.11
4	−OH	2.65 ± 0.12	4.60 ± 0.19	30	0 → 5	4.78 ± 0.10

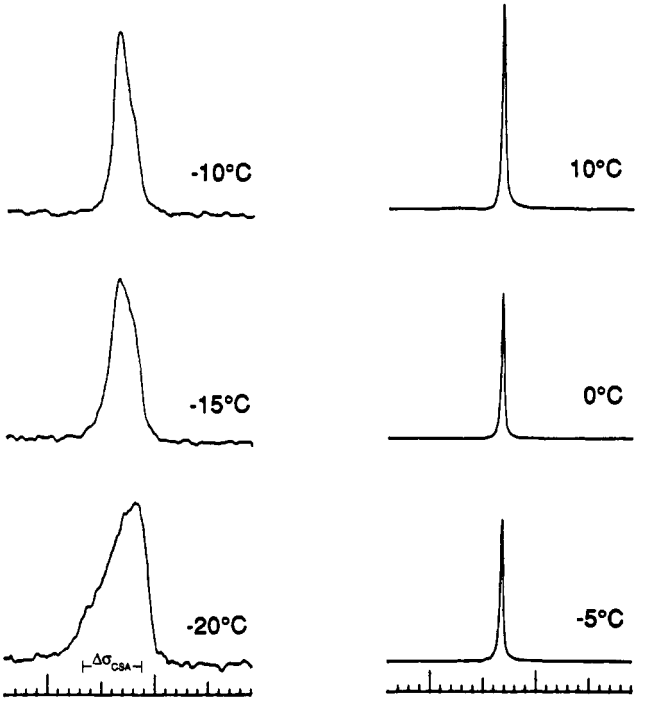


FIGURE 2: Proton-decoupled ^{31}P NMR spectra of an aqueous/ethylene glycol dispersion of the 2-methoxy substituted alkyllysophospholipid **2**. Temperatures are indicated adjacent to the spectra. The scale of the NMR spectra is given in increments of 5 ppm per division.

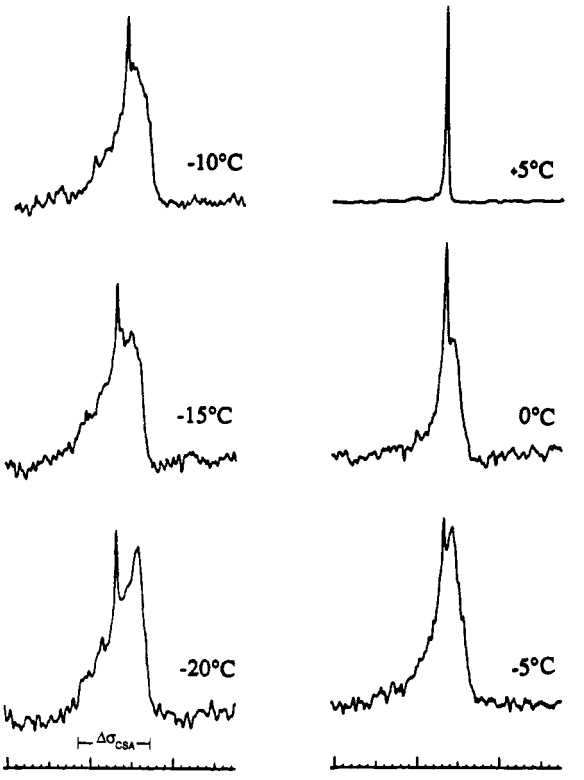


FIGURE 3: Proton-decoupled ^{31}P NMR spectra of an aqueous/ethylene glycol dispersion of the 2-deoxy alkyllysophospholipid **3**. Temperatures are indicated adjacent to the spectra. The scale of the NMR spectra is given in increments of 5 ppm per division. Note that an isotropic component is present even at −20 °C.

shape arises from the motional averaging of a multitude of phosphorus orientations in the choline head group moieties aligned both parallel and perpendicular to the bilayer normal and is diagnostic of a lamellar structural organization (Seelig, 1978).

The temperature-dependent ^{31}P NMR spectra depicted in Figure 2 for the 2-methoxy derivative are generally representative of the spectra obtained for the other ALP analogs (however, vide infra). As noted above, the spectrum acquired for this lipid at −20 °C is consistent with the notion that the individual lipid molecules are packed into a lamellar array. As the temperature is raised to −15 °C and beyond, the anisotropic signal gradually collapses to a completely isotropic resonance. The phase transition is complete between −10 and −5 °C, which is in the range of the T_m (−5.2 °C) obtained from differential scanning calorimetry. The transition temperature range (ΔT_m) values derived from the ^{31}P NMR experiments for the four lipids are provided in Table I. In addition, while the four ALPs in this study did exhibit a powder pattern at low temperatures, the spectra of 2-deoxy and 2-benzyloxy analogs displayed an isotropic component even at −30 °C (shown for the 2-deoxy derivative in Figure 3).

The width of the powder patterns ($\Delta\sigma_{CSA}$; measured at −20 °C) from the ^{31}P NMR spectra range from -25 to -45 ppm. Lipids **1**, **2**, and **4** all exhibit relatively narrow powder patterns. In contrast, the 2-deoxy derivative (**3**) displays a somewhat broadened ($\Delta\sigma_{CSA}$ = −45 ppm) powder pattern.

cmc Determination by Fluorescence Spectroscopy. Critical micelle concentrations were determined by fluorescence spectroscopy employing PATMAN as the fluorescent probe (Lakowicz, 1983). A plot of relative fluorescence intensity versus ALP concentration is provided in Figure 4 for the 2-methoxy derivative. A distinct break in the resulting titration curve is apparent, which provides an accurate and highly reproducible determination of the cmc. Analogous plots were obtained for the other lipids as well. The cmc values for all four lipids are given in Table I.

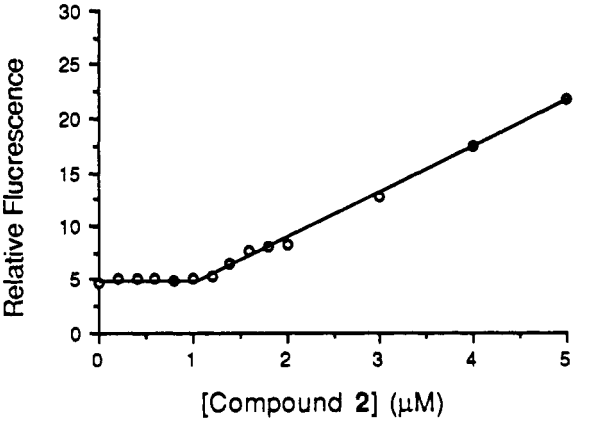


FIGURE 4: Fluorescence titration of PATMAN with the 2-methoxy substituted alkyllysophospholipid **2** at 25 °C.

The cmc value for the 2-benzyloxy analog (0.15 μM) is markedly lower than those obtained for the other lipids (1.10–4.78 μM). This is in agreement with results obtained from the differential scanning calorimetry studies. In short, the 2-benzyloxy derivative displays a pronounced tendency to occupy the micellar state relative to its ALP counterparts.

DISCUSSION

The basic physicochemical properties of the medicinally valuable alkyllysophospholipids have not been evaluated in a systematic fashion. Indeed, only a relatively few studies have even been reported on the naturally occurring acyllysophospholipids (Arvidson et al., 1985; Casal et al., 1984; Huang et al., 1984; Kramp et al., 1984; Nakagaki et al., 1986; Stafford et al., 1989; Van Echteld et al., 1981; Wu et al., 1982, 1983). The latter have been found to undergo a lamellar-to-micellar transition at characteristic temperatures and concentrations. This is in contrast to the structural behavior of diacylphospholipids, which assemble to principally generate lamellar-based aggregates. The temperature-dependent transitions typically exhibited by diacylphospholipids are gel to liquid crystalline phase changes where the lamellar nature of the aggregate is retained. Of the two reports that have appeared on the corresponding ether lipids, light-scattering experiments suggest that 1-alkylpropanediol-3-phosphorylcholines form micellar structures (Weltzien et al., 1977). In contrast, experiments on 1-octadecyl-2-benzyl-*rac*-glycero-3-phosphocholine indicate that this ALP generates large liposomal-like aggregates in solution (Weltzien et al., 1976). Since the phase behavior of these asymmetric ether lipids could prove informative in evaluating their biological mechanism of action, we have initiated a detailed study of the structural behavior of ALPs in solution. This investigation focuses on the 1-hexadecyl substituted lysophospholipids.

Differential scanning calorimetric studies revealed that lipids **1**–**4** undergo a transition near 0 °C (Figure 1 and Table I). The 2-benzyloxy derivative exhibits the lowest T_m, suggesting that this ALP has a special predilection for the micellar state. This observation is in agreement with the results of the cmc and ^{31}P NMR experiments discussed below. In addition, although the transition profiles for all of the ALPs are endothermic, the enthalpy associated with the thermotropic transition for the 2-benzyloxy derivative is less than those exhibited by the other lysolipids. In short, the 2-benzyloxy analog undergoes the observed phase transition with relative ease. Furthermore, for the limited number of lipids investigated in this study, there does appear to be an overall trend which suggests that relatively large substituents at position 2 (i.e., methoxy and benzyloxy) facilitate the thermotropic structural reorganization. However, this generalization is not strictly adhered to since the 2-deoxy derivative **3**, which possesses the least sterically imposing substituent, is not the most recalcitrant in terms of its tendency to undergo the phase transition. In short, lipids **3** (2-deoxy) and **4** (2-hydroxy) exhibit behavior, relative to each other, which is contrary to that expected for the above generalization. The data currently available on alkyl lysophospholipids are, as yet, not extensive enough to formulate a cogent hypothesis to explain this observation. Nevertheless, it is evident that lipid **3** is different from its three counterparts in that it lacks an oxygen substituent at position 2, and therefore this site cannot serve as a hydrogen-bond acceptor. However, lipid **4** is also structurally distinct from the other three ALPs. Since this lysolipid contains a free hydroxyl moiety at the 2 position, it is the only ALP in this study which can serve as a hydrogen-bond donor. While it remains to be determined if the observed thermodynamic behavior of these two lipids, and other related analogs, is a reflection of either of these structural features, it is clear from the ^{31}P NMR experiments ($\Delta\sigma_{CSA}$ values) described below that the lamellar phase properties of the deoxy derivative (**3**) are quite different from those of its counterparts. This anomalous structural arrangement below the T_m may account for the observed thermodynamic behavior of the ALP **3** derivative.

^{31}P NMR studies were undertaken to ascertain the structural nature of the aggregates on both sides of the phase transition. The asymmetric resonance obtained for lipids **1**–**4** (shown for the 2-methoxy derivative in Figure 2) below the T_m is diagnostic of a lamellar structural organization (Seelig, 1978). Alternatively, Linblom and Arvidson (Arvidson et al., 1985; Eriksson et al., 1987) have shown that some LPCs form micellar aggregates (cubic phase) that exhibit asymmetric resonances as well. However, the formation of a cubic liquid crystalline phase is extremely unlikely in this case since we employed low concentrations of lipid (95% water content) in our studies (Linblom et al., 1989). Interestingly, there is an apparent reversal of line shape asymmetry in the spectra at −10 and −15 °C for the 2-methoxy species. This is most likely due to the incomplete disruption of the bilayer at these temperatures (producing the upfield shoulder in these spectra). In the ^{31}P NMR spectra of the 2-benzyloxy (**1**) and 2-deoxy (**3**) derivatives, an isotropic component is still observed at temperatures well below T_m. Although we stored ALPs **1** and **3** at −20 °C for up to 1 week prior to these experiments, it is possible that the incubation period was insufficient to allow for complete conversion to the lamellar phase. In addition, we note that the phase transition temperature ranges derived from these NMR studies are in good agreement with those obtained from the differential scanning calorimetry experiments (Table I). The isotropic resonances exhibited by these ALPs at temperatures greater than their T_m have been previously observed for acyllysophospholipids, and this behavior has been ascribed to lipids arranged in a micellar state (Huang et al., 1986; Wu et al., 1982, 1983). However, isotropic ^{31}P NMR signals can actually arise from lipids contained within several distinct lipid phases, including (1) small unilamellar vesicles (SUVs), (2) micelles, (3) inverted micelles, and (4) the cubic phase (Gennis, 1989). As noted above, the existence of a cubic phase is not likely under the conditions of our experiments. For lipids contained within SUVs, not all of the lipid head groups are exposed to the aqueous milieu, and consequently not all of these head groups are available for interaction with exogenously added reagents. Therefore, when lipid aggregates in this phase are treated with a lanthanide shift reagent, more than one ^{31}P NMR resonance should be produced (Kumar et al., 1986). However, upon addition of 6 mM $PrCl_3$ to a 50 mM solution of either ALP **1** or **2**, we observed a single isotropic resonance. Furthermore, a downfield shift of the ^{31}P resonance was observed for both compounds **1** (10.1 ppm) and **2** (12.5 ppm) relative to the initial signal obtained in the absence of $PrCl_3$. Although both micelles and inverted micelles should provide one isotropic resonance, only in the former case should a chemical shift of the ^{31}P signal occur (in addition, it is highly unlikely that inverted micelles will form under the conditions that we employed for both the DSC and the ^{31}P NMR experiments). Consequently, we conclude that the lanthanide shift experiments and most consistent with the existence of a micellar state. Furthermore, this conclusion was substantiated by freeze–fracture electron microscopy, which revealed small

(average diameter of 104 Å) irregularly shaped particles that are most consistent with micellar aggregates (unpublished data[2]).

The chemical shift anisotropy values ($\Delta\sigma_{CSA}$, Table I) obtained for lipids **1**, **2**, and **4** are nearly identical to those which have been previously reported for lysophosphatidylcholines (Huang et al., 1986). The reduction in the magnitude of the $\Delta\sigma_{CSA}$ values for lysolipids (~20–25 ppm) relative to those obtained for diacyl lipids has been primarily attributed to an increase in rotational freedom about the C(1)–C(2) bond of the glycerol backbone in the former (Van Echteld, 1981; Wu et al., 1984). In marked contrast, and as noted above, a larger $\Delta\sigma_{CSA}$ value was observed for the 2-deoxy derivative (**3**). This may indicate that **3** can assume a more rigid structural organization than that of its counterparts at temperatures below the T_m. This relatively ordered array may be due to the lack of a substituent at position 2 of the propanediol backbone, which should afford tighter packing of the individual lipid molecules. Alternatively, the unusually large $\Delta\sigma_{CSA}$ value for **3** may be due to a restriction in movement of the phosphocholine head group (Allegrini et al., 1983) or to the formation of exceptionally large and/or extended aggregates (Burnell et al., 1980).

The initial experiments conducted to obtain critical micelle concentrations for lipids **1**–**4** utilized the anionic fluorescent probes 2-*p*-toluidinylnaphthalene-6-sulfonic acid (TNS) and 8-anilino-1-naphthalenesulfonic acid (ANS) as well as the cationic probe rhodamine 6G. Unfortunately, although these compounds exhibited a slightly enhanced (TNS and ANS) or diminished (rhodamine 6G) fluorescence upon titration with the ALPs in this study, the changes were linear and yielded little information regarding cmc values. These results are consistent with those reported by Weltzein et al. (1977), who employed UV–vis spectroscopy (with methyl orange) and light-scattering experiments in an attempt to determine cmcs for 1-alkylpropanediol-3-phosphocholines. These investigators found that it was difficult to acquire accurate values when the alkyl chain length exceeded 14 carbon atoms. The explanations which may account for these difficulties are (1) the presence of micellar aggregates which are so stable that the probe molecule is not readily incorporated or (2) inherent structural limitations with the probe molecules themselves. In order to investigate the former possibility, we incubated the fluorophores mentioned above with variable concentrations of lipid (1 μM to 15 mM) for time intervals of up to 1 week. These experiments were performed at elevated (60–70 °C), ambient (25 °C), and subfreezing (–20 °C) temperatures. In the latter case, by employing a temperature below the T_m, we reasoned that, upon warming to 25 °C, the fluorophore would be readily incorporated into the micelle upon completion of the phase transition. Unfortunately, each of these studies failed to produce the requisite break point in the titration curve. It occurred to us that the structural features of the three fluorescent probes might preclude facile accommodation by the micellar structure. Consequently, we employed PATMAN (Lakowicz et al., 1983) as the fluorescent probe. This disubstituted napthalene contains a palmitoyl substituent, which is of comparable length to the hexadecyl chains contained within the ether lipid micelles. The predicted molecular compatibility between this fluorophore and lipids **1**–**4** appears to be consistent with the highly reproducible biphasic titration curves obtained in each of the cmc assays (shown for ALP **2** in Figure 4). To the best of our knowledge, this study represents the first use of a fatty acid-based fluorophore to obtain lysophospholipid cmc values. The low micromolar cmc values for ALPs **2**–**4** are comparable to those previously reported for the corresponding palmitoyllysolipids (Nakagaki et al., 1986; Kramp et al., 1984). In addition, Hanahan and his colleagues have employed the Wilhelmy plate equilibrium method, which is based on surface tension measurements, to obtain a cmc value (3.30 μM) for compound **4** (Kramp et al., 1984). This is in good agreement with our own results for this particular ALP. Finally, the trend in cmc values parallels the results obtained from the DSC studies, namely, the apparent propensity for micelle formation runs from **1** > **2** > **3** > **4**.

In summary, this investigation is the first systematic study of the physicochemical properties of the medicinally valuable cytotoxic ether lipid family. These hexadecyl-based lysolipids undergo a lamellar to micellar phase transition at freezing or subfreezing temperatures. ^{31}P NMR studies have confirmed the presence of both a lamellar and a micellar structural organization below and above the T_m, respectively. The results of the DSC, ^{31}P NMR, and cmc experiments are consistent in terms of assessing the relative tendencies of the ALPs in this study to assume a micellar structural state. Finally, we have found that the palmitoyl-based fluorophore, PATMAN, is a particularly useful probe in acquiring critical micelle concentrations for these lysophophatidyl ether lipids.

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[2] Freeze-fracture electron microscopy experiments were performed by Dr. Sek Wen Hui (Roswell Park Memorial Institute, Buffalo, NY).

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