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Phase behavior of cerebroside and its fractions with phosphatidylcholines: calorimetric studies

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Bovine brain cerebroside and its kerasin (β-D-galactosyl-*N*-acyl-D-sphingosine) and phrenosin (β-D-galactosyl-*N*-(2-D-hydroxyacyl)-D-sphingosine) fractions were mixed with diacylphosphatidylcholines (PCs) to form fully hydrated lamellar phases. These mixtures were examined by differential scanning calorimetry, and phase diagrams for cerebroside/diacylPC mixtures were constructed from the data. Cerebroside was found to be miscible with egg PC at low mole fractions X of cerebroside; the mixture behaves non-ideally for $X > 0.25$. The non-ideal behavior appears to be a superposition of separate interactions of kerasin and phrenosin with egg PC. Strikingly, phrenosin mixes nearly ideally with egg PC. Kerasin mixed with egg PC yields a peritectic phase diagram. Cerebroside and phrenosin were found to be immiscible with dimyristoylphosphatidylcholine (DMPC) in the gel state in low proportions. Both stable and metastable gel phases of kerasin were detected in different endotherms of kerasin/PC mixtures. Kerasin in the stable and metastable gel states exhibits discontinuous and continuous ranges of miscibility, respectively, with DMPC. The stable gel phase of kerasin does not segregate in natural cerebroside. Natural kerasin was found to act isomorphic to semi-synthetic (natural configuration) D-kerasins but not completely to synthetic DL-kerasins of single acyl chain lengths.

Introduction

Cerebroside is a major lipid component both of myelin, where it occurs in a 2:1 ratio to acylphosphatidylcholines (PC) [1], and of various plasma membranes. Various studies suggest that cerebrosides enhance membrane stability through the formation of lateral hydrogen bonding between lipid species [2–4]. Since the dominant characteristics of cerebroside, compared to PC, are the long acyl chain and the large number of hydrogen-bonding groups in the headgroup region [5], an understanding of the compatibility of these diverse structures in particularly cerebroside/PC mixtures becomes important in attempts to assign specific functions to designated membrane lipid components.

The two cerebroside fractions, phrenosin and kerasin, are differentiated by the presence of a

Abbreviations: kerasin, β-D-galactosyl-*N*-acyl-D-sphingosine; phrenosin, β-D-galactosyl-*N*-(2-D-hydroxyacyl)-D-sphingosine; NPGS, β-D-galactosyl-*N*-palmitoyl-D-sphingosine; NLGS, β-D-galactosyl-*N*-lignoceryl-D-sphingosine; C_{18}-DL-kerasin, β-D-galactosyl-*N*-stearoyl-DL-dihyrosphingosine; C_{24}-DL-kerasin, β-D-galactosyl-*N*-lignoceryl-DL-dihydrosphingosine; egg PC, egg phosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine.

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hydroxyl group in phrenosin at the two-position of the acyl chain. This hydroxyl substitution imparts different packing properties to the anhydrous and hydrated bilayer forms [6,7]. For an anhydrous, synthetic phrenosin, X-ray diffraction studies demonstrate an intricate intermolecular hydrogen-bonding network at the surface of the bilayer [5]. Vibrational Raman spectroscopic comparisons of anhydrous and fully hydrated bilayers of natural bovine brain phrenosin show that hydration alters considerably the lipid headgroup conformation, induces a large decrease in hydrocarbon chain order and expands the lipid lattice [7]. These results indicate that hydration modifies the hydrogen-bond network at the polar interface of the phrenosin bilayer.

For kerasin, in contrast to phrenosin, the Raman spectral parameters associated both with the polar headgroup region and with changes in chain conformation and packing are virtually unaltered upon hydration (see also Discussion). That is, the fully hydrated kerasion gel exhibits significantly more ordered and denser packed chains than the phrenosin gel [7]. Accordingly, kerasin gels display a much higher enthalpy change, 15.8 kcal/mol at 72° C, upon transformation to the liquid crystalline phase, in comparison to phrenosin gels, which yield 7.7 kcal/mol at 66.5° C. Kerasin easily forms a metastable gel phase structure between 25° C and 55° C. For this phase the gel to liquid crystalline phase transition occurs at 56° C with an enthalpy change of 7 kcal/mol [8]. Since the metastable gel phase exists at physiological temperature and may be stabilized either by other lipids or by surface interactions with proteins, we also discuss its characteristics in the present study. Additionally, the gel phase of brain cerebroside, an approximately 40:60 mixture of kerasin and phrenosin ([9]; Cammer, W., personal communication), most closely resembles that of phrenosin, not only in the value of its gel to liquid crystalline phase transition enthalpy of 6.7 kcal/mol at 66° C, but also in its chain conformation and packing properties as reflected by Raman spectral analysis [7].

With the above structural and thermodynamic information at hand, we report here the phase diagrams for mixtures of cerebroside, kerasin, or phrenosin with diacylphosphatidylcholines in hydrated bilayers [10] and compare our results with recent studies, including those of semisynthetic kerasins of natural configuration. We also examine calorimetrically the behavior of synthetic DL-kerasins of uniform chain length as possible model cerebroside systems.

Methods

Bovine brain cerebroside, kerasin (β-D-galactosyl-*N*-acyl-D-sphingosine), and phrenosin (β-D-galactosyl-*N*-(2-D-hydroxyacyl)-D-sphingosine) were used as obtained (more than 98% pure) from P-L Biochemicals, Inc., Milwaukee, WI. Synthetic, β-D-galactosyl-*N*-stearoyl-DL-dihydrosphingosine (C_{18}-DL-kerasin) and β-D-galactosyl-*N*-lignoceryl-DL-dihydrosphingosine (C_{24}-DL-kerasin) were obtained from Miles-Yeda (Miles Labs., Inc., Elkhart, IN). Dimyristoylphosphatidylcholine (DMPC) and egg phosphatidylcholine (egg PC) were obtained from Calbiochem., Inc., San Diego, CA, and P-L Biochemicals, Inc., respectively. The molecular weights of these compounds used for computations were as follows: cerebroside, 822; kerasin, 814; phrenosin, 830, assuming the lignoceryl form for the cerebrosides; DMPC monohydrate, 696; egg PC, 800; C_{18}-DL-kerasin, 728; and C_{24}-DL-kerasin, 812.

The differential scanning calorimetric methods, including sample preparation and construction of phase diagrams, have been, in part, previously described [11]. Lipid samples mixed in chloroform/methanol solutions were thoroughly dried, hydrated with mechanical mixing above the gel to liquid crystalline transition temperatures, and then annealed by thermal cycling from −20° C through the thermal phase transition. Thermal annealing was found necessary for achieving reproducible calorimetric behavior. The average sample contained 1.1 mg lipid and 60% by weight water. All samples contained excess, freezable water. A Perkin-Elmer DSC-2 differential scanning calorimeter (Perkin-Elmer, Norwalk, CO) was employed at scan rates from 2.5 to 10 Cdeg/min; the survey scan rate for the figures presented here was usually 10 Cdeg/min. Effects of scan rate were examined at various mole fractions. Scans were also occasionally interrupted for several minutes at temperatures between the phase boundaries to test

for further equilibration. Phase diagrams were constructed from endotherms and exotherms, and phase transition enthalpies were obtained from standardized areas under peaks, as previously described [11].

Results

Pure cerebrosides

The present paper examines the miscibility of natural cerebroside fractions with acylPCs using simple calorimetric analyses. The natural cerebroside fractions contain acyl chains whose lengths are heterogeneous [12] (note the breadth of transition for X equal to 1 in Figs. 1 and 3). To justify the use of these lipid fractions, the pattern of phase transitions for anhydrous bovine brain kerasin, phrenosin, and cerebroside was determined calorimetrically and compared to the phase diagrams obtained from X-ray diffraction studies for synthetic cerebrosides of natural configuration and containing only C_{18} acyl chains [6].

All the natural fractions were found to give transition patterns analogous to those of the corresponding synthetic fractions, with the modification that the observed calorimetric transitions occurred up to 10 °C but usually less, below those reported for the synthetic compounds. These results support the treatment here which considers the cerebroside fractions as single components.

We also examined calorimetrically the synthetic, commercially available kerasins, *N*-stearoyl-DL-dihydrogalactocerebroside (C_{18}-DL-kerasin) and *n*-lignoceryl-DL-dihydrogalactocerebroside (C_{24}-DL-kerasin), as possible model kerasin bilayer systems. The molecules, unlike natural kerasin, have no double bond at the 4,5 position of the sphingosine moiety, and are racemic about the second carbon of sphingosine. Neither anhydrous C_{18}-DL-kerasin nor C_{24}-DL-kerasin gave a calorimetric pattern entirely analogous to those reported for C_{18}-D-kerasin from X-ray analysis [6].

For both hydrated C_{18}-DL-kerasin and C_{24}-DL-kerasin, two endotherms at different temperatures were observed in different scans (Table I). These results suggest that DL-kerasin, like D-kerasins, form two gel phases. Only the lower-temperature transition of C_{24}-DL-kerasin shows a high enthalpy, which is lower, however, than that of kerasin of natural configuration (Table I). To test further for isomorphic behavior of the gels, the synthetic DL-kerasins were also mixed with equimolar quantities of bovine brain kerasin (see Table I). The mixture containing C_{18}-DL-kerasin shows a single endotherm at a temperature intermediate to its own and to that of the metastable phase of natural kerasin, possibly mixing well with the latter phase (Table I). C_{24}-DL-kerasin did not mix ideally with bovine brain kerasin; rather, values of ΔH for the two endotherms of the mixture are close to those for C_{24}-DL-kerasin (Table I), suggesting isomorphism of each gel formed by the mixture to the corresponding C_{24}-DL-kerasin gel, but not to the natural kerasin. The deviations from isomorphic behavior with natural kerasin render the DL-kerasins unsuitable for studies with lipids from natural membranes.

Calorimetric studies of cerebrosides mixed with phosphatidylcholines

Endotherms for mixtures of bovine brain cerebroside and its kerasin and phrenosin frac-

TABLE I

THERMODYNAMIC DATA FOR PHASE TRANSITIONS OF SYNTHETIC DL-KERASINS AND MIXTURES WITH NATURAL CONFIGURATION KERASIN, IN EXCESS WATER

The symbol X denotes mole fraction of DL-kerasin; the second component consists of bovine brain kerasin of natural configuration.

Synthetic kerasin	$X = 1.0$		$X = 0.5$		$X = 0.0$	
	T (°C)	ΔH (kcal/mol)	T (°C)	ΔH (kcal/mol)	T (°C)	ΔH (kcal/mol)
C_{18}-DL-kerasin	74.3	6.0	not observed		71.8	15.8
	68.0	6.0	60.2–62.5	7.0	56.0	7.0
C_{24}-DL-kerasin	76.2	7.9	75.2	8.2	71.8	15.8
	70.3	11.2	66.3	11.1	56.0	7.0

tions with DMPC and with egg PC were obtained over the entire range of mole fractions. Temperature/composition plots, constructed as previously described [11], were drawn as binary phase diagrams. The cerebroside/phosphatidylcholine systems contain three lipids, kerasin, phrenosin, and diacylPC. However, the ratio of kerasin to phrenosin in the natural cerebroside of the present experiments is fixed; therefore, these systems are pseudobinary. If the components were pure, the maximum number of coexisting lipid phases at constant pressure, with zero degrees of freedom, would be four. Cerebroside and egg PC contain more than one hydrocarbon chain length, permitting multiple phases to coexist. However, both cerebroside and egg PC occur as single phases in excess water by themselves, as determined by X-ray analysis [6,13].

The gel to liquid crystalline transition enthalpies, determined here from the endotherms, were found to be nearly ideal sums of the partial molar enthalpies, within 5% for cerebroside/DMPC and within 10% for cerebroside/egg PC, suggesting that structural changes as a function of composition were not easily detected by these parameters; hence further studies of these quantities were not pursued here.

Cerebroside / DMPC mixtures

For cerebroside/DMPC mixtures, the pretransition of DMPC, centered at 15 °C, survives up to a mole fraction of cerebroside X_c equal to 0.3 (Fig. 1). The pretransition is sensitive to impurities; its occurrence is compatible with the immiscibility of cerebroside with DMPC in the gel phase. The solidus for the main DMPC transition remains horizontal up to X_c equal to 0.46, indicating the domain of immiscibility of cerebroside and phosphatidylcholine in the gel phase (Fig. 2). At about X_c equal to 0.4, the shape of the endotherms alters from one exhibiting a DMPC-like transition below 25 °C plus a higher-temperature peak to one exhibiting two peaks above 35 °C ($0.4 < X_c < 0.8$). The new, lower-temperature peak in this region decays to a shoulder at X_c equal to 0.65 (Fig. 1). The complexity of the shapes of the endotherms indicates that more than one phase may exist within the endotherm boundaries over a large composition range.

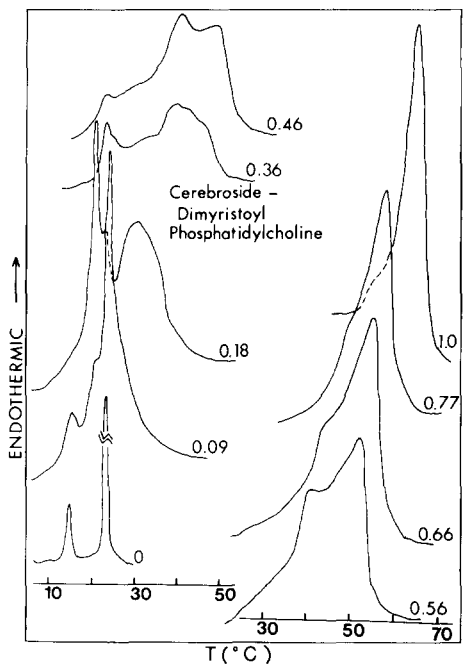

Fig. 1. Some of the endotherms for bovine brain cerebroside/DMPC mixtures. Two complete sets of samples were scanned. Mole fraction X of cerebroside is marked beside each curve. Scan rates varied from 2.5 to 10/min in all figures. In this figure and Figs. 3, 6, and 8, full baselines, all of which were flat, have not been traced in order to conserve space.

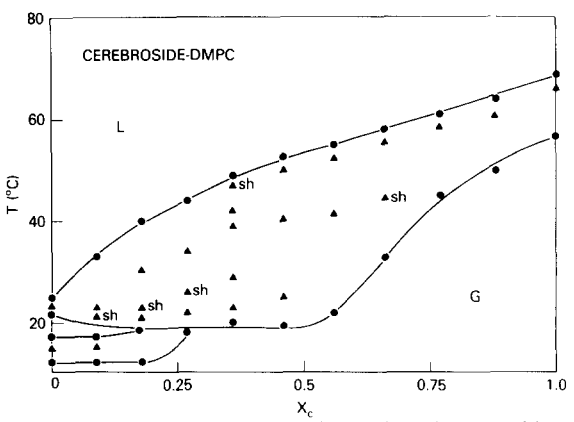

Fig. 2. Plot of temperature vs. mole fraction of cerebroside X for cerebroside/DMPC mixtures. Lower and upper edges of endotherms are plotted (●); peak and shoulder temperatures (▲) are also noted, where sh denotes shoulder. The letters L and G denote liquid crystalline and gel phases, respectively, in this and subsequent figures; the phase boundary for the region of demixing in the gel below 20 °C is not marked.

Mixtures of kerasin / DMPC and phrenosin / DMPC

Because the cerebroside/DMPC mixtures exhibited complex endotherms, the calorimetric behavior of mixtures of DMPC with kerasin or phrenosin was also examined (Figs. 3–5). With excess water present, these systems are treated as containing two lipid components, one glycosphingolipid and one phospholipid. The maximum number of coexisting phases at constant pressure and zero degrees of freedom is three.

The phase boundaries obtained for phrenosin/DMPC are similar in shape to those obtained for cerebroside/DMPC, particularly with regard to the horizontal solidus implying gel phase immiscibility for values of X below 0.5 (compare Figs. 2 and 4). For $X > 0.5$, the solidus for the phrenosin/DMPC mixture lies several degrees higher than for kerasin/DMPC (compare Figs. 4 and 5).

In the case of kerasin/DMPC mixtures, two temperature-composition diagrams were obtained, reflecting whether kerasin assumed the form of either the stable or metastable gel phase (high and low transition enthalpy forms, respectively). For kerasin/DMPC above 0.25 X we assume from the equilibrium phase diagram that the kerasin gel forms the stable phase structure (solid lines, Fig. 5). We base this on the following grounds. The alternate phase diagram (dashed lines, Fig. 5) was most easily obtained by cooling the mixture from the liquid crystalline state. Further, prolonged

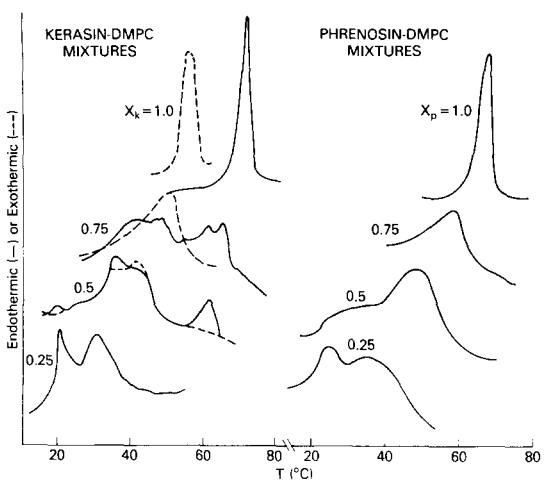

Fig. 3. Endotherms (———) and exotherms (- - - - - -) for mixtures of kerasin/DMPC and phrenosin/DMPC. Mole fraction of kerasin X_k or of phrenosin, X_p, is marked on each tracing. Endotherms and exotherms for kerasin/DMPC mixtures have been superposed for clarity. Where no dotted line is visible (kerasin/DMPC mixtures) the exotherms are coincident with endotherms.

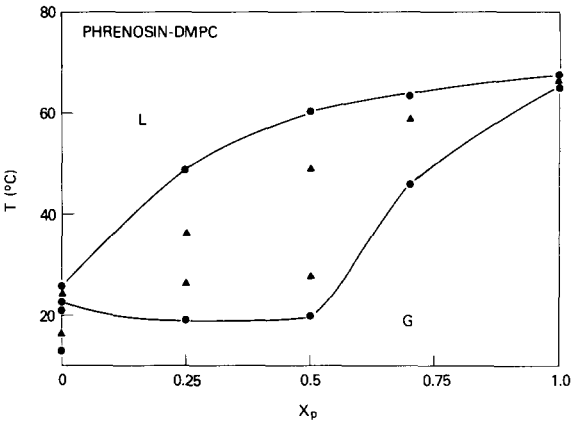

Fig. 4. Plot of temperature vs. mole fraction X_p for phrenosin/DMPC mixtures. Peak temperatures (▲) are shown. Again, a region of demixing in the gel phase existing below the horizontal portion of the solidus has not been delineated.

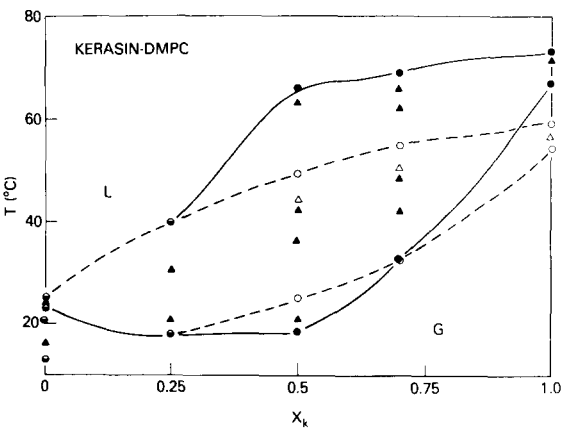

Fig. 5. Plot of temperature vs. X_k for kerasin/DMPC mixtures. The upper liquidus and lower solidus (———) are sketched from points (●) obtained from heating curves beginning at 40 °C or below for liquidus, or 5 °C for solidus. The lower liquidus and upper solidus segments (- - - - - -) are sketched from points (○) obtained from cooling curves. Where dashed line segments are not shown, they are coincident with solid lines. Peak temperatures recorded in heating scans (▲) and cooling scans (△) are also noted. For simplicity, pre-transition of DMPC seen at low values of X_k is not shown. Scan rate was 2.5/min. The gel phase G existing below the dashed solidus is presumably different from that occurring below the solid-line solidus. A region of demixing occurs, for $0.25 < X < 0.5$ (solid-line solidus) below 20 °C, and possibly for X_k less than 0.25; these regions are not delineated.

equilibration of the mixtures at intermediate mole fraction compositions within the temperature range of the broad phase transition promoted separation of the higher-melting kerasin/DMPC phase, as seen upon continued thermal scanning. This melting was sometimes preceded by a crystallization isotherm.

When assuming the conformation reflecting the metastable gel phase, kerasin is miscible with DMPC over a wide range, $0.25 < X_k < 1.0$. Below 0.25, the solidus sags, suggesting non-ideality but miscibility in both solid and liquid crystal phases. The low liquidus in this region implies that the gel phase mixture cannot be equivalent in structure to that of the high-enthalpy (stable) gel phase of kerasin. The phase diagram incorporating the metastable gel phase (dashed lines, Fig. 5) is thus near-ideal. (See Ref. 14 for typical phase diagrams.)

With kerasin transformed into the stable gel conformation, the kerasin/DMPC mixture exhibits a level solidus and a corresponding sharp rise in the slope of the liquidus for $0.25 < X_k < 0.5$. This region denotes contact of the solidus with a region of gel phase separation of a phase of composition X_k equal to 0.25 and one highly enriched in kerasin. The high liquidus is consistent with the disparities in transition temperatures and enthalpies (15.8 vs. 6.8 kcal/mol) between kerasin in the stable gel state and DMPC. (For an illustration of the shape of the phase diagram ensuing from such a disparity, see the ideal curve sketched in Fig. 7.) The middle points at intermediate values of X_k also suggest the presence of another phase smoothly increasing in kerasin content (intermediate solid black triangles, Fig. 5). The same liquid crystalline phase is attained above either the ideal liquidus, implying that it is well-mixed, or non-ideal liquidus (dotted and solid lines, Fig. 5).

The lower-temperature and higher temperature peaks in cerebroside/DMPC mixtures are nearly coincident with, respectively, those of kerasin/DMPC and phrenosin/DMPC mixtures for high values of X (Table II). These reflect the nearly additive behavior of the cerebroside fractions in the ternary mixtures. The locus of highest peaks directly under the liquidus (Fig. 5) for kerasin/DMPC mixtures (Fig. 5) does not appear in the cerebroside/DMPC phase diagram (Fig. 2) (or thus in Table II), suggesting that the phase which is very high in kerasin content and in transition enthalpy does not separate in the cerebroside/DMPC mixture.

Mixtures of cerebroside / egg PC

The temperature/composition diagram obtained from endotherms (Fig. 6) for mixtures of cerebroside with egg PC, a more representative phosphatidylcholine found in natural cell membranes, is shown (solid lines) in Fig. 7. The dotted lines (Fig. 7) which sketch the shape of the diagram expected for ideal mixing of two lipids were computed using the 'equal-G' method [14] and the method outlined by Lee [15]. For this figure the higher-melting cerebroside was assigned the observed phase transition enthalpy of 6.7 kcal/mol; egg PC, 1.3 kcal/mol. The convexity in the theoretical liquidus reflects closely the disparity in enthalpies. The theoretical and experimental liquidi are closely superposed, indicating miscibility of these components in the liquid crystalline phase. For $X_c < 0.25$, the solidi of theoretical and actual curves are also superposed. For $X_c > 0.25$, the actual solidus has a lower slope than the theoretical one, following the locus of a low-temperature peak, at $X_c = 0.25$, which appears as a shoulder at higher mole fractions.

TABLE II

COMPARISON OF TRANSITION PEAK TEMPERATURES FOR CEREBROSIDE/DMPC MIXTURES TO THOSE OF THE COMPONENTS KERASIN AND PHRENOSIN MIXED WITH DMPC

X denotes mole fraction of cerebroside, kerasin, or phrenosin component. sh denotes peak shoulder.

X	Peak temperatures (°C)		
	cerebroside/ DMPC	kerasin/ DMPC	phrenosin/ DMPC
0.5	41.2 (sh)	41.9 [a]	
		43.8 [b]	
	50.4		48.8
0.7	47.4	48.3 [a]	
		50.1 [b]	
	56.9		58.8

[a] These temperatures refer to the higher-temperature profile of Fig. 5.

[b] These temperatures refer to the lower-temperature profile of Fig. 5.

Kerasin / egg PC and phrenosin / egg PC

The relation of the behavior of cerebroside/egg PC mixtures to the kerasin and phrenosin content was investigated by calorimetric scans of kerasin/egg PC and phrenosin/egg PC mixtures at higher mole fractions (Fig. 8). Again, two temperature-composition plots are obtained with kerasin present (Fig. 9). Mixtures containing kerasin, which assume the stable gel form, show a very broad phase transition region which fully encompasses the liquidus and solidus of the cerebroside/egg PC diagram. The horizontal segment of the solidus at higher mole fractions (solid black circles, Fig. 9) indicates the separation of a solid phase of composition 0.20 ± 0.05 mole fraction of kerasin (see Fig. 7). (In Fig. 9, only points for $X_k > 0.5$ are shown, but the elevation and slope of the solidus are consistent with the prior points being of positive slope.) The high liquidus (Fig. 9) is consistent with the higher transition enthalpy of the stable gel phase of kerasin relative to egg PC and with the melting of a solid phase highly enriched in kerasin.

Mixtures of kerasin and egg PC in which kerasin assumes the metastable gel state show a transition region nearly as broad as that of cerebroside-egg PC but with a slightly lower liquidus.

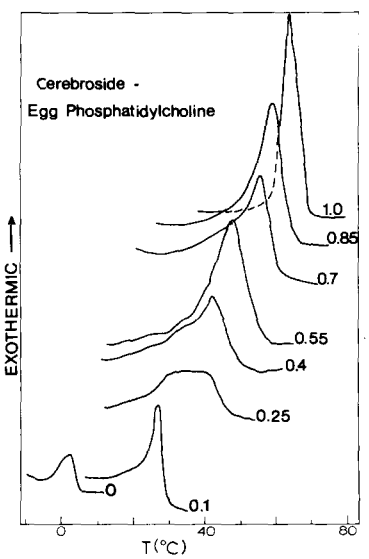

Fig. 6. Exotherms for cerebroside-egg PC mixtures. Endotherms free of interference by ice were also obtained by scanning from above 0 ° C for all samples except for X equal to 0. Numbers beside curves denote X for cerebroside.

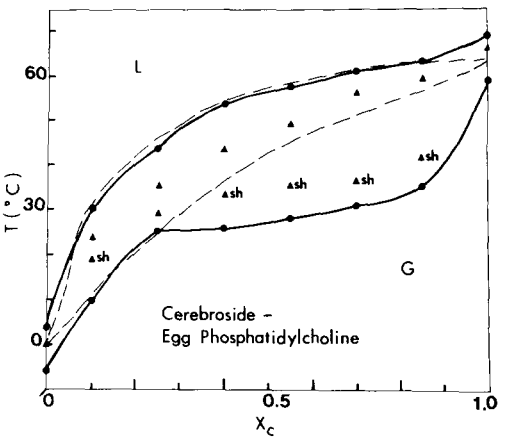

Fig. 7. Plot of temperature vs. X for cerebroside/egg PC. Solid lines are solidus and liquidus determined from experimental points (●). Dashed lines sketch the solidus and liquidus calculated for an ideal mixture of lipids, where the enthalpy for the gel to liquid crystalline phase transition of cerebroside is much greater than that of egg PC (see text). Peak and shoulder temperatures are also noted; sh denotes shoulder.

For phrenosin/egg PC mixtures, the liquidus most closely approaches that of cerebroside/egg PC mixtures, while the solidus is much higher and nearly superposable with the ideal solidus shown in Fig. 7. (The near-equality of the gel to liquid crystalline phase transition enthalpies for phrenosin and natural cerebroside allows this direct comparison.) That is, phrenosin and egg PC are miscible in both the gel and liquid crystalline phases.

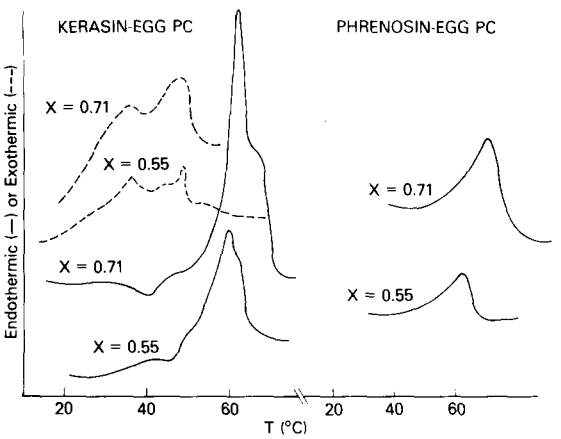

Fig. 8. Endotherms (———) and exotherms (- - - - -) for kerasin/egg PC and phrenosin/egg PC mixtures at higher values X of the cerebroside component.

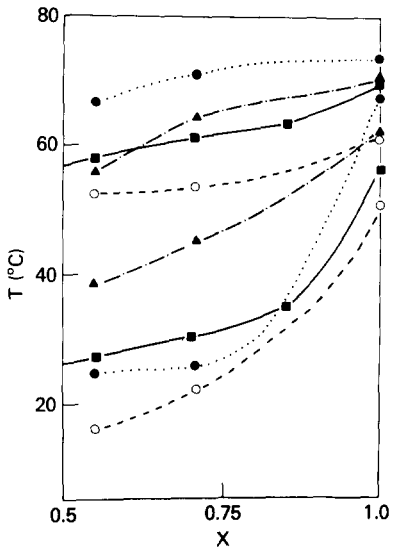

Fig. 9. Comparison of phase boundaries obtained for mixtures of cerebroside/egg PC to those for kerasin/egg PC and phrenosin/egg PC at high values of X. Upper and lower lines of each like pair denote liquidus and solidus, respectively, for kerasin/egg PC (●●●) obtained from data from heating scans (●); kerasin/egg PC (------) from cooling scans (○); phrenosin/egg PC (●———●) from heating scans (▲); and cerebroside/egg PC (———) from heating scans (■).

Discussion

One of the most interesting results of this study is the detection of different intreactions of phrenosin and kerasin with phospholipid for various lipid mixtures. Our previous thermodynamic and Raman spectroscopic studies established that natural cerebroside assumes a gel phase structure nearly isomorphic to that of phrenosin and less ordered than that of kerasin gels [7,8]. In the present work, the deviations of the solidi in the phase diagrams for cerebroside/phosphatidylcholine mixtures from those for phrenosin/phosphatidylcholine mixtures at high mole fractions are attributed to the direct interaction of kerasin with the phosphatidylcholine species, as described above.

A second noteworthy result is the near-ideal mixing of phrenosin with egg PC in both the gel and liquid crystalline phases. This is in accord with our previous determination that the integrity of the hydrogen bond lattice in the plane of the headgroup for gel phase phrenosin lessens in comparison to the anhydrous phase (see Ref. 7 and above); thus the barrier to mixing with PC would be expected to be reduced. The miscibility is also remarkable in that bovine brain phrenosin contains a large fraction of 24-carbon acyl cahins [6,12], compared to 65% 18-carbon chains in egg PC [16]. In a well-ordered gel phase the chains are interdigitated (vide infra).

Complex phase behavior has been previously observed for mixtures of two lipids which are each chemically homogeneous (see for example, Refs. 15 and 17). In contrast, natural membranes or extracted membrane lipids usually exhibit broad, featureless phase transitions, reflecting the heterogeneity in lipid species and lipid chain length. The well-defined calorimetric behavior of cerebrosides with phosphatidylcholines, observed in this study, supports the behavior of the cerebroside/egg PC mixtures being interpreted in terms of single components of phospholipid and each glycosphingolipid.

Our calorimetric results can be compared to several new studies of cerebrosides (see also two recent reviews, Refs. 18 and 19). Curatolo and London (personal communication) have found, using a fluorescence quenching technique, an approximately 20 mol% solubility limit for bovine brain cerebroside with egg yolk phosphatidylcholine at 23° C. This is in agreement with our results. Bovine brain cerebroside, in contrast, was found to be immiscible with 1-palmitoyl-2-oleoylPC (POPC) in the gel phase up to 70 mol% [20], perhaps because the saturated chain of POPC is about two carbons shorter than the average egg PC chain.

Bovine brain cerebroside was found to be immiscible with dipalmitoylPC (DPPC) in the gel state up to 46 mol%; phrenosin revealed similar behaviour [21]. These properties are similar to those found here for the cerebroside and phrenosin mixed with DMPC. However, the transition enthalpies of the cerebroside-DPPC system were reported to fall from approx. 8 kcal/mol at $X_c = 0$ to a minimum of approx. 1.5 kcal at $X_c = 0.8$ [21]. These results are in contrast to the near-ideal enthalpies found for cerebroside/DMPC mixtures (see results reported above).

The cerebroside/DPPC study [21] was conducted, however, with very dilute lipid (less than

1% by weight) suspensions. The morphology of these bilayers is probably different (see Refs. 18 and 22) from that of 50% lipid suspensions. Bovine brain kerasin/DPPC mixtures, also in a dilute suspension, exhibited a phase diagram [21] comparable to that of the stable gel phase kerasin/DMPC system reported here, except that approx. 6 kcal/mol transition enthalpy was reported for pure kerasin [21], implying that kerasin attains only the metastable gel state in a dilute suspension. In the latter case a near-ideal phase diagram is perhaps expected (see Fig. 5).

Our results with natural kerasin/DMPC mixtures are supported by recent work with new semi-synthetic kerasins in 30 wt.% aqueous suspensions. Mixtures of β-D-galactosyl-*N*-palmitoyl-D-sphingosine (NPGS), a semi-synthetic kerasin of natural configuration [23], with DPPC were examined by calorimetric and X-ray techniques [24]. The miscibility limit of this kerasin was found to be 20 mol% of kerasin in the gel phase of DPPC. Both this phase and a kerasin-rich phase were confirmed by X-ray analysis to co-exist at higher mole fractions of NPGS [24]. This result agrees well with ours for kerasin/DMPC for $0.25 < X_k < 0.5$, where for $X_k = 0.25$, the liquidus changes slope. Enthalpies for NPGS/DPPC mixtures were roughly ideal for all mole fractions similar to those found here for mixtures with DMPC and egg PC.

Similar limited solubility in the DPPC-rich phase was observed with dihydrolactosylceramides having saturated, 16, 18, or 24 carbons acyl chain lengths [25]. Also, 18 mol% of a C_{16}-glucokerasin was reported to be soluble in DMPC at 25°C by Skarjune and Oldfield [26]; the solubility limit was not determined. In the present case of kerasin/DMPC, three sets of calorimetric peaks for each $X_k > 0.25$ (Fig. 5) may reflect the existence of more than one gel phase enriched in kerasin. For $0.25 \leq X < 1$, most of the kerasin is in a phase (large intermediate peaks, Fig. 3) with PC gel-like packing, as determined from five order parameters obtained from Raman spectra (Fig. 7, Ref. 7). The multiple phases may result from the heterogeneity of the long acyl chains of the natural kerasin, including unsaturation at the C-15–C-16, position, and their differing abilities to mix with the shorter chains of DMPC.

The C-15–C-16 double bond present in half of bovine kerasin may aid, for example, in chain interdigitation and hence the solubility of DMPC in kerasin in the metastable gel phase at high values of X_k. These high values correspond to the naturally occurring proportion of cerebroside to acylPC, which is about two to one in myelin [1]. Also, kerasin contains three times as many unsaturated chains as phrenosin [12], possibly accounting for the grater miscibility of kerasin that phrenosin in DMPC for $X_k < 0.25$.

The shape of the phase diagram for kerasin/egg PC is similar overall to that reported for DPPC/NPGS [24]. Both systems are peritectic with a gel phase miscibility upper limit of about $X = 0.25$.

X-ray diffraction studies of hydrated NPGS [27] and β-D-galactosyl-*N*-lignoceryl-D-sphingosine (NLGS) [28] show that both the stable and metastable gel phases of these kerasins have highly ordered hydrocarbon chains, where the stable phase shows the shorter lamellar repeat (compare 54.5 Å to 58 Å for NPGS [27]). The repeat distances are consistent with tilted chains in the stable phase and vertical chains in the metastable phase. These kerasins also show the 8–9 kcal/mol disparity in ΔH between the two phase types [27,28] exhibited by bovine brain kerasin [8]. This supports our conclusion of isomorphism among these components. This disparity has been attributed to differences in the hydrogen-bonding and hydration properties of the headgroups [27–29]. In addition, Shipley and coworkers identify the metastable gel phase of NPGS as being isomorphic to the solid phase [27]. The Raman spectral order parameter $1/[\Delta\nu_{1/2}\ (1450)]$ for this phase is much lower than for the stable gel phase of kerasin (Fig. 4, Ref. 7), reflecting less hydrocarbon chain order and implying a lower barrier to miscibility with other lipids, as found here with PC.

More than half of the chains in kerasin and phrenosin are 24 carbons long. In a crystal structure of bilayer form, these chains can be accommodated only by interdigitation with the much shorter sphingosine chains. Supportive X-ray data for chain interdigitation are given by Dahlén and Pascher for a solid phase of a ceramide, tetracosanoylphytosphingosine [30], and Raman spectral data for hydrated cerebrosides [7] and a

sphingomyelin, DL-*erythro*-*N*-lignocerylsphingosylphosphocholine, by Levin and coworkers [31] also indicate interdigitated bilayers. Except in cases of fortuitous isomorphism, these dense, near-crystalline packings of gel phase sphingolipids must rearrange upon mixing with other lipids. Regions where this occurs in the present study include the kerasin/egg PC gel for $X < 0.25$ and the fraction of miscible gel phase kerasin/DMPC for $X > 0.5$. In such mixtures, chain interdigitation occurs in a less dense central zone of the bilayer.

In summary, we have demonstrated that kerasin and phrenosin exhibit differing abilities to mix with PC either as a binary or ternary mixture. We suggest that these differences may persist under physiological conditions in, for example, myelin, a system of high lipid and limited water content which exists partly in the gel state at 37°C.

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