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GANGLIOSIDE HEADGROUP DYNAMICS

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Summary

Gangliosides, spin-labelled specifically on *N*-acetylneuraminic acid residues or on random-headgroup sugars, have been used to extend previous studies of headgroup behaviour. Headgroup sugar mobility is seen to be homogeneous and relatively unrestricted in a range of systems including three lines of cultured cells. The effects of temperature and pH have been considered. Binding of small quantities of the lectin, wheat germ agglutinin, was found to increase average headgroup mobility for gangliosides in lipid bilayers, most likely as a result of a disordering effect on ganglioside clusters.

Introduction

Gangliosides comprise a family of complex glycosphingolipids bearing one or more of the negatively charged sugar residue, *N*-acetylneuraminic acid. They represent an important class of specific recognition site at the eucaryotic cell surface [1—5]. Clearly, both the type and sequence of headgroup sugars must be important determinants of the role of a particular ganglioside as a receptor in a given recognition event. However, it is becoming increasingly obvious that for receptors in general, arrangement, exposure, and interaction with other surface components are equally important (e.g., Refs. 6—10). Furthermore, although very little is known about the basic characteristics of receptor behaviour, a key aspect of current thinking is that their response to a specific contact may be very dynamic [1,6,8,11—13].

The ganglioside headgroup is a single oligosaccharide chain extending up to 2.5 nm from the membrane/water interface. Its behaviour may be expected

Abbreviations: CHO, Chinese hamster ovary; CMC, critical micelle concentration; TIBS, triisopropylbenzenesulphonyl chloride. TEMPO, 2,2,6,6-tetramethylpiperidine-*N*-oxyl; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid.

to be sensitive to the nature of the lipid bilayer in view of its attachment to a ceramide backbone which is capable of rapid lateral and rotational diffusion. One would also expect the headgroup to be subject to control by exogenous factors including ionic conditions, pH, presence of other surface structures, and specific binding events. Several of these points have been addressed previously by us, using spin-labelled derivatives [14], and by others using NMR spectroscopy [15]. The picture obtained is one in which headgroup carbohydrate residues move with uniformly high freedom of motion in an aqueous environment when gangliosides are dispersed in buffer or in simple phospholipid bilayers. In this report, we describe an extension of such work to more complex systems including intact cells.

Materials and Methods

L-α-Dimyristoyl and L-α-dipalmitoyl phosphatidylcholine and cholesterol were obtained from Sigma. Egg phosphatidylcholine was purchased from Sigma (Type III-E) and further purified by column chromatography on silicic acid. Phosphatidylserine was obtained from Serdary Res., London, Canada. All phospholipids were pure, as judged by thin-layer chromatography on silica gel G (Stahl). Gangliosides were isolated from bovine brain by using a modification of the method of Kanfer [16] in which gangliosides obtained from the initial Folch extraction were purified by chromatography on silicic acid (Bio Rad, 200-400 mesh, eluting with $CHCl_3/CH_3OH$) and checked for purity by thin-layer chromatography (TLC) [16]. Ca^{2+} and Mg^{2+} were added as their chlorides, and EDTA as the disodium salt. Hepes was obtained from Sigma. The spin-labelled gangliosides illustrated in Fig. 1a and b were prepared as described previously [14,17].

Gangliosides labelled on NANA residues (Fig. 1c) have not been reported previously and were prepared by a modification of a method we employed earlier for labelling the NANA-rich glycoprotein, glycophorin [18]. Typically, 10 mg of purified gangliosides were incubated at 0° C in 10 ml of 0.1 M sodium acetate buffer, pH 5.0, containing 0.15 M NaCl. To this were added with stirring 10 ml of ice-cold 10 mM $NaIO_4$. The reaction was stopped after 10 min by adding excess ethylene glycol and stirring for a further 10 min. The reaction mixture was then concentrated to 1 ml on a rotary evaporator and dialysed at 4°C, first against phosphate-buffered saline (pH 7.4) and then against distilled water. The contents of the dialysis bag were then added to 4 ml of Na_2CO_3 buffer (pH 9.5) containing 10% Me_2SO and 16 mg of 4-amino-2,2,4,4-tetramethylpiperidine-1-oxyl (Aldrich). After stirring for 90 min at 0° C, 0.5 ml of 0.6 M $NaBH_4$ in 0.01 M NaOH was added and stirring was continued for 30 min. The reaction mixture was dialysed exhaustively, first against Na_2CO_3 buffer (pH 9.5) and subsequently against distilled water prior to being evaporated to dryness. The product comigrated with native gangliosides on TLC plates (eluent 55 : 45 : 2 : 8 $CHCl_3/CH_3OH/NH_4OH/H_2O$), It was stored in 1 : 1 $CHCl_3/CH_3OH$ at −20° C.

Lipid mixtures were made by dissolving appropriate amounts of each in $CHCl_3/CH_3OH$ and pumping extensively under vacuum to remove traces of solvent. Dried lipid mixtures were suspended in buffer by using a vortex

mixer.

EPR spectra were recorded on a Varian E12 spectrometer equipped with a TM_{110} cavity (using aqueous sample flat cells for maximum signal-to-noise ratio when the samples consisted of whole cells), and interfaced with a Nicolet 1180 data system.

Cell culture

Chinese hamster ovary cells (CHO) (Pro^-3) were grown in suspension culture at 37°C in complete alpha medium supplemented with 10% fetal bovine serum albumin.

Myoblasts used in this work were a clone of Yaffe's L6 designated L6 9/1 from rat skeletal muscle. The cell line was routinely maintained at 37°C in monolayer cultures (in Falcon tissue-culture flasks) in Dulbecco's modified Eagle's medium supplemented with 10% horse serum albumin. The incubation atmosphere was 95% air/5% CO_2. For the experiments described here the cells were grown in roller bottles.

HeLa cells were grown both in monolayer culture (roller bottles) and in suspension culture, using Dulbecco's modified Eagle's medium supplemented with bovine serum albumin.

In each case, the signal-to-noise ratio was optimized by using at least 200 μl of packed cells per sample in a quartz flat cell. Prior to an experiment, fresh cells were washed twice with 10 ml of phosphate- or Hepes-buffered saline (with Ca^{2+} and Mg^{2+}) containing 1 mM $K_3Fe(CN)_6$. Washed cells were exposed to 0.02 mg of spin-labelled ganglioside in 1 ml of the above buffer at 37°C for 2 h. Following this incubation, cells were washed extensively to remove unincorporated labelled ganglioside. Cell viability remains high subsequent to this treatment. In the absence of $K_3Fe(CN)_6$, appreciable spin-label reduction occurred over a period of several hours. Cells grown in monolayer culture were harvested as gently as possible (see Table I) to minimize damage to the glycocalyx.

Results and Discussion

Spin-labelled gangliosides

In spectroscopic-probe studies of complex systems, there are great advantages to be gained from localizing the probe to regions of interest. In this case, we have attached spin labels covalently to gangliosides oligosaccharide chains. We have previously reported two methods of achieving this (structures in Fig. 1a, b [14,17]), both of which take advantage of the relative reactivity of primary alcohols to esterification in the presence of triisopropylbenzene-sulphonyl chloride (TIBS). Although every headgroup sugar has one primary alcohol function, we have controlled reagent ratios so as to introduce (presumably randomly) an average of one or fewer spin labels per ganglioside. The third method (Fig. 1c) has not been applied to gangliosides previously and involves specific labelling of *N*-acetylneuraminic acid residues, once again keeping label density at one or fewer per ganglioside.

Each of our three labels has certain distinctive features which may, depending upon the circumstances, be advantageous or disadvantageous. The first

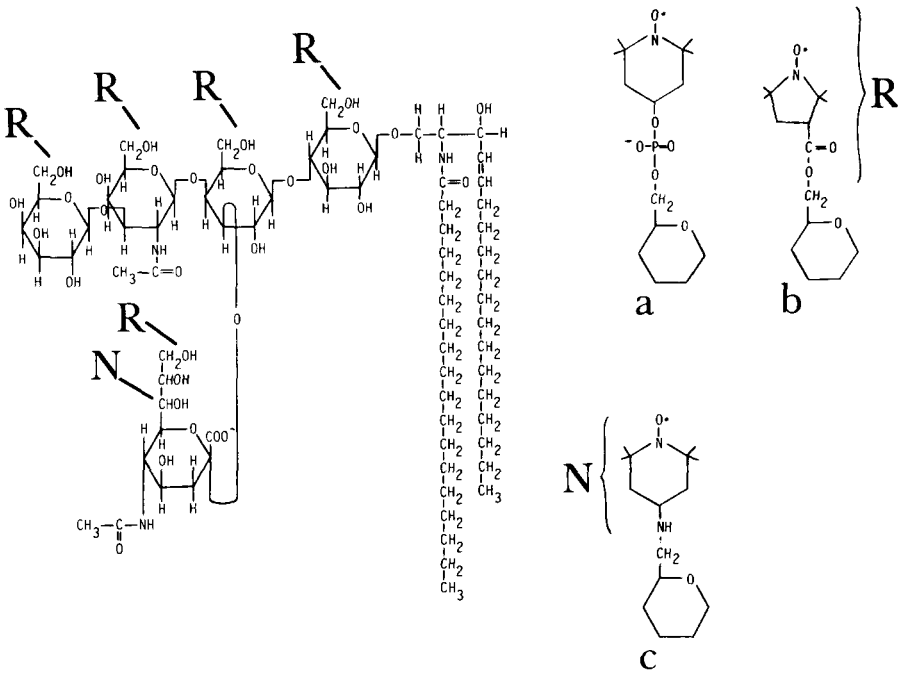

Fig. 1. Possible nitroxide ring attachment points for various spin-labelling procedures referred to in the text. a and b have been described previously and introduce the portion, R, (as shown on the right) without specificity for any particular sugar [14,17]. c has high selectivity for coupling the group, N, to NANA residues as illustrated.

two (a and b of Fig. 1) are characterized by approximately random labelling of headgroup sugars. Hence, their spectra reflect average behaviour of all sugars in the gangliosides. The phosphate-coupled label (Fig. 1a) introduces an additional negative charge to the headgroup region. Obviously this is undesirable, although ganglioside headgroups already possess from one to three negative charges, and we have tended to use the alternative derivatives. The carboxyl-coupled label (b of Fig. 1) does not introduce a charge, and is more tightly coupled to oligosaccharide dynamics [14] since the nitroxide ring is separated from the labelled sugar ring by only two bonds about which free rotation can occur.

The previously unreported label illustrated in Fig. 1c does not modify headgroup charge and is arrived at by an extremely well characterized route known to label selectively NANA residues [18—22].

General considerations

The nitroxide spin-label ring is similar in dimensions to a monosaccharide. In our derivatives, it is attached by two or three bonds about which free rotation can occur to a sugar residue. Hence, not only would one expect the EPR spectra obtained to reflect the behaviour of the oligosaccharide chains, but also it seems likely that the tumbling rate for the nitroxide ring will roughly approximate that of a terminal headgroup sugar. Such an assumption could be importantly in error due to the presence of sugar hydroxy groups which have no counterpart in the spin-label ring. However, Bernstein

et al. [23] have reported very good agreement between values of effective correlation time for sugar residues on soluble glycoproteins as measured by NMR of deuterated residues and EPR of spin-labelled ones.

Parameters which are potentially useful in interpreting the spectra obtained are: the factor, $W_0[(h_0/h_{-1})^{1/2} - 1]$, which is inversely related to headgroup mobility * [24]; hyperfine splitting (peak separation), which is directly related to the dielectric constant of the headgroup environment [25,26]; and peak width, which is inversely related to headgroup mobility and directly related to the extent of interaction between labelled gangliosides (spin-exchange broadening [27]). Since we have kept the degree of spin labelling low, and ganglioside oligosaccharide chains are fairly extensive, we tend to see spin-exchange effects only when labelled gangliosides are particularly densely packed.

Table I presents typical spectral data for headgroup-labelled gangliosides in a variety of samples selected to illustrate basic features of headgroup behaviour. These data will be referred to at intervals throughout the manuscript, but certain aspects are worth remarking upon at the outset because they are common to all samples. Firstly, the headgroup manifests appreciable freedom of motion (mobility being inversely related to τ_c). The correlation time for headgroup sugars is about 2.5 times greater when gangliosides are incorporated into unsonicated egg phosphatidylcholine bilayers than when dissolved as monomers [28] in the organic solvent, dimethylformamide (DMF). Presumably, this reflects the effect of preventing ceramide backbone tumbling. A further reduction in mobility is seen when the lipid bilayer is in the gel phase (compare egg to dipalmitoyl phosphatidylcholine at 23°C), no doubt because reduced ceramide backbone translational and rotational motion is transmitted to the sugar residues. Headgroup mobility is also subject to control by other structures occupying the headgroup region, e.g., in glycolipid micelles, lipid bilayers containing other components with oligosaccharide headgroups, and of course in real cells. Although we have not included spectra here, all of those recorded to date (except when lectin binding was involved) have shown three homogeneous, narrow lines. Furthermore, the values of τ_c obtained for the different labelled derivatives are very similar in spite of the fact the labels must occupy a variety of different positions along the oligosaccharide chains. This is in agreement with the conclusion of Harris and Thornton [15] from NMR studies of ganglioside suspensions, that all headgroup sugars have approximately the same mobility.

Secondly, the hyperfine splitting, a_N, is always such as to indicate that the oligosaccharide chains occupy a totally aqueous environment with no sign of carbohydrate residence in hydrophobic domains. This is probably not

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* The correlation time, τ_c, may under certain circumstances be written:

$$\tau_c = 6.5 \cdot 10^{-10} W_0[(h_0/h_{-1})^{1/2} - 1]$$

where W_0 is the linewidth of the mid-field line and h_0 and h_{-1} are the heights of the mid- and high-field spectral lines, respectively. This equation assumes rapid, isotropic tumbling of the nitroxide label (approximations which presumably diminish in validity as the ganglioside headgroups interact with other species at a membrane surface). We use the above relationship qualitatively and often plot $[(h_0/h_{-1})^{1/2} - 1]$ instead of τ_c because difficulty in very precise measurements of W_0 leads to point scatter.

TABLE I

CHO-COOH-NO designates the gangliosides spin-labelled on random headgroup sugars with a carboxyl-linked nitroxide ring (Fig. 1b). NANA-NH_2-NO designates the gangliosides spin-labelled on *N*-acetylneuraminic acid residues (Fig. 1c). PC, phosphatidylcholine; PS, phosphatidylserine. τ_c is the correlation time for nitroxide radical reorientation as outlined earlier. It is best used for semiquantitative comparison within a group of experiments, but clearly does bear a relation to actual headgroup-sugar behaviour. Values of a_N for the (free) six-membered nitroxide ring system used for spin-labelling gangliosides on NANA are 16.80 G (in buffer) and 14.50 G (in hexane). Values of a_N for the (free) five-membered nitroxide ring system used for spin-labelling gangliosides on random sugar residues are 16.15 G (in buffer) and 14.00 G (in hexane).

Sample	Conditions	τ_c ($\pm 0.5 \cdot 10^{-10}$ s)	a_N (± 0.05 G)
CHO-COOH-NO	0.01 mg in 100 μl DMF	4.8	—
NANA-NH_2-NO	0.01 mg in 100 μl DMF	4.4	—
CHO-COOH-NO	1 mol% in egg PC in Hepes-buffered saline with 2 mM Ca^{2+}, Mg^{2+}	13.3	16.12
NANA-NH_2-NO	1 mol% in egg PC in Hepes-buffered saline with 2 mM Ca^{2+}, Mg^{2+}	13.0	16.80
CHO-COOH-NO	0.01 mg in 100 μl Hepes-buffered 5 mM EDTA + 0.1 mg globoside	19.9	16.25
CHO-COOH-NO	0.01 mg in 100 μl Hepes-buffered 5 mM EDTA + 0.1 mg ganglioside	28.8	16.45
CHO-COOH-NO	1 mol% in dipalmitoyl PC in Hepes-buffered saline with 2 mM Ca^{2+}, Mg^{2+}	15.0	16.12
CHO-COOH-NO	1 mol% in 9:1 egg PC/PS in Hepes-buffered saline with 2 mM Ca^{2+}, Mg^{2+}	14.2	16.12
CHO-COOH-NO	1 mol% in pure PS in Hepes-buffered 5 mM EDTA	15.5	16.12
CHO-COOH-NO	1 mol% in pure PS in Hepes-buffered 10 mM $CaCl_2$	17.7	16.12
CHO-COOH-NO	1 mol% in 3:3:1.5:1.5 cholesterol/egg PC/globoside/sphingomyelin in Hepes-buffered saline with 2 mM Ca^{2+}, Mg^{2+}	19.10	16.25
CHO-COOH-NO	1 mol% in 3:3:0.75:0.75:1.5 cholesterol/egg PC/globoside/ganglioside/sphingomyelin in Hepes-buffered saline with 2 mM Ca^{2+}, Mg^{2+}	18.4	16.30
CHO-COOH-NO *	in rat myoblasts (L6)	15.6	16.12
CHO-COOH-NO	in HeLa cells (grown on surface)	15.4	16.50
CHO-COOH-NO	in HeLa cells (grown on surface) (after trypsinization)	15.1	16.50
CHO-COOH-NO	in Chinese hamster ovary cells (from spinner culture)	18.7	16.50
CHO-COOH-NO	in Chinese hamster ovary cells (from spinner culture) (after trypsinization)	17.3	16.50
NANA-NH_2-NO *	in rat myoblasts (L6)	17.2	16.75
NANA-NH_2-NO	in HeLa cells (grown on surface)	18.1	17.30
NANA-NH_2-NO	in HeLa cells (grown on suface) (after trypsinization)	18.2	17.25
Integral membrane glycoprotein (glycophorin) spin-labelled on NANA residues and assembled into fluid lipid bilayers		10.0	—
Integral membrane glycoprotein (glycophorin) spin-labelled on random sugar residues and assembled into fluid lipid bilayers **		10.4	—

* Values of τ_c quoted for intact cells may tend to be somewhat too low since it is difficult to be sure that all loosely adhering spin-labelled ganglioside has been washed away prior to running the spectra.

** Unpublished results of similar experiments with headgroup-labelled integral membrane glycoproteins.

surprising, although it has been pointed out [29] that such considerations should not be neglected. In cases of very dense headgroup packing such as ganglioside micelles or intact cells, the dielectric constant of the headgroup

environment rises somewhat above the value for pure water, presumably due to proximity to charged groups.

Gangliosides in intact cells

Since it is known [30—33] that up to a few percent of gangliosides dissolved in the surrounding medium is incorporated into cell membranes, we have been able to obtain spectra of our labelled derivatives in intact cells. This approach involved incubating viable cultured cells with labelled gangliosides dispersed below their critical micelle concentration (CMC). The data obtained from such experiments are included in Table I. We have kept the amount of new ganglioside incorporated into cells below concentrations known to exist naturally, with the result that the signal-to-noise ratio became an important consideration. However, by employing large quantities of cells as described in Materials and Methods, we were able to obtain very good spectra which could be accurately investigated for any sign of unusual behaviour (such as the appearance of highly immobilized subpopulations). In fact, though, the spectra were all homogeneous, and consistent with our model system findings. Note that trypsinization had relatively little effect on the measured correlation times for headgroup sugars.

Collisional encounters at the bilayer/water interface

The spectra of labelled gangliosides in intact cells have been compared to those of various model systems (e.g., Table I) in order to decide what factors may be operating at the cell surface. In this regard, an instructive experiment is to take advantage of ganglioside micelle formation as an inducible clustering event. It is known that gangliosides exhibit a CMC of about 0.2 mg/ml. Below this concentration they exist in aqueous solution as monomers or very small clusters. Any increase in concentration above 0.2 mg/ml will go into forming large micelles the correlation time of which is very long on the EPR time scale [15,34]. Gangliosides in these large micelles would be expected to exhibit decreased overall mobility (by virtue of being part of a large slowly tumbling structure), and also dramatically increased headgroup interactions (headgroup packed extremely densely). This system should provide a situation representing the maximum contribution of crowding to oligosaccharide-chain motional restriction: all the carbohydrates residues being tightly packed in a thin (2.5 nm) surface layer. Such a system might, for instance, be viewed as an upper limit of conditions in a dense patch of receptors at a cell surface. The model suffers though from an artificially high enrichment in the negatively charged NANA residue relative to intact cells. The effect of such enrichment seems to be even greater headgroup immobilization, presumably due to involvement of the charged ganglioside headgroups in ionic bonding (note, for instance, that the labelled gangliosides show less motional restriction in micelles of the complex neutral glycosphingolipid, globoside, which are larger than ganglioside micelles [35]).

Fig. 2A is a plot of a parameter related to headgroup immobility for ganglioside headgroups when a small amount of spin-labelled ganglioside (below the CMC) in 10 mM Hepes buffer (pH 7.0) is made progressively more concentrated by addition of unlabelled ganglioside of the same type. $[(h_0/h_{-1})^{1/2} - 1]$

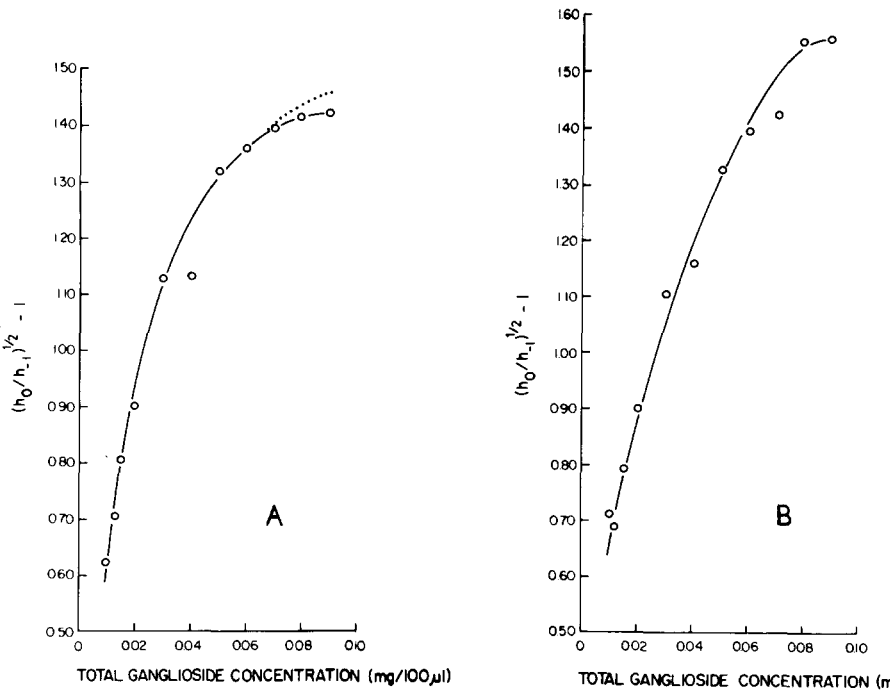

Fig. 2. Reduction in headgroup sugar mobility as a result of forcing a small amount of spin-labelled ganglioside (below CMC) into ganglioside micelles by increasing the concentration of unlabelled material. A, in 10 mM Hepes, pH 7.4 (dotted curve shows effect of phosphate-buffered saline or Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline. B, in 10 mM Hepes, pH 7.4, containing 100 mM $CaCl_2$.

increases steeply as the ganglioside concentration passes through the CMC, and then levels off to some sort of limiting value. Presumably, this reflects a steady concentration-dependent incorporation of gangliosides into large micelles with resultant immobilization as described above. A superimposable curve is obtained when this experiment is carried out in isotonic buffer (phosphate-buffered saline or Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline) except that in the isotonic case the highest points are slightly, but reproducibly, higher (dotted portion of Fig. 2A). Fig. 2B shows the result of a similar experiment in the presence of a very high concentration of Ca^{2+}.

The values found for τ_c in real cells range between the relatively greater freedom characteristic of lipid bilayers and the reduced freedom of structures such as globoside micelles.

Temperature dependence of headgroup behaviour

The effect of temperature on headgroup-labelled gangliosides is shown in Figs. 3—5. Arrhenius-type plots are a useful format for handling the data from such experiments since $1/\tau_c$ is a rate-related parameter. The slope of these plots is determined by the activation energy for spin-label reorientation (5.8 kcal/mol, linear correlation factor 0.9975, in Fig. 3a over the physiological temperature range); and any change in slope should indicate a change in the mechanism of reorientation. We have already demonstrated that the ganglioside headgroup is relatively motionally unrestricted and disordered in lipid

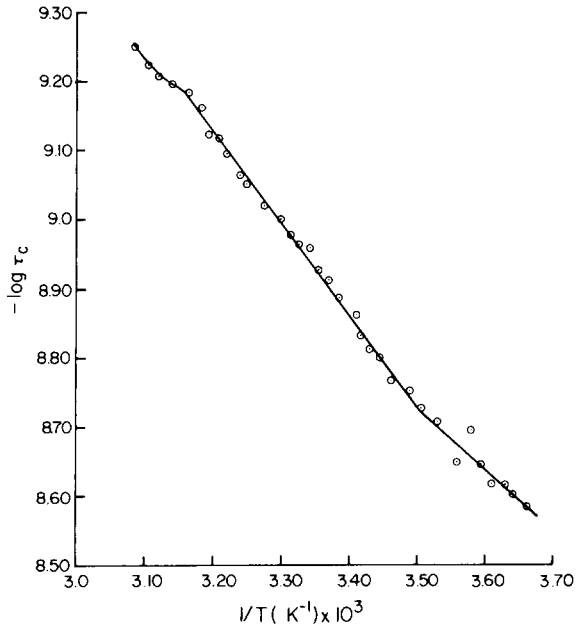

Fig. 3. Arrhenius treatment of data obtained with unsonicated egg phosphatidylcholine bilayers containing 1 mol% spin-labelled gangliosides (carboxyl-coupled) in normal saline buffered with 5 mM Hepes, pH 7.4, (with 2 mM Ca^{2+}, Mg^{2+}).

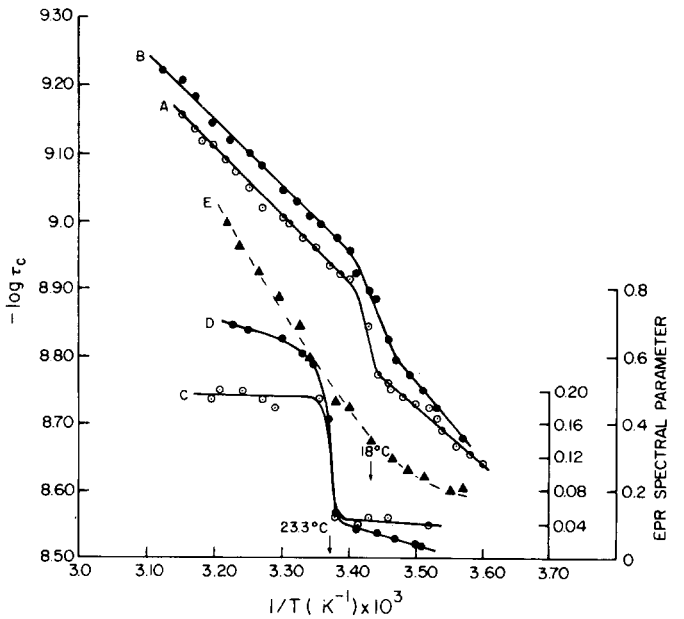

Fig. 4. Same as Fig. 3 but in dimyristoyl phosphatidylcholine. (A) Heating up; (B) cooling down; (C and D) using right-hand axes are TEMPO [37] curves used to measure the phase transition of dimyristoyl phosphatidylcholine bilayers without and with, respectively, 1 mol% unlabelled gangliosides; (E) Same as A but in the presence of 100 mM $CaCl_2$.

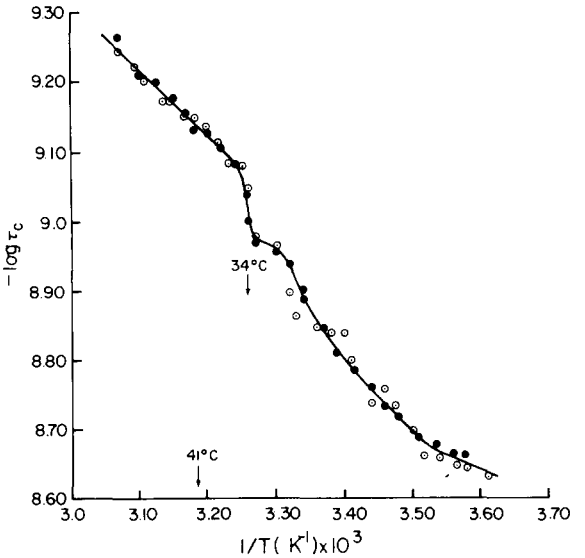

Fig. 5. Same as in Fig. 3 but in dipalmitoyl phosphatidylcholine (filled circles, heating up; hollow circles, cooling down).

bilayers (at 23°C). As a result, one would expect more or less linear Arrhenius plots. This expectation is seen to be borne out for gangliosides in bilayers of egg phosphatidylcholine (a lipid of which the bilayer fluidity varies smoothly with temperature). On the other hand, given the measurable dependence of headgroup dynamics on bilayer fluidity (Table I), one would expect a break of some sort in curves for gangliosides in any lipid matrix possessing a sharp phase transition. This expectation is also seen to be borne out (Figs. 4 and 5). Note that these experiments were carried out on bilayer structures containing only 1 mol% ganglioside so that the bulk phase behaviour of the host phospholipid is unaltered.

One aspect of these curves which is worth remarking upon is the fact that the slope changes presumably associated with the host matrix fluid-gel transition do not occur at the expected phase transition temperatures of 23°C for dimyristoyl phosphatidylcholine and 41°C for dipalmitoyl phosphatidylcholine [36,37]. Rather, a comparison of Figs. 4 and 5 with Fig. 3 shows the most striking changes to occur about 6—9°C lower than the phase transition of the host matrix. Experiments with the small spin label, 2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMPO) [37], readily confirm that 1 mol% ganglioside does not disrupt the bulk phase transition.

Clearly, the immediate environment of a ganglioside in rigid phospholipid bilayers becomes disordered at a temperature substantially below that needed to fluidize the bulk matrix. This would be the expected result if the gangliosides were to show a tendency toward phase separation. Bilayer regions relatively enriched in the ganglioside (i.e., local concentration considerably greater than 1 mol%) would have a lower, broadened melting range. The thermodynamic aspects of such behaviour have been considered by Bunow and Bunow [38] who have used calorimetry to derive phase diagrams for aqueous disper-

sions of gangliosides and phospholipids. These authors have pointed out the potential importance of the packing characteristics of ceramide backbone and sugar headgroups, the length of acyl chains, and attractive headgroup interactions amongst glycolipids in determining the enthalpy of demixing in such systems. Consistent with this concept, note (Fig. 4E) that in the presence of a high concentration of Ca^{2+} (conditions which should predispose toward ganglioside headgroup cross-linking [14]), the effect of the phospholipid phase transition is largely eliminated.

Binding of wheat germ agglutinin

The lectin, wheat germ agglutinin, is known to bind to NANA residues [39] and we have investigated the effect of this event on the ganglioside headgroup. For instance, the result of adding increasing amounts of wheat germ agglutinin to lipid bilayers bearing 5 mol% ganglioside is shown in Fig. 6. In the absence of wheat germ agglutinin, the headgroup mobility is characteristic of values already seen in Table I. In the presence of a large quantity of the lectin, one sees a reduction in the ganglioside headgroup mobility; an effect to be expected in the face of massive binding and cross-linking by a tetravalent macromolecule. However, at very low concentrations of wheat germ agglutinin, the effect is one of increased headgroup mobility. This effect occurs at concentrations such that the vast bulk of the ganglioside in the bilayer is unbound by lectin. In other words, one is seeing a general increase in headgroup motional freedom upon binding to a small number of gangliosides. Qualitatively and quantitatively, the same result was obtained using the ganglioside labelled on random sugar residues. The insert to Fig. 6 shows that this is in marked contrast to a binding event involving the (spin-labelled) glycoprotein, glycophorin [18]. This experiment suggests once again that the

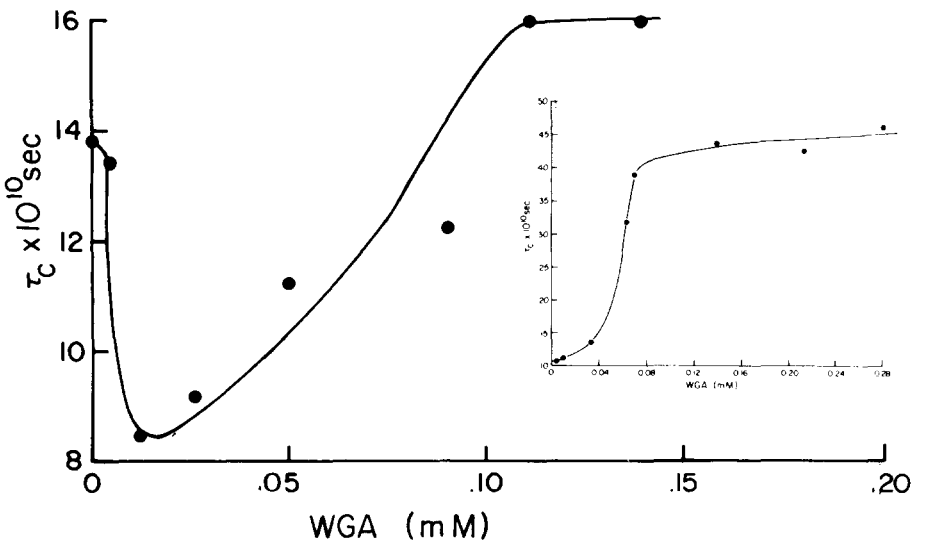

Fig. 6. Effect of a specific binding event on ganglioside headgroup mobility. Unsonicated egg phosphatidylcholine bilayers containing 4.8 mol% NANA-labelled ganglioside in Hepes-buffered saline, pH 7.4 (no Ca^{2+}, Mg^{2+}). WGA, wheat germ agglutinin.

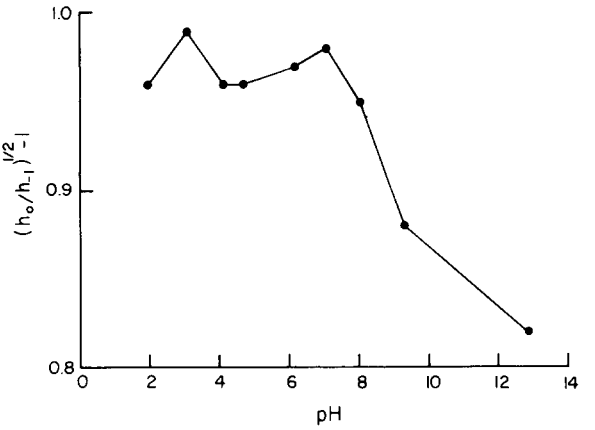

Fig. 7. Effect of pH on ganglioside headgroup mobility. Unsonicated egg phosphatidylcholine bilayers containing 1 mol% NANA-labelled ganglioside were made up in the following buffers (no Ca^{2+}, Mg^{2+}): 0.1 M KCl/HCl (pH 2.00), 0.1 M glycine-HCl/KCl (pH 3.13), 0.1 M sodium acetate (pH 4.18), 0.1 M sodium acetate (pH 4.75), 0.1 M sodium phosphate (pH 6.21), 0.1 M sodium phosphate (pH 7.11), 0.1 M sodium phosphate (pH 8.10), 0.1 M sodium carbonate (pH 9.36), 0.1 M KOH (pH 12.94).

ganglioside exists in phase-separated patches (rather than uniformly distributed throughout the bilayer) so that a relatively small number of binding events can lead to a general effect. A logical mechanism for the low concentration effects of wheat germ agglutinin would be that headgroup disruption caused by the binding event disrupts the (patches of) ganglioside leading to increased motional freedom. Such an explanation is consistent with the observation of Bunow and Bunow [38] that massive binding of wheat germ agglutinin to 1 : 1 mole ratio ganglioside : phospholipid mixtures leads to mixing.

pH considerations

The effect of pH on ganglioside headgroups is illustrated in Fig. 7. Note that the *Y*-axis parameter is related to headgroup immobility. The peak of relative immobilization between pH 2 and 3 is presumably attributable to electrostatic bonding involving NANA residues ($pK_a = 2.6$), and has a counterpart in similar studies of the NANA-rich glycoprotein, glycophorin (Lee, P.M. and Grant, C.W.M., unpublished observation). Variation in τ_c as a function of pH is about 20% over the range indicated.

Acknowledgements

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