PHASE BEHAVIOR OF GANGLIOSIDE-LECITHIN MIXTURES

RELATION TO DISPERSION OF GANGLIOSIDES IN MEMBRANES

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ABSTRACT Ganglioside G_{M1} and mixed brain gangliosides were mixed with 1-stearoyl-2-oleoyl lecithin (SOPC) and examined by differential scanning calorimetry as a function of ganglioside content and temperature. Low mole fractions of ganglioside G_{M1} and of mixed brain gangliosides are shown to be miscible with SOPC in the gel phase up to $X = 0.3$, with the possible exception of a small region of immiscibility for the mixed brain gangliosides system centered around $X = 0.05$. Above $X = 0.3$, the low-temperature phases demix into a (gel) phase of composition $X = 0.3$ and a (micellar) phase of composition $X = 1.0$. Above the endothermic phase transition temperature, no phase boundaries are discerned. It is pointed out that phase structures need to be determined in each domain delineated in the phase diagrams, and that cylindrical phases may exist at higher temperatures and intermediate compositions. The effects of addition of wheat germ agglutinin, which binds to ganglioside G_{M1}, on a ganglioside G_{M1}-SOPC mixture ($X = 0.5$), are described and interpreted in terms of partial demixing of ganglioside and lecithin. Behavior of the ganglioside-SOPC system is discussed with respect to the kinetics of cholera toxin action in lymphocytes, as well as to other physiological roles of gangliosides in membranes.

INTRODUCTION

Gangliosides, which are sialic acid-bearing glycosphingolipids, function in the cell membrane as cell surface and hormone receptors, immunogenic groupings, and ligands for cell-cell contacts, although their roles may be inferior to those of glycoproteins (1–6). In particular, the cell membrane ganglioside G_{M1} (galactosyl-*N*-acetylgalactosaminyl [*N*-acetylneuraminyl] galactosylglycosylceramide) has been shown to bind cholera toxin with a stoichiometry of five or six ganglioside molecules per cholera toxin oligomer (7–9). The delay time observed for cholera toxin activation or for cell capping to occur after addition of toxin to cells has been interpreted as a lateral diffusion time, during which each toxin oligomer locates and binds to a full complement of ganglioside molecules, which apparently are initially well-mixed with other lipids in the cell surface (10–12). In addition, ganglioside G_{M1} binds wheat germ agglutinin (WGA) (13, 14), a lectin used to study surface binding and information transduction at cell membrane interfaces. Agglutinins by definition have more than one ligand; lectins are specific for oligosaccharide moieties of glycoproteins or glycolipids (13, 14).

These biological phenomena raise questions of the miscibility and phase behavior of gangliosides when combined with other membrane lipids. We describe here the behavior of some ganglioside-lecithin phases, treated as binary lipid mixtures, which resemble a ganglioside-containing membrane or local area of a cell membrane. Phase diagrams and latent heats of endothermic phase transitions for the lipid mixtures are determined by differential scanning calorimetry. The effects of addition of WGA are also examined.

METHODS

L-α-1-stearoyl-2-oleoyl lecithin (SOPC) was obtained from Applied Science Labs., Inc. (State College, Pa.); monosialoganglioside G_{M1}, disialoganglioside G_{D1a} (*N*-acetylneuraminylgalactosyl-*N*-acetylgalactosaminyl [*N*-acetylneuraminyl] galactosyl-glycosylceramide), and mixed bovine brain gangliosides from Supelco, Inc. (Bellefonte, Pa.); and purified WGA from Miles-Yeda, Inc. (Rehovot, Israel). The main component of beef brain gangliosides is usually G_{D1a} (footnote 1, 15, 16). Purity of lipids was checked by TLC in the solvent chloroform:methanol:0.25% $CaCl_2$ (60:35:8, vol:vol:vol). Lipids were dried thoroughly under N_2 and then under vacuum at 30°C. Lipid mixtures of given composition were prepared by redissolving components of known dry weight in solvent, usually chloroform-methanol, using mol wt of 788, 1,503, 1,780, and 1,700 for SOPC, monosialoganglioside G_{M1}, disialoganglioside G_{D1a}, and mixed gangliosides, respectively, to calculate mole fractions. The mixtures were again dried to remove all traces of solvent, and rehydrated in 50 mM phosphate buffer (K^+ as cation), pH 7.2, to 75% water content.

WGA supplied in saline solution was dialyzed overnight at 4°C against dilute phosphate buffer. The protein concentration before and after dialysis was monitored by absorbance at 280 nm. When WGA was added to dilute lipid suspensions prepared as described above, samples were equilibrated at 30°C for 15 min and then lyophilized to reduce the aqueous volume in preparation for calorimetric scanning.

Samples containing 1–2 mg lipid were placed in tared aluminum pans and hermetically sealed by cold welding. Wet weights of samples were determined before and after calorimetric scanning and were invariant; dry weights were determined after scanning by pricking holes in pans and drying samples at 70°C to constant weight. The samples were annealed before calorimetric scanning by cycling for several hours between −20°C and the upper transition temperature. A Perkin-Elmer DSC-2 differential (Perkin-Elmer Corp., Instrument Div., Norwalk, Conn.) was used to study the thermal properties of the lipids. The usual scan rate was 10°C/min; rates down to 2.5°C/min were used to search for rate-dependent effects. All samples contained freezable water, and calorimetric scans were reproducible over periods up to a week; hence lipid samples were fully hydrated and fully mixed.

Van Dijck and co-workers reported that overnight cooling below the phase transition temperature is essential for equilibration to construct accurate heating curves for mixed lecithins (18). In light of this report, the phase diagram for ganglioside G_{M1}-SOPC, constructed from a set of heating scans collected after 72-h equilibration below 0°C, without any intervening warming, was compared to the phase diagram constructed from data omitting low-temperature equilibration (Fig. 4). A significantly lower liquidus was found after low-temperature equilibration for the mole fractions $X_{GM1} = 0.5$ and $X_{GM1} = 0.7$; other points were comparable. By analogy, the upper edges of heating curves in the same region of the phase diagram for mixed brain gangliosides-SOPC (Fig. 5), obtained without the low-temperature equilibration, may be about 3° high.

Transition enthalpies were obtained from areas under the heat uptake/release curves measured with a planimeter. Phase transition boundaries were taken as the intercept of the straight sides of transition peaks with the baseline. The endothermic peak area of the main, gel-to-liquid crystalline phase transition of fully hydrated dimyristoyl lecithin was used as an enthalpy standard. The observed value of 6.4 kcal/mol, obtained by standardization against an indium transition, may be compared to the average

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[1]Formisano, S., M. L. Johnson, G. Lee, S. M. Aloj, and H. Edelhoch. 1979. Critical micelle concentrations of gangliosides. Submitted to *Biochemistry*.

of reported values of 6.6 kcal/mol (18, 19). The value of 4.3 kcal/mol similarly obtained for pure SOPC is rather low for a lecithin with C_{18} acyl chains (cf. 6.4 kcal for DML with C_{14} chains).

RESULTS

Pure Gangliosides

Mixed bovine brain gangliosides, which form a micellar phase in excess water (17), exhibit two endothermic peaks, at 27 and 46°C (Figs. 3 and 5, $X_G = 1.0$). The two peaks apparently do not reflect disproportionation into two types of ganglioside structures of different average charge per headgroup, because monosialated ganglioside G_{M1} also exhibits two peaks of comparable size located at 26 and 43°C (Figs. 3 and 4, $X_{GM1} = 1.0$). Disialated ganglioside G_{D1a}, however, exhibits one very broad peak centered at 12°C, with a minor one at 40°C (not shown).

A 1:1 mole ratio, ganglioside G_{M1}:ganglioside G_{D1a} mixed phase exhibits two peaks at 24 and 40°C (not shown), resembling those of G_{M1} alone. The molar transition enthalpies for G_{M1}, G_{D1a}, and the 1:1 mixed phase, determined from the heating curves, are 2.6, 3.0, and 2.7 kcal/mol, respectively. The transition enthalpy for mixed brain ganglioside micelles was found to be 3.9 kcal/mol. Cooling curves gave consistently lower enthalpies. Because of peak breadth, peak areas, although reproducible, have an error of ~10% due to uncertainty in baseline.

Phases Containing Gangliosides and SOPC

In these experiments, L-α-1-stearoyl-2-oleoyl lecithin (SOPC) was used as a lecithin component of chain length and unsaturation both representative of lecithin in cell membranes and compatible with that of gangliosides. Monosialogangliosides contain primarily sphingosine and fatty acid moieties of C_{18} chain length (20). Disialogangliosides, the main species in mixed bovine brain gangliosides (footnote 1, 15, 16), and trisialogangliosides have a somewhat higher C_{20}-sphingosine content (16). Preliminary calorimetric experiments showed, as anticipated from the disparity in chain lengths, that dimyristoyl lecithin does not mix with low mole fractions of gangliosides.

The heat uptake curves obtained for the two systems examined here, ganglioside G_{M1}-SOPC and mixed brain gangliosides-SOPC, are shown in Figs. 1–3. Phase diagrams were constructed from these curves, with the assumption that ganglioside G_{M1} and mixed brain gangliosides each behave as a single lipid component and that the glycolipid-phospholipid systems can thus be treated as binary lipid systems (Figs. 4 and 5). In this treatment, with water present in excess and pressure constant, the maximum number of lipid phases (with zero degrees of freedom) is given as three by the phase rule. In fact, the upper limit may possibly be greater than three for the ganglioside G_{M1}-SOPC system because of slight ganglioside chain heterogeneity, and greater than three in the mixed brain gangliosides-SOPC system primarily because of ganglioside headgroup heterogeneity.

For ganglioside G_{M1}-SOPC mixtures, the positive slope of the solidus at mole fractions of G_{M1}, X_{GM1}, below 0.32 indicated miscibility of glycolipid and phospholipid in the gel phase in this region (Fig. 4). For mixed brain gangliosides-SOPC mixtures, the solidus in the phase diagram has a positive slope below $X_G = 0.32$, with a possible but uncertain short horizontal segment at $X_G = 0.05$ (Fig. 5), indicating miscibility in the gel phase throughout most of this

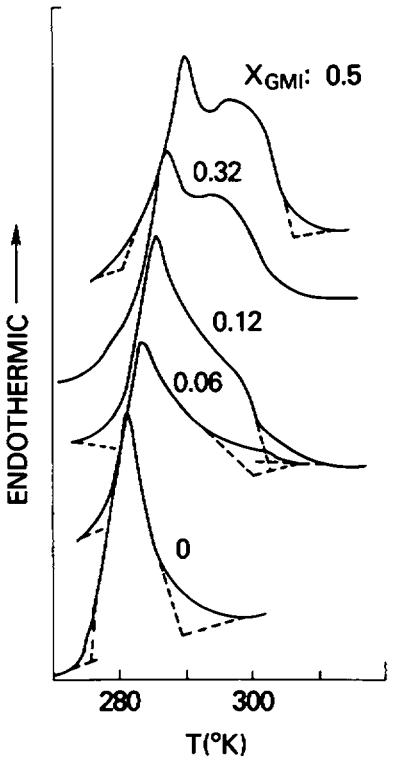

FIGURE 1

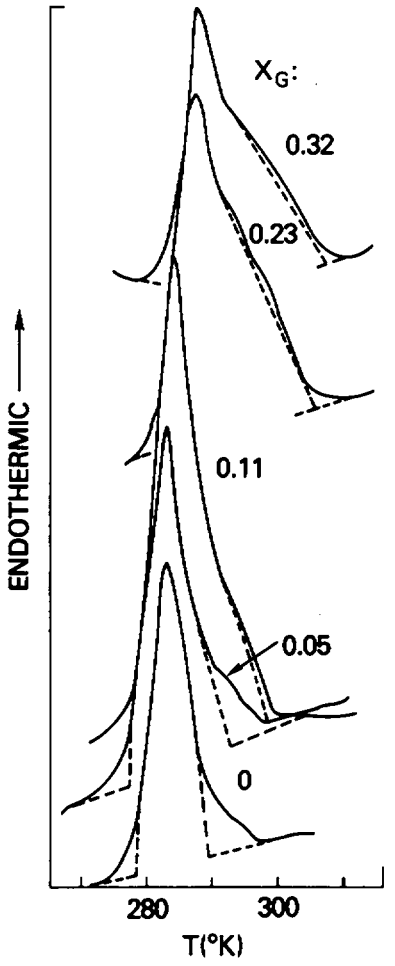

FIGURE 2

FIGURE 1 Heat uptake curves for ganglioside G_{MI}-SOPC mixtures for low mole fractions, X_{GMI}, of ganglioside G_{MI}. Values for X_{GMI} are given on each curve. Scan rate for all scans shown was 10°C/min. Dotted lines show intersections of peak sides with baseline, used for construction of Figs. 4 and 5. Curves shown are not normalized to sample weight.

FIGURE 2 Heat uptake curves for mixed brain gangliosides-SOPC mixtures for low values of X_G. For explanation see legend to Fig. 1.

region. The positive slope of the solidus in both systems also reflects the fact that the partial molar transition enthalpies of the gangliosides are roughly comparable to, i.e., not much smaller than, that of the lecithin, and thus the gangliosides, upon mixing with SOPC, exert experimentally significant shifts in the endotherms. The molar transition enthalpies for ganglioside G_{MI} and mixed brain gangliosides (in the fully hydrated micellar phases) were found to be 60 and 90%, respectively, that of SOPC (in the fully hydrated lamellar phase); the partial molar enthalpies of gangliosides may be increased when gangliosides are incorporated at low mole fractions into extended lecithin lamellae.

For both ganglioside-SOPC systems, the positive slope of the solidus transforms to zero

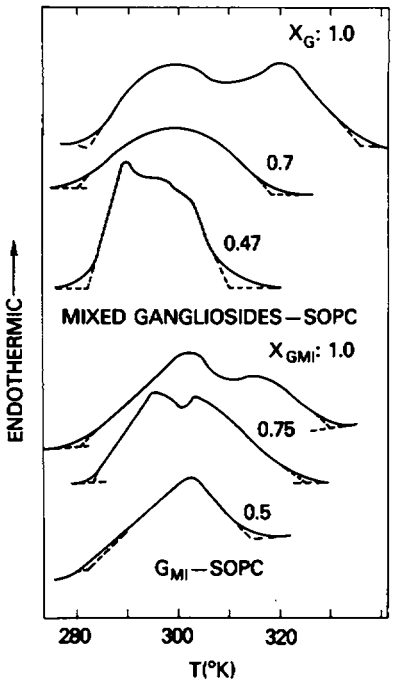

FIGURE 3

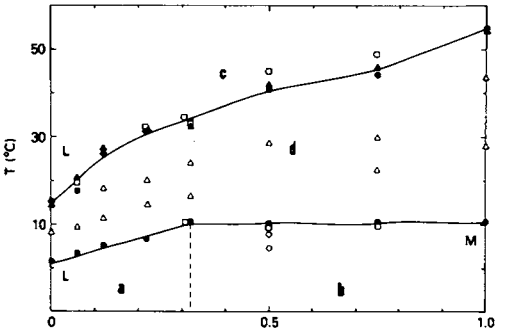

FIGURE 4

FIGURE 3 Heat uptake curves for mixed brain gangliosides-SOPC mixtures (upper three curves) and for ganglioside G_{MI}-SOPC mixtures (lower three curves) for high values of X. For explanation see legend to Fig. 1.

FIGURE 4 Phase diagram plotted as a function of X_{GMI} for mixtures of ganglioside G_{MI}-SOPC suspended in 50 mM phosphate buffer, pH 7., obtained as in Fig. 1. The experimental points are from (□) heating curves obtained after extensive sample annealing, and (▲) heating and (●) cooling curves obtained after sample annealing followed by equilibration for 72 h at −20°C. Peak temperatures (△) are also noted. Temperatures were corrected for calorimetric scanning rate; no correction was made for finite transition widths for pure components. Open circles (○): solidus points from heating curves after addition of WGA at mole ratios, WGA:SOPC, of 6:100 (upper circle) and 12:100 (lower circle). Lipid phases in each domain are: (*a*) gel: (*b*) gel (X_{GMI} = 0.32) plus low-temperature micellar (X_{GMI} = 1.0); (*c*) incompletely known: liquid crystalline for low values of X_{GMI}, and probably micellar (*M*) for high values of X_{GMI}; (*d*) incompletely known (see text). The letter *L* in domains *a* and *c* indicates lamellar phase for low X_{GMI}.

slope at X = 0.3 (Figs. 4 and 5). The simplest explanation for this behavior is that the more ordered (lower-temperature) lipid phases are demixing above X = 0.3 into two phases of composition, X = 0.3 and X = 1.0.

As the mole fraction of gangliosides increases above 0.1, the width of the phase transition increases and each transition peak develops a shoulder and gradually assumes double-peak character (Figs. 1–5). Temperatures of peak shoulders and maxima are noted in Figs. 4 and 5. The heat uptake curves revert briefly to symmetric, single-peak character at X_{GMI} = 0.5 and X_G = 0.7 (Figs. 4 and 5, respectively), suggesting more complicated phase behavior. In the case of the mixed gangliosides-SOPC system, the broad single peak can be deconvoluted into two peaks, so the single-peak appearance may be deceptive. But for the ganglioside G_{MI}-SOPC system, it appears that the lower transition is lost at X_{GMI} = 0.5.

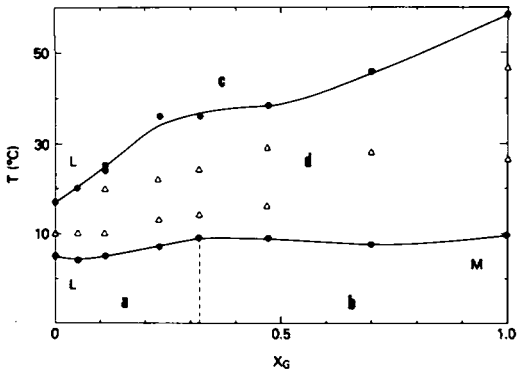

FIGURE 5 Phase diagram plotted as a function of X_G for mixed brain gangliosides-SOPC, suspended in 50 mM phosphate buffer, pH 7. The experimental points (●) are obtained from heating curves. Peak temperatures (△) are also noted. Phases in domains *a-d* are as explained in Fig. 4.

Above the liquidus, the higher-temperature phases are known to be lamellar for very low X, and micellar for high X (see Discussion). The resolution of curve shape for the liquidus is not good enough to speculate on the exact location of the lamellar-micellar phase boundary for the higher-temperature phases. The two-peak nature of the endotherms, including those of pure gangliosides, suggests there may be additional phase boundaries within the transition boundaries drawn in Figs. 4 and 5, as well.

The total transition enthalpies (the areas under the heat uptake curves) have been measured and plotted as a function of mole fraction (Figs. 6 and 7). The accuracy of the measured enthalpies is about 5% for small mole fractions of gangliosides because of narrower

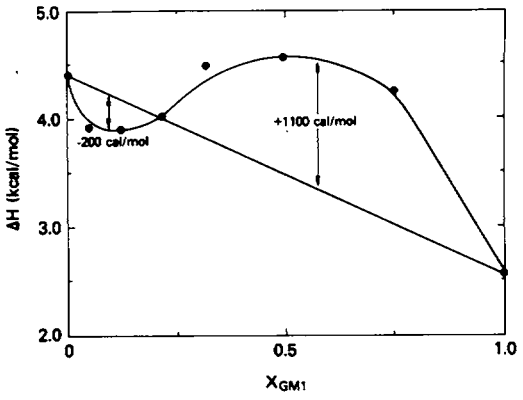

FIGURE 6

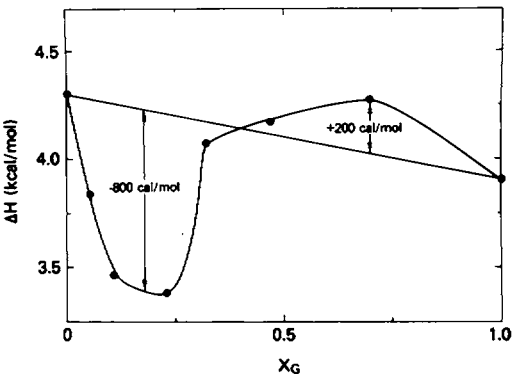

FIGURE 7

FIGURE 6 Transition enthalpies in the ganglioside G_{M1}-SOPC system as a function of X_{GM1}. Experimental conditions are the same as in Fig. 4. The straight line is the theoretical transition enthalpy curve for an ideal mixture, i.e., the enthalpy calculated by assuming additivity of the molar enthalpies of the pure components weighted by their mole fractions in the mixtures. Maximum observed values of the net excess enthalpy, $H_{net}^{ex}(X)$, are noted on the plot.

FIGURE 7 Transition enthalpies in the mixed brain gangliosides-SOPC system as a function of X_G. Experimental conditions are the same as in Fig. 5. See Fig. 6 for explanation.

peaks and better determined baselines, compared with the high-ganglioside regime, where the error is ~10%, as mentioned earlier. The measured transition enthalpies represent the enthalpy differences between lipid phases above and below the endothermic phase transitions. The straight lines plotted in Figs. 6 and 7 are the sums of partial molar transition enthalpies for ideal lipid mixtures (with zero heats of mixing) undergoing a single endothermic phase transition at all mole fractions. The deviation of the measured transition enthalpies from the straight line plots for ideal mixtures gives the net excess enthalpy as a function of mole fraction,

$$H^{ex}_{net}(X) = H^{ex}_{h}(X) - H^{ex}_{l}(X), \tag{1}$$

where ex = excess, h = higher-temperature phase, and l = lower-temperature phase. The quantities on the right-hand side of Eq. 1 are not separately experimentally determined. These excess enthalpies represent the sum of processes occurring with changing mole fraction, including heats of mixing that compose part of the excess enthalpies.

For the two ganglioside-SOPC systems, the net excess enthalpy is negative below X_{GM1} = 0.22 ± 0.07 and X_G = 0.35 ± 0.07 (Figs. 6 and 7, respectively). At these mole fractions, a change in sign occurs. These points correspond adequately with the independently determined break points of the solidi in the phase diagrams of Figs. 4 and 5; the agreement is better for the mixed gangliosides-SOPC system. These points indicate the approximate values of X at the boundaries between regions containing different phase structures.

For low X_{GM1} (Fig. 6), the maximum value of H^{ex}_{net}, at X_{GM1} = 0.12, is −200 ± 200 cal/mol. This value of H^{ex}_{net}, if assigned as a positive heat of mixing of the gel phase, is consistent within experimental error with total miscibility of ganglioside G_{M1} and SOPC in the gel phase, in agreement with the positive slope of the solidus in the phase diagram (Fig. 4). The small loss of total transition enthalpy in this region is also consistent with maintenance of a lamellar phase about as well ordered as that of pure SOPC.

For the mixed gangliosides-SOPC system (Fig. 7), the maximum value of H^{ex}_{net} of −800 cal/mol at X_G = 0.18 is too large to be due wholly to the difference between the excess heats of mixing of higher- and lower-temperature phases, as explained below. Rather, the large loss in transition enthalpy can be interpreted as reflecting progressive loss of order, especially with respect to hydrocarbon chain packing in the gel phase, as X_G increases. Part of H^{ex}_{net} can be assigned to a positive excess heat of mixing centered about X_G = 0.05 for the gel phase, consistent with the small but uncertain region of immiscibility apparent in Fig. 5.

To clarify the relation between the excess heat of mixing and the shape of the phase diagram, we have fitted by computer the phase diagrams in the region of biological interest, that of low ganglioside content. To focus on this region (below X = 0.3), the ganglioside-SOPC systems were idealized to be lamellar for all ganglioside contents. Nonideal mixing in the gel phase is described by an excess enthalpy of mixing, $h_{mix}(X)$, a function with a single maximum similar to that used by van Dijck and co-workers (18). Phase diagrams were constructed using both the equal-G technique of Oonk and Sprenkels (21) and an approach similar to that of Lee (section IIC of reference 22). The solidus is shown by this model to be sensitive to small changes in the value of h of the gel phase (Fig. 8). Assignment of h = 50 cal/mol to the gel phase produces a wholly miscible system (Fig. 8 *a*). This phase diagram up to X = 0.3 satisfactorily models the behavior of G_{M1}-SOPC system. Raising h to 100 cal/mol

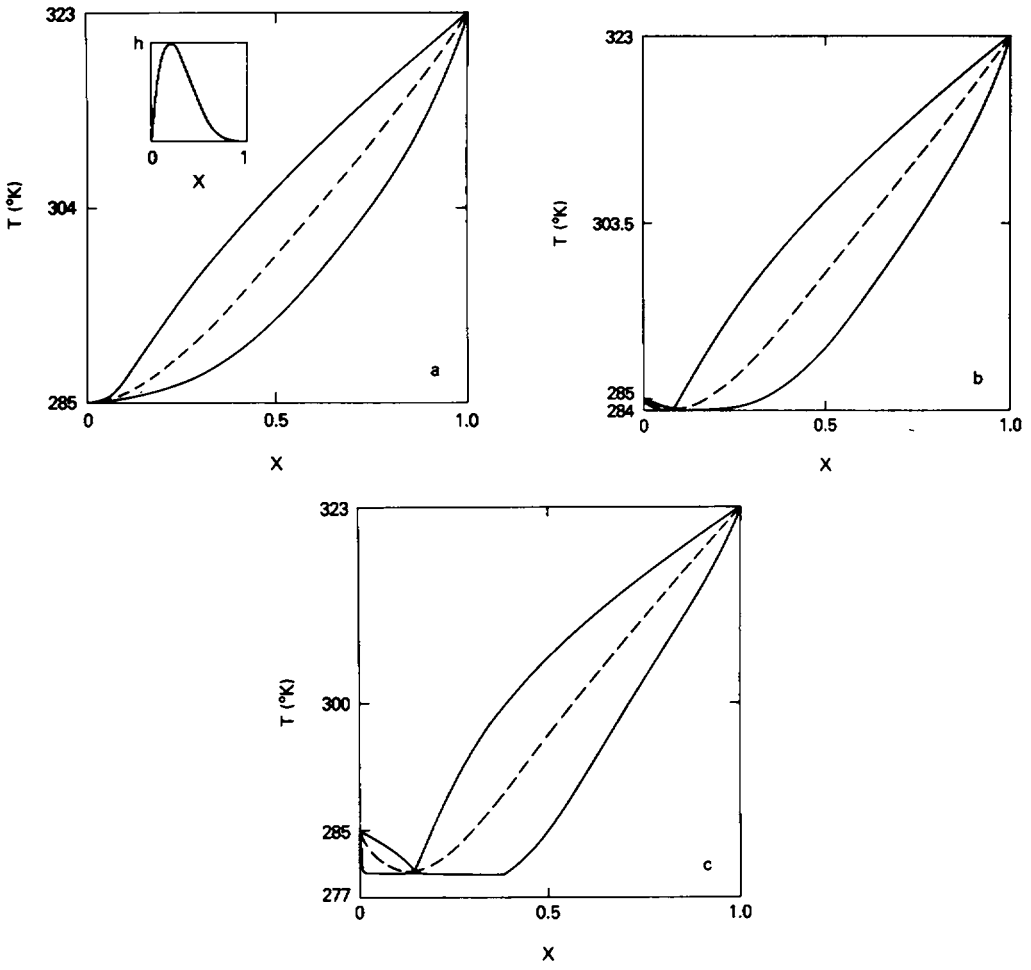

FIGURE 8 Variation of shape of phase diagram, temperature vs. X, with the excess heat of mixing $h_{mix}(X)$. The liquid crystalline phase is assumed ideal ($h_{mix} = 0$). For the gel phase, $h_{mix}(X)$ is represented by the function $hf(m, n)\ X^n\ (1 - X)^m$, where h is the maximum value of $h_{mix}(X)$, and $f(m,n)$ is a normalization factor. The pure lecithin and pure ganglioside phases have been assigned transition enthalpies of 6.5 and 5.0 kcal/mol, respectively; for the excess function, $n = 1$ and $m = 4$. Values of h are set at (*a*) 50 kcal/mol. Inset: the function $h_{mix}(X)$. (*b*) 100 cal/mol. (*c*) 200 cal/mol. Dotted lines are equal-G curves (21).

yields a phase diagram exhibiting a minimum at $X = 0.1$ and a peritectic equilibrium between $X = 0.12$ and 0.18 (Fig. 8 *b*). An intermediate value of $h = 75$ cal/mol (not shown) produces a phase diagram with a peritectic equilibrium similar to that found near $X = 0.1$ for mixed gangliosides-SOPC (compare Fig. 5). A eutectic equilibrium between $X = 0.03$ and $X = 0.4$ at a temperature below that of the phase transition temperature of pure SOPC is produced when h is raised to 200 cal/mol (Fig. 8 *c*). This behavior is unlike that observed in the ganglioside-SOPC systems, setting an upper limit on the amount of excess heats that can be identified as heats of mixing.

At higher ganglioside contents ($X > 0.3$), the total transition enthalpies deviate positively

from ideality, with maxima in H_{net}^{ex} of about 1,100 cal/mol at $X_{GM1} = 0.5$ and 200 cal/mol at $X_G = 0.7$ (Figs. 6 and 7). The maximum value of the transition enthalpy for both systems in this region is 4.3 kcal/mol. The mole fractions for these maxima correspond to those points at which the endotherms reduce to single-peak character, as previously noted.

Effects of WGA on G_{M1}-SOPC Phases

Samples of ganglioside G_{M1}-SOPC suspensions ($X_{GM1} = 0.5$) were titrated with WGA at molar ratios, WGA:ganglioside, of 3:100, 6:100, and 12:100, respectively. At the lowest ratio of WGA to ganglioside, no significant changes in the endotherms were seen. For the two higher ratios, the solidus was shifted to lower temperatures, from 10 to 7.4 and 4.5°C, respectively (average from heating and cooling curves, Fig. 10 *a*). The heating curves exhibited slightly less depression of the solidus (Figs. 5 and 9). The centers of the endothermic peaks and the liquidus points were unshifted, i.e., the transitions were broadened. The endotherms also exhibit shoulders, especially pronounced for the WGA:G_{M1} ratio of 12:100 (Fig. 9). This behavior is consistent with formation of a phase enriched in lecithin upon partial segregation of ganglioside by lectin, so that the endothermic transition in the WGA-containing system encompasses a lipid mixture heterogeneous in local composition.

In addition, in the latter two samples, the heats of transition per milligram of lipid were found to increase by 37 and 72%, respectively, to 6.2 and 7.7 kcal/total moles lipid (Fig. 10 *b*). These large enthalpy values, greater than those found at any point in the phase diagram for ganglioside G_{M1}-SOPC (compare Fig. 6), imply that both ganglioside G_{M1} and bound lectin are well-organized components of the bilayer and that both contribute substantially to the transition enthalpy. It is possible that the addition of WGA induces a disproportionation of the phase structures towards a higher lamellar phase content at the expense of cylindrical or

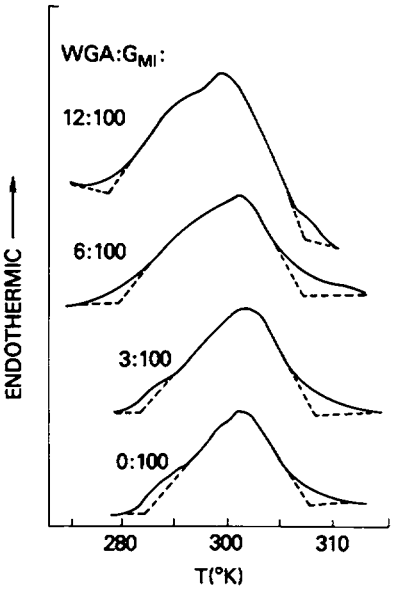

FIGURE 9 Heat uptake curves for G_{M1}-SOPC mixtures ($X_{GM1} = 0.5$) upon addition of WGA at molar ratios, WGA:G_{M1}, shown on the curves.

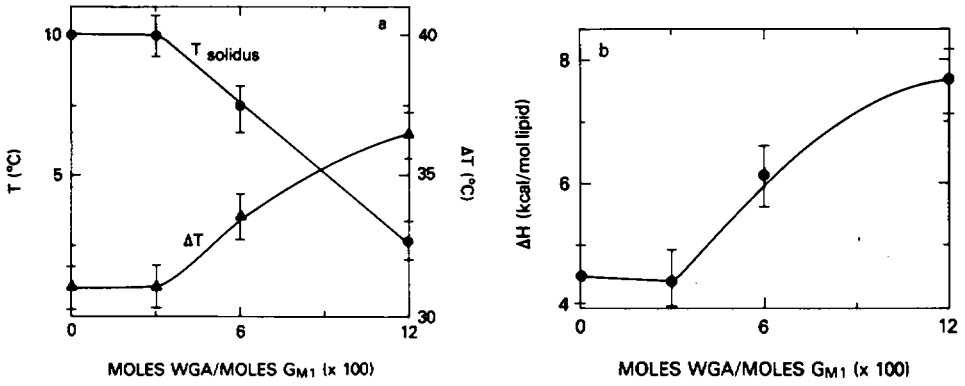

FIGURE 10 (*a*) Lowering of solidus (●) and increase in phase transition width (▲) in °C for ganglioside G_{M1}-SOPC mixtures (X_{GM1} = 0.5) upon addition of WGA. The system was suspended in phophate buffer, pH 7.2, at a final concentration of 50 mM buffer. (*b*) Increase in transition heats of ganglioside G_{M1}-SOPC mixtures, upon addition of WGA.

micellar structure, that is, that part of the increased transition enthalpy is contributed by the melting of a phase with characteristically better-ordered chains.

DISCUSSION

Ganglioside Phases

The double enthalpic peak we observed for mixed brain gangliosides and for G_{M1}, seen also by Curatolo and co-workers by differential scanning calorimetry, persists in the mixed brain gangliosides-water system at lower hydrations at which these gangliosides form a cylindrical phase (17). The higher-temperature enthalpic peak (above 35°C) in the cylindrical phase is associated with a gradual increase in the x-ray diffraction spacing of the hydrocarbon chains from 4.2 to 4.5 Å, that is, with observable increase in chain disorder (17). At full hydration, the higher peak is still presumably associated with chain melting in the micelle. No structural changes are known for the lower peak. The headgroup comprises half the bulk of the ganglioside molecule; changes in packing efficiency and hydrogen-bonding in the polar half of the molecule may be reflected in the lower peak.

Phases Containing Gangliosides and SOPC

The structures of ganglioside-lecithin phases at intermediate compositions have been little studied, mostly at temperatures lying within the endothermic phase transition range. The present work, which maps these phase transition boundaries, indicates those domains that lie either completely above, within, or below the phase transition range and for which phase structure determinations are much needed.

Fully hydrated lecithin (X = 0) undergoes a gel-to-liquid crystalline phase transition and, as described above, pure gangliosides (X = 1.0) appear to be micellar and undergo at least one transition involving increased chain disorder. For low values of X, the ganglioside-lecithin structure is likely to remain lamellar. It follows that the demixed, higher-ganglioside content phases at low temperatures of composition X = 0.3 and X = 1.0 (Figs. 4 and 5) have gel and

micellar structures, respectively. In a study of suspensions of mixed brain gangliosides-egg lecithin, Hill and Lester identified lamellar, cylindrical, and micellar phases with increasing ganglioside content by sedimentation studies in 10 mM salt, electron microscopic appearance, and phase texture (23). A fully sedimentable lamellar phase was found by these workers to exist in the mixed lipid system up to $X_G = 0.3$, in excellent agreement with the conclusions drawn here.

The phase structures at higher temperatures are less certain. The following data suggest that at higher temperatures a stable cylindrical phase exists at intermediate compositions. In excess aqueous solution at 23°C, the lipid phases studied here appear turbid below $X = 0.3$, clear and nonviscous above $X = 0.75$, but clear and highly viscous between 0.3 and 0.75. Further, as previously mentioned, a hexagonal phase has been observed for mixed brain gangliosides at water contents between 18 and 50% (17). A cylindrical phase is also seen for fully hydrated gangliosides upon addition of cholesterol (24). A metastable cylindrical phase was also seen by Hill and Lester (23) in mixed brain gangliosides-egg lecithin at $X_G = 0.5$ at a temperature lying within the phase transition range. The acquisition of more phase structural data at controlled temperatures is strongly needed.

The functional dependence of transition heats upon composition, for gangliosides-SOPC, is shown here to aid in location of phase boundaries and to indicate large changes in state. Excess heats of mixing functions, as employed above, are commonly used as parameters for construction of phase diagrams for nonideal binary lipid mixtures. For these mixtures, assignment of a value of h to the gel phase at most 200 cal/mol larger than assigned to the liquid crystalline phase has been found sufficient to represent demixing in the gel phase evident at phase transition temperatures (18, 22). Our calculated phase diagrams agree with these results.

WGA has previously been noted to bind to and induce fusion of lecithin vesicles containing more than a 5 mol % threshold concentration of ganglioside G_{M1} (13). The glycolipid was specifically required for these effects (13, 14). A patching phenomenon (aggregation of ganglioside molecules in the plane of the bilayer) was postulated to occur before vesicle aggregation and fusion (13). This concept is supported by the heterogeneity in local lipid composition deduced from the endotherms of the WGA-ganglioside G_{M1}-SOPC system described here (Figs. 9 and 10).

The partial demixing of gangliosides and lecithin shown here upon the lowering of temperature or the addition of WGA has the following physiological implications. Receptor molecules dispersed in a membrane surface are efficient sensors of agents suspended in the external medium (25). Patching or demixing converts the population of dispersed receptors into fewer, condensed sites, often of high cooperative binding affinity. Both stable and induced clustering of ganglioside molecules in cell membranes has been inferred, supporting the association with a receptor-type role. In the present study, ganglioside G_{M1} was found to be miscible with SOPC below $X_{GM1} = 0.3$ This result is consistent with the deduction that ganglioside G_{M1} molecules, which act as cholera toxin receptors, are well mixed with other classes of lipids in lymphocyte membranes, resulting in the lateral diffusion time delay seen for activation of cholera toxin after addition of toxin to cells (10–12).

Gangliosides appear to accumulate in regions of the outer surface of certain specialized cell membranes, such as in microvillous protrusions of absorptive epithelial cells of small intestine,

and portions of synaptic clefts (26). The fatty acid residues of brain gangliosides, as used here, are typically C_{18}, whereas gangliosides of other tissues contain a higher proportion of C_{22} and/or C_{24} fatty acids (20, 27). The longer-chain character, and correspondingly large van der Waals chain interactions, should exert pressure towards ganglioside segregation in plasma membranes of the latter character, as well as towards association of ganglioside molecules with other longer-chain components, especially with sphingomyelin and cerebrosides. In a separate paper, we demonstrate by calorimetric measurements that longer chain cerebrosides are immiscible at low mole fractions with some lecithins;[2] however, in this case, as shown by Raman spectroscopic measurements, very strong headgroup interaction among cerebroside molecules also contributes to demixing of lipids.[3] In the general case, for both C_{18} and longer-chain gangliosides, the presence of external ligands can induce or maintain clustering of ganglioside molecules, as exemplified by the effects of WGA and cholera toxin.

One of us (Dr. M. R. Bunow) would like to thank Dr. N. L. Gershfeld for use of the calorimeter and excellent critical advice, and Dr. I. W. Levin for laboratory supplies.

Received for publication 21 August 1978 and in revised form 12 March 1979.

REFERENCES

1. HAKOMORI, S. I., and R. W. JEANLOZ. 1970. Glycolipids as membrane antigens. *In* Blood and Tissue Antigens. D. Aminoff, editor. Academic Press, Inc. N.Y. 149.
2. EDELMAN, G. M. 1976. Surface modulation in cell recognition and cell growth. *Science* (*Wash. D.C.*). **192**:218.
3. FISHMAN, P. H., and R. O. BRADY. 1976. Biosynthesis and function of gangliosides. *Science* (*Wash. D.C.*). **194**:906
4. FISHMAN, P. H., and R. O. BRADY. 1975. Modification of membrane glycolipids by oncogenic agents. *In* Modification of Lipid Metabolism. E. G. Perkins and L. A. Witting, editors. Academic Press Inc., N.Y. 105.
5. LAINE, R. A., K. STELLNER, and S. I. HAKOMORI. 1974. Isolation and characterization of membrane glycosphingolipids. *Meth. Membrane Biol.* E. D. Korn, editor. **2**:205.
6. MULLIN, B. R., T. PACUSZKA, G. LEE, L. D. KOHN, R. O. BRADY, and P. H. FISHMAN. 1978. Thyroid gangliosides with high affinity for thyrotropin: potential role in thyroid regulation. *Science* (*Wash. D.C.*). **199**:77.
7. CUATRECASAS, P. 1973. Interaction of *Vibrio cholerae* enterotoxin with cell membranes. *Biochemistry*. **12**:3547.
8. HOLMGREN, J., I. LÖNNROTH, and L. SVENNERHOLM. 1973. Tissue receptor for cholera: postulated structure from studies with G_{M1} ganglioside and related glycolipids. *Infect. Immun.* **8**:208.
9. FISHMAN, P. H., J. MOSS, and J. C. OSBORNE, JR. 1978. Interaction of choleragen with the oligosaccharide of ganglioside G_{M1}: evidence for multiple oligosaccharide binding sites. *Biochemistry*. **17**:711.
10. RÉVÉSZ, T., and M. GREAVES. 1975. Ligand-induced redistribution of lymphocyte membrane gangliosides G_{M1}. *Nature* (*Lond.*). **257**:103.
11. CRAIG, S. W., and P. CUATRECASAS. 1975. Mobility of cholera toxin receptors on rat lymphocyte membranes. *Proc. Natl. Acad. Sci. U.S.A.* **72**:3844.
12. SEDLACEK, H. H., J. STAERK, F. R. SEILER, W. ZIEGLER, and H. WIEGANDT. 1976. Cholera toxin induced redistribution of sialoglycolipid receptor at the lymphocyte membrane. *FEBS* (*Fed. Eur. Biochem. Soc.*) *Lett.* **61**:272.
13. REDWOOD, W. R., and T. G. POLEFKA. 1976. Lectin-receptor interactions in liposomes. II. Interaction of wheat germ agglutinin with phosphatidylcholine liposomes containing incorporated monosialoganglioside. *Biochim. Biophys. Acta.* **455**:631.
14. BOLDT, D. H., S. F. SPECKART, R. L. RICHARDS, and C. R. ALVING. 1977. Interactions of plant lectins with glycolipids in liposomes. *Biochem. Biophys. Res. Commun.* **74**:208.

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[2]Bunow, M. R. 1979. Manuscript in preparation.

[3]Bunow, M. R., and I. W. Levin. 1979. Manuscript in preparation.

15. BARTON, N. W., and A. ROSENBERG. 1973. Action of *Vibrio cholerae* neuraminidase (sialidase) upon the surface of intact cells and their isolated sialolipid components. *J. Biol. Chem.* **248**:7353.
16. YOHE, H. C., D. E. ROARK, and A. ROSENBERG. 1976. C_{20}-sphingosine as a determining factor in aggregation of gangliosides. *J. Biol. Chem.* **251**:7083.
17. CURATOLO, W., D. M. SMALL, and G. G. SHIPLEY. 1977. Phase behavior and structural characteristics of hydrated bovine brain gangliosides. *Biochim. Biophys. Acta.* **468**:11.
18. VAN DIJCK, P. W. M., A. J. KAPER, H. A. J. OONK, and J. DEGIER. 1977. Miscibility properties of binary phosphatidylcholine mixtures. A calorimetric study. *Biochim. Biophys. Acta.* **470**:58.
19. LEE, A. G. 1977. Lipid phase transitions and phase diagrams. I. Lipid phase transitions. *Biochim. Biophys. Acta.* **472**:237.
20. LEDEEN, R. 1966. The chemistry of gangliosides: a review. *J. Am. Oil Chem. Soc.* **43**:57.
21. OONK, H. A. J., and A. SPRENKELS. 1969. Types of TX phase diagrams derived from Gibbs functions with the help of the equal-G curve. *Rec. Trav. Chim. Pays-Bas Belg.* **88**:1313.
22. LEE, A. G. 1977. Lipid phase transitions and phase diagrams. II. Mixtures involving lipids. *Biochim. Biophys. Acta.* **472**:285.
23. HILL, M. W., and R. LESTER. 1972. Mixtures of gangliosides and phosphatidylcholine in aqueous dispersions. *Biochim. Biophys. Acta.* **282**:18.
24. MCCABE, P. J., and C. GREEN. 1977. The dispersion of cholesterol with phospholipids and glycolipids. *Chem. Phys. Lipids.* **20**:319.
25. BERG, H. C., and E. M. PURCELL. 1977. Physics of chemoreception. *Biophys. J.* **20**:193.
26. HANSSON, H. A., J. HOLMGREN, and L. SVENNERHOLM. 1977. Ultrastructural localization of cell membrane G_{M1} ganglioside by cholera toxin. *Proc. Natl. Acad. Sci. U.S.A.* **74**:3782.
27. SLOMIANY, A., C. ANNESE, and B. L. SLOMIANY. 1976. The glycosphingolipids of rat sublingual and submaxillary glands. *Biochim. Biophys. Acta.* **441**:316.