N-Parinaroyl Glycosphingolipids: Synthesis and Characterization of Novel Fluorescent Probes of Membrane Structure†

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ABSTRACT: *N*-Parinaroylceramides and -glucocerebrosides were synthesized and characterized. These fluorescent glycolipids were found to be nonperturbing membrane lipid probes, which partitioned preferentially into fluid-phase phosphatidylcholine (PC) in liposomes containing both fluid and solid-phase PC. *N*-Parinaroylglucocerebroside, parinaroyl-PC, and free parinaric acid were used to analyze the motion and distribution of glucocerebroside and ganglioside G_{M1} in liposomes composed of these glycosphingolipids (GSL) and 1-stearoyl-2-oleoyl-PC (SOPC). Steady-state fluorescence anisotropy of these probes indicated that the neutral glucocerebroside formed solid-phase domains in SOPC liposomes; these domains contained little or no PC. In contrast, the negatively charged ganglioside G_{M1} was miscible with fluid-phase PC. Incorporation of G_{M1} into SOPC liposomes resulted in an increase in the transition temperature of the mixture; no transition was observed in either of the pure GSL used over the temperature range from 5 to 70 °C. These data indicate that the glucocerebroside probes may be specific for sphingolipid domains in mixed PC/GSL membranes.

Glycosphingolipids (GSL)[1] are ubiquitous components of eukaryotic plasma membranes and are found predominantly in the extracellular leaflet of the plasma membrane bilayer [for review, see Hakomori (1981)]. This location makes GSL excellent candidates for mediation of extracellular messages. These membrane components have been implicated as receptors or modulators of receptors for toxins (Fishman & Brady, 1976), hormones (Fishman & Brady, 1976), growth factors (Bremer et al., 1984), and lymphokines (Liu et al., 1982). Additionally, the reappearance of fetal-type GSL in transformed cells raises the possibility that these molecules are also involved in growth control and metastasis (Kinders et al., 1982; Hakomori, 1981). However, in contrast to the wealth of information presently available regarding the function of glycoprotein receptors, very little is known on the molecular level about the function of most GSL as membrane receptors. An important first step toward the acquisition of this information is a detailed study of the motion and distribution of these molecules in model membrane systems.

Bunow and Bunow (1979) reported that ganglioside G_{M1} is miscible in fluid-phase SOPC lamellae at concentrations up to 30 mol % ganglioside. However, simple neutral glycolipids behave differently than negatively charged species such as gangliosides. Reports of special relevance to the present study include the observation by Correa-Friere et al. (1979) that domains of glucosylcerebroside might exist in liquid-crystalline (fluid) phosphatidylcholine bilayers. Tillack et al. (1982), using DSC and freeze-etch electron microscopy, reported that the neutral species known as asialo-G_{M1} also exist in clusters in fluid-model phosphatidylcholine membranes. Similarly, Grant and co-workers (Sharom et al., 1976), using galactosylcerebroside containing a nitroxide spin-label in the carbohydrate moiety, concluded that this molecule is clustered in fluid phosphatidylcholine model membranes. In contrast, a recent study using a similar probe labeled in the acyl chain concludes that these molecules are randomly distributed (Utsumi et al., 1984). A possible explanation for this discrepancy might be that the probe labeled in the acyl chain may not interact with neighboring lipid molecules in a normal manner. It is known that GSL interactions in the plane of the bilayer depend heavily on hydrogen bonds involving hydroxyl groups on the sphingosine chain (Boggs, 1980). Such hydrogen bonding might well be disrupted by the introduction of a bulky nitroxide probe. In conclusion, it is known that neutral glycolipids in model and biological systems (Curtain et al., 1980) probably exist in domains in fluid bilayers; the physiological ramifications of the resulting phase-separated membranes are numerous [for review, see Grant (1983)].

Experimental Procedures

Reagents. Ganglioside G_{M1} was obtained from Supelco (Bellefonte, PA) and was further purified by silicic acid chromatography before use (Irwin & Irwin, 1979). This step was necessary to remove a variety of phosphate-containing lipids from the commercial preparations (Carter et al., 1972). 1,2-Dielaidoylphosphatidylcholine (DEPC), 1,2-dimyristoylphosphatidylcholine, and 1-stearoyl-2-oleoylphosphatidylcholine (SOPC) were obtained from Sigma (St. Louis, MO) and were used without further purification. 1,2-Dipalmitoylphosphatidylcholine (DPPC) and 1-palmitoyl-2-*cis*-parinaroylphosphatidylcholine were the generous gifts of

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[1] Abbreviations: G_{M1}, Gal(β1-3)GalNAc(β1-4)[NAcNeu(α2-3)]-Gal(β1-4)GlcCer [the ganglioside designation corresponds to the nomenclature of Svennerholm (1973)]; PC, phosphatidylcholine; cPnA, *cis,trans,trans,cis*-9,11,13,15-octadecatetraenoic acid; tPnA, *all-trans*-9,11,13,15-octadecatetraenoic acid; DEPC, dielaidoylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; PDPC, 1-palmitoyl-2-docosahexanoylphosphatidylcholine; SOPC, 1-stearoyl-2-oleoylphosphatidylcholine; GSL, glycosphingolipid; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*'-2-ethanesulfonic acid; TLC, thin-layer chromatography; DSC, differential scanning calorimetry.

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Dr. R. Welti, Department of Biochemistry, Kansas University School of Medicine, Kansas City, KS. *cis*- and *trans*-parinaric acids were the generous gifts of Dr. R. D. Simoni, Biological Sciences, Stanford University, Stanford, CA. Sphingolipid standards, sphingosine, glucopsychosine, and ethyl[3-(dimethylamino)propyl]carbodiimide were purchased from Sigma. Unisil silicic acid was from Clarkson (Williamsport, PA), thin-layer chromatography (TLC) plates were from Analtech, and all solvents were reagent-grade from Fisher.

Preparation of Liposomes. Phospholipid (200 nmol), with or without varying amounts of glycolipid, was dried under nitrogen. The residue was resuspended in 10 μL of absolute ethanol and slowly injected into 1.0 mL of buffer (50 mM HEPES, 100 mM KCl, pH 7.2) while vortexing (Batzri & Korn, 1973). These unilamellar liposomes were then used for measurements of fluorescence anisotropy as described by Welti and Silbert (1982). Analysis of liposomes prepared by this technique indicated that the final preparations contained 95–100% of the phosphatidylcholine (as assayed by phosphate content) and 98–100% of the glycolipid (as assayed by sphingosine content). For analysis of the phase-transition temperatures of various pure phosphatidylcholines (Table I) or for calorimetric analysis (Table II), multilamellar liposomes were prepared as previous described (Rintoul et al., 1979).

Fluorescence Anisotropy Measurements. Liposomes, prepared as described above, were placed in a 0.5-mL quartz cuvette in the thermostated, temperature-controlled cuvette chamber of a Spex Fluorolog spectrofluorometer. The spectrofluorometer was equipped with quartz polarizers obtained from Spex. The limiting fluorescence anisotropy (for fluorescein) in alkaline glycerol at 10 °C) was 0.35 for this experimental arrangement (Chen & Bowman, 1975). Probes were added either before the injection into buffer (for the parinaroyl phospholipids and glycolipids) or after injection (for the free fatty acid probe species). Final probe/lipid (mol/mol) ratios were 1/200, except where otherwise noted. Excitation wavelength was 320 nm (band-pass 5.0 nm) for *trans*-parinaric acid and probes containing this fluorophore and 325 nm (band-pass 5.0 nm) for *cis*-parinaric acid and probes containing this fluorophore. Fluorescence emission was monitored at 420 nm (band-pass 20.0 nm) for all probes used. Fluorescence emission parallel and perpendicular to the vertically polarized excitation was monitored as the sample was cooled at a rate of 0.75 °C/min, as controlled by a Neslab circulating bath and a linear temperature programmer. Data were analyzed on the computer smoothing and curve-fitting program previously described (Welti et al., 1981). Fluorescence anisotropy of *trans*-parinaric acid (r) is correlated with membrane lipid order or negatively correlated with "fluidity". The anisotropy (r) is defined as

$$\frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}}$$

where $I_{\parallel}$ and $I_{\perp}$, respectively, refer to fluorescence intensity parallel and perpendicular to the vertically polarized excitation beam (Lentz et al., 1979). This ratio can vary from a theoretical minimum of 0 (completely anisotropic motion of the probe) to a theoretical maximum of 0.40 (essentially no molecular motion of the probe during the lifetime of the excited state). No corrections for scattering depolarization were required (Lentz et al., 1979), since all samples prepared by the techniques described above had absorbances of <0.05 at the excitation wavelengths. Data obtained while the samples were heated were equivalent to those obtained while they were being cooled over the same temperature range, implying that the structures formed were equilibrium mixtures of the components. Additionally, liposomes stored for several days at 4 °C gave identical fluoresence anisotropy profiles when compared to freshly made liposomes, implying that the structures were stable with regard to time. Calculation of partition coefficients ($K_p^{s/f}$) in binary PC liposomes was performed as previously described by Sklar et al. (1979) and by Welti and Silbert (1982), using both quantum yield measurements and fluorescence polarization.

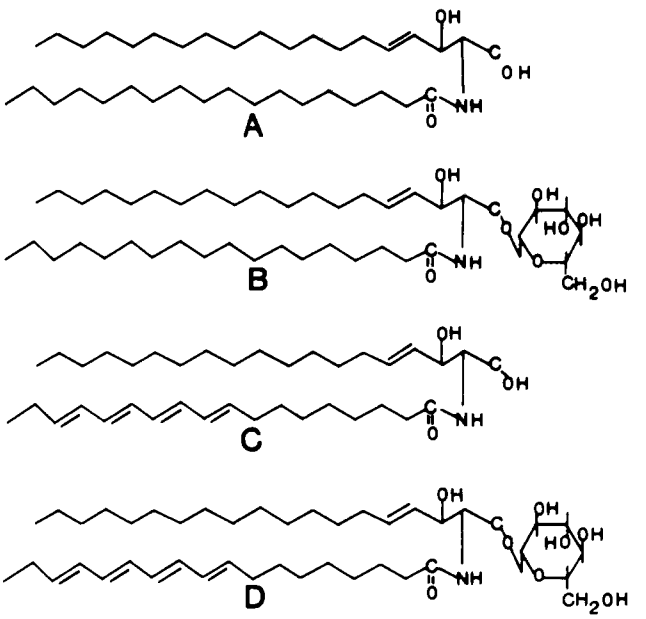

FIGURE 1: Structures of *N*-stearoyl and *N-trans*-parinaroyl sphingolipids: (A) *N*-stearoylceramide; (B) *N*-stearoylglucocerebroside; (C) *N-trans*-parinaroylceramide; (D) *N-trans*-parinaroylglucocerebroside.

Synthesis of N-Parinaroyl Glycosphingolipid Probes. The method described by Hammarstrom (1971) for the synthesis of *N*-acylceramides was used essentially as described by the author. Purification of the reaction products was also performed as described, with the exception that an atmosphere of N_2 was maintained in the separatory funnel at all times. *N*-Parinaroylglucocerebrosides were synthesized by substituting glucopsychosine for sphingosine in the reaction mixture and purified by an additional silicic acid column chromatography step with chloroform/acetic acid (95/5) as the eluting solvent. Purity of the isolated reaction products was verified by TLC in a number of solvent systems on 250-μm silica gel G plates. Plates were sprayed with 50% H_2SO_4 and charred overnight at 120 °C. In no cases were detectable amounts of free parinaric acid isomer found. Yields routinely ranged from 78 to 87% on the basis of the sphingosine base content. Chemical analysis of the composition of these novel fluorescent probes indicated that the purified products contained from 0.95 to 1.02 mol of sphingosine/mol of fatty acid (Figure 1).

Differential Scanning Calorimetry. Differential scanning calorimetry (DSC) was performed on a Perkin-Elmer DSC-1B in the laboratory of Dr. W. Klopfenstein, Department of Biochemistry, Kansas State University. Liposomes were prepared as previously described (Rintoul et al., 1979) in fluorescence buffer (50 mM HEPES, 100 mM KCl, pH 7.4) and centrifuged for 10 min at 10000*g* in a Beckman microgfuge. These liposomes contained DPPC and various concentrations of either heterogeneous glucocerebroside (Sigma, from Gaucher's spleen) or *N-trans*-parinaroylglucocerebroside prepared as described above. Liposome preparations containing 5–8 mg of phospholipid were scanned at the rate of

Table I: Phase-Transition Onset Temperatures of Multilamellar Phosphatidylcholine Liposomes As Determined by Fluorescence Anisotropy of *N-trans*-Parinaroylglucocerebroside[a]

phospholipid	T_o (Silvius, 1982)	T_o (tPnA glucocerebroside)
DPPC	41.5	42.0
DMPC	23–24	24.0
DSPC	54.5	54.0
DEPC	9.5–13.0	12.0
SOPC	6.3–13.0	11.0

[a] Multilamellar liposomes were prepared by agitating a dry film of 500 nmol of the indicated phospholipid in 2.5 mL of buffer (50 mM HEPES, 100 mM KCl, pH 7.2) at a temperature above the reported phase-transition temperature. A 1.0-nmol aliquot of *N-trans*-parinaroylglucocerebroside, synthesized as described in the text, was added to this suspension; fluorescence anisotropy of the probe was measured and calculated as described in the text. Transition onset temperatures (T_o) were determined to be the temperature where the rate of increase in the anisotropy (during cooling of the suspension) exceeded 0.07/°C, as determined from the computer-smoothed data.

5 °C/min from recorder tracings of instrument output, with the water melting endotherm as a reference point.

Analytical Methods. Phosphate was analyzed according to the method of Ames (1966), sphingosine was analyzed according to a modification of the method of Naoi et al. (1974), and GSL carbohydrates were analyzed by gas–liquid chromatography (GLC) of the alditol acetates on a 4-ft column of SP-2340 (3% on Supelcoport 100/120, Supelco, Bellefonte, PA) as described by Yang and Hakomori (1971). Parinaric acid concentrations were determined by absorbance with known extinction coefficients (Sklar, 1976). Phospholipid and GSL fatty acids were analyzed by GLC of the methyl esters as previously described (Rintoul et al., 1979). Sialic acid was determined by the method of Cassidy et al. (1966).

Results

Synthesis and Characterization of Parinaroyl Glycosphingolipids. In order to further analyze the structure and molecular dynamics of mixtures of phospholipid and glycosphingolipids, we synthesized *N*-parinaroylceramides and *N*-parinaroylglucocerebrosides by adapting the method first described by Hammarstrom (1971). The synthesis of these probes is described under Experimental Procedures, and chemical characterization of these novel fluorescent probes was performed as described. Component analyses indicated that the compounds were pure and contained fatty acids, sphingosine, and carbohydrate in nearly the theoretical ratios.

In order to determine if these probes are indeed nonperturbing analogues of naturally occurring GSL, we determined the phase-transition onset temperatures (T_o) of multilamellar liposomes composed of pure phosphatidylcholine, using fluorescence anisotropy measurements. Representative data are shown in Table I. These data indicate that the parinaroyl GSL probes detect gel–liquid-crystalline phase transitions in these pure PC liposomes; the onset of the phase transition in all cases corresponds closely to that observed by a variety of other techniques (Silvius, 1982). In model PC membranes, then, these GSL molecules behave as probes of PC phase behavior.

We then analyzed the fluorescence anisotropy of one of these parinaroylglucocerebrosides as a function of probe/lipid molar ratio in DPPC liposomes; these data are shown in Figure 2. These experiments indicate that the probe is nonperturbing at ratios of probe/lipid of 1/100 or less; some probe clustering is seen at 1/50, as indicated by the broadening of the DPPC phase transition. The data in Figure 2 indicate that *N-trans*-parinaroylglucocerebroside is a nonperturbing probe, as judged by the amplitude and width of the gel–liquid-crystalline phase transition of DPPC, at probe/DPPC ratios less than 1/100. The gel–liquid-crystalline phase transition of a pure phosphatidylcholine bilayer is a highly cooperative event, occurring over a narrow temperature range. The lipid used in these studies, dipalmitoylphosphatidylcholine (DPPC), undergoes a thermally induced transition from solid to fluid at 41.5 °C, as detected by many independent techniques (Silvius, 1982). Transition magnitude and width are sensitive indicators of the cooperativity of the transition; a decrease in magnitude or an increase in width of the transition would indicate perturbation of the lipid bilayer structure (Sklar, 1976). Transition width (Figure 2B) was determined as the peak width, at half-height, of the first derivative of the anisotropy vs. temperature curves; transition magnitude (Figure 2C) was determined as the ratio of the fluorescence anisotropy value below the transition at 35 °C to that above the transition at 45 °C (r_{35}/r_{45}). The raw data (Figure 2A), and the analyses of transition width and magnitude, indicate that probe/lipid ratios of 1/100 or 1/200, which are normally used in this laboratory, have little or no effect on DPPC bilayer structure in this sensitive assay.

Table II: Phase-Transition Temperatures and Widths of Multilamellar Liposomes Containing Glucocerebroside As Determined by Differential Scanning Calorimetry[a]

glucocerebroside	GSL/PL (mol/mol)[b]	T_m (main transition)	T_m (pretransition)	transition width
	0	41.9 ± 0.15	35.5 ± 0.50	1.00 ± 0.10
Gaucher's spleen	1/500	41.9 ± 0.00	35.0 ± 0.40	1.06 ± 0.10
	1/200	41.8 ± 0.20	35.1 ± 0.50	1.06 ± 0.20
	1/50	42.3 ± 0.50	ND[c]	1.06 ± 0.10
N-trans-parinaroyl	1/500	42.1 ± 0.15	34.9 ± 0.50	1.06 ± 0.15
	1/200	42.2 ± 0.15	35.7 ± 0.50	1.20 ± 0.20
	1/50	41.7 ± 0.25	ND	1.06 ± 0.10

[a] Data shown are results of triplicate analyses of DPPC liposomes prepared as described in the text. T_m of the main transition and pretransition was determined with the water endotherm as a reference. [b] GSL/PL = mole ratio of glycosphingolipid to phospholipid. [c] ND = not detected.

Similar percentages of either *N-trans*-parinaroylglucocerebroside or heterogeneous glucocerebroside were added to multilamellar DPPC liposomes and analyzed by DSC; these data are shown in Table II. These data indicate that low concentrations of glucocerebroside do not alter either transition temperature or transition width of a pure phospholipid species.

Next, we determined the solid/fluid partition coefficients ($K_p^{s/f}$) of the *N-trans*-parinaroylceramide and the *N-trans*-parinaroylglucocerebroside, using the binary PC model membrane system first described by Sklar et al. (1979). These data are reported in Table III. The partition coefficients of both isomers of parinaric acid and the partition coefficient of 1-palmitoyl-2-*cis*-parinaroyl-PC, determined by the same methods, are in good agreement with published values (Sklar et al., 1979; Welti, & Silbert, 1982). Both tPnA glycosphingolipids have partition coefficients below 1.0, implying that they would be preferentially located in a fluid-phase domain in membranes containing both fluid and solid regions. These data indicate that the two parinaroyl glycosphingolipids tested partitioned similarly and exhibited a general preference for fluid-phase PC. Partition coefficients of other probes used in this study were also determined; the results were in good agreement with previous reports (Sklar et al., 1979; Welti & Silbert, 1982). The partition coefficient of the *N*-tPnA glucocerebroside probe is similar to that determined for an un-

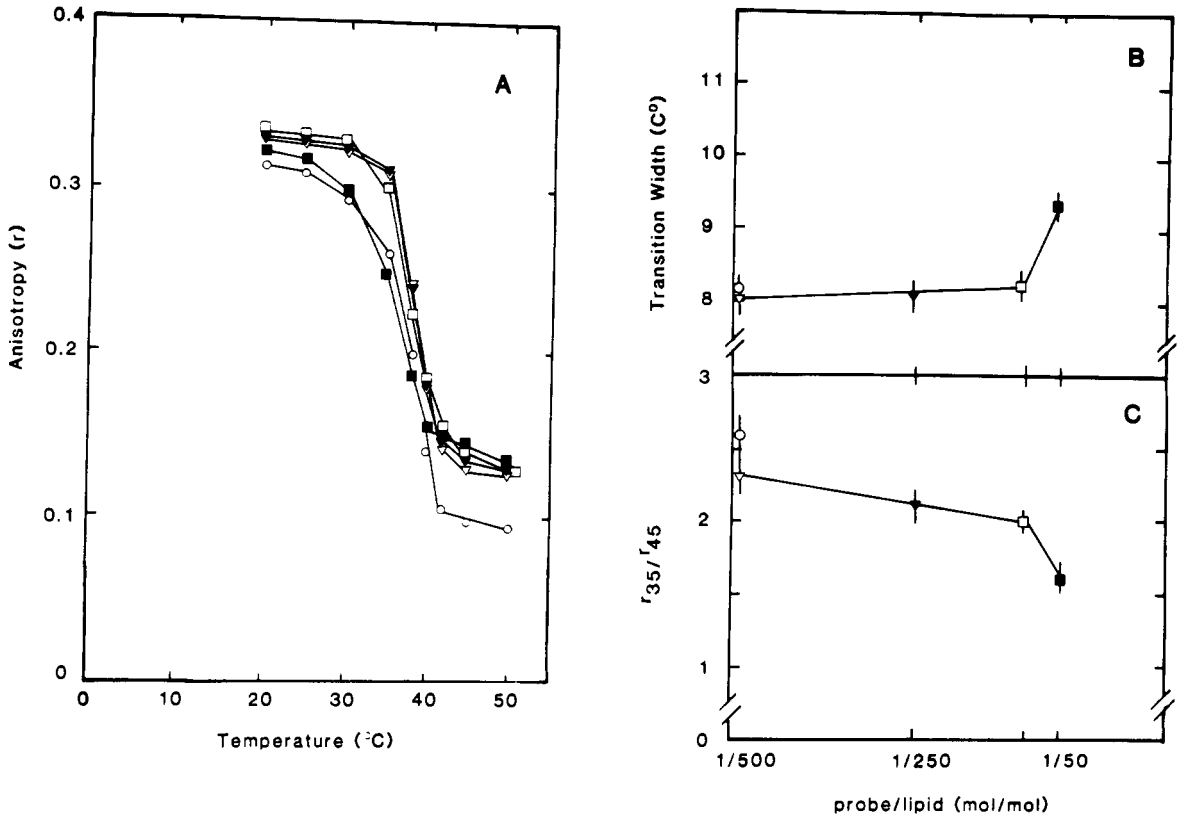

FIGURE 2: Fluorescence anisotropy of *N-trans*-parinaroylglucocerebroside in DPPC liposomes—effects of probe/lipid ratio. (Panel A) Fluorescence anisotropy $r = (I_{\parallel} - I_{\perp})/(I_{\parallel} + 2I_{\perp})$, where $I_{\parallel}$ and $I_{\perp}$, respectively, refer to fluorescence intensity parallel and perpendicular to the vertically polarized excitation beam, of parinaric acid probes in DPPC liposomes as a function of temperature. Lipid samples of the indicated composition, containing a total of 500 nmol of phospholipid in all cases, were dried under N_2, resuspended in 10–15 mL of ethanol, and injected into 2.5 mL of buffer at 56 °C as described in the text. These liposomes were cooled at a rate of 0.75 °C/min in a Spex Fluorolog; fluorescence emission parallel ($I_{\parallel}$) and perpendicular ($I_{\perp}$) to the vertically polarized excitation beam was monitored simultaneously. The data were smoothed with the computer program previously described, and anisotropy values were calculated for the points indicated. (○) cPnA, probe/lipid = 1/500; (▽) tPnA GC, probe/lipid = 1/500; (▼) tPnA GC, probe/lipid = 1/250; (□) tPnA GC, probe/lipid = 1/100; (■) tPnA GC, probe/lipid = 1/50. (Panel B) The first derivatives of the smoothed data shown in panel A were calculated with the computer program described above. The width of the peak (in °C) at half-height was taken as the transition width. It should be noted that the transition widths are greater in ethanol-injection vesicles than would be the case for multilamellar dispersions (Sklar, 1976). Symbols as in panel A. (Panel C) Anisotropy values from the data in panel A were determined at temperatures above r_{45} and below r_{35} the DPPC transition (41 °C). The ratio of the low-temperature anisotropy to the high-temperature anisotropy is a rough indicator of the magnitude of the transition and should be a function of the cooperativity of the transition (Sklar, 1976). Symbols are as in panel A.

Table III: Solid/Fluid Phase Partition Coefficients[a]

probe	mean $K_p^{s/f}$ (from polarization data)	mean $K_p^{s/f}$ (from quantum yield data)
tPnA	5.2 ± 1.9 (33)	5.8 ± 3.0 (33)
tPnA[b]	4.9 ± 2.3	5.3 ± 4.1
tPnA[c]	5.1 ± 1.2	3.3 ± 0.65
cPnA	0.70 ± 0.18 (34)	0.65 ± 0.19 (29)
cPnA[b]	0.68 ± 0.88	0.49 ± 0.24
cPnA[c]	0.59 ± 0.12	0.82 ± 0.33
1-16:0-2-cPnA-PC	0.63 ± 0.45 (61)	0.61 ± 0.6 (61)
1-16:0-2-cPnA-PC	0.70 ± 0.40	0.63 ± 0.19
N-tPnA glucosylcerebroside	0.66 ± 0.08 (78)	0.72 ± 0.29 (78)
N-tPnA ceramide	0.59 ± 0.40 (64)	0.45 ± 0.25 (64)

[a] Shown are means and standard deviations, calculated as described by Sklar et al. (1979) and Welti and Silbert (1982). Numbers in parentheses are the number of data points used in the calculations. [b] From Welti and Silbert (1982). [c] From Sklar et al. (1979).

saturated (1-oleoyl-2-tPnA-PC) rather than a saturated (1-palmitoyl-2-pTnA-PC) phosphatidylcholine (Welti & Silbert, 1982 36); this could indicate that the sphingosine backbone of the GSL probe does not pack properly in a pure PC bilayer (Boggs, 1980).

Fluorescence Anisotropy of Parinaroyl Probes in Mixtures of Phosphatidylcholine and G_{M1} or Glucocerebroside. The availability of parinaroyl phospholipid and glycosphingolipid probes with similar partition coefficients enabled us to test the hypothesis that neutral GSL are not miscible in PC membranes. We analyzed the fluorescence anisotropy of the *cis*-parinaroyl phospholipid, *trans*-parinaroylglucocerebroside, and cPnA in mixtures of SOPC and either 25 mol % G_{M1} or 25 mol % glucocerebroside. These data are shown in Figure 3. It can be noted that all three probes detected the major gel–liquid-crystalline transition of SOPC at or near 11 °C. In mixtures of SOPC and the neutral glucocerebroside, the three probes detected dissimilar thermotropic transitions. The *cis*-parinaroyl phospholipid detected a transition near 11 °C, which was very similar to that of the SOPC alone (Figure 3A). The *N-trans*-parinaroylglucocerebroside detected a higher temperature transition near 20 °C (Figure 3B). The cPnA probe showed an increase in fluorescence anisotropy at an intermediate temperature (Figure 3C). Thus, three probes that had similar $K_p^{s/f}$'s in the pure PC system detected different phase behavior in the PC/GSL mixtures. We interpret these results to indicate that the PC and neutral glucocerebroside are undergoing a phase separation and that at temperatures from 11 °C to at least 20 °C the SOPC coexists in a fluid phase with solid glucocerebroside domains. At these temperatures, the PC probe appears to be completely excluded from the GSL phase, as indicated by the observation that in this mixture the PC probe detects the same transition as it

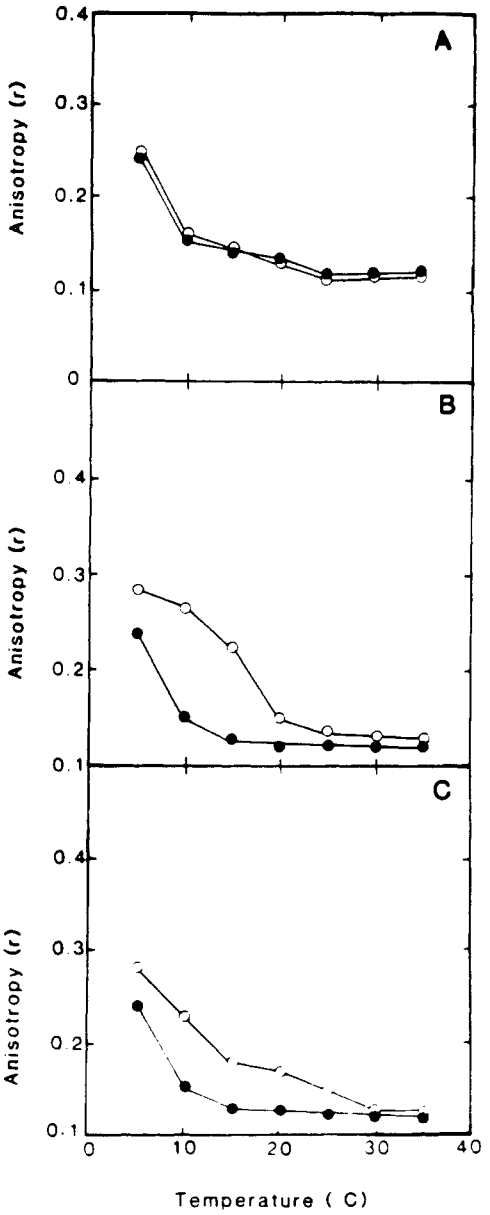

FIGURE 3: Fluorescence anisotropy of parinaroyl probes in SOPC and SOPC plus 25 mol % glucocerebroside. (Panel A) Fluorescence anisotropy of 1-palmitoyl-2-cPnA-PC in SOPC (filled symbols) and SOPC containing 25 mol % glucocerebroside (open symbols). A total of 200 nmol of lipid was used to prepare liposomes as described in the text; fluorescence anisotropy as a function of temperature was calculated as described in the text. Probe/lipid was 1/200 (mol/mol). (Panel B) Fluorescence anisotropy of *N*-tPnA glucocerebroside in SOPC (filled symbols) and SOPC + 25 mol % glucocerebroside (open symbols). Data were obtained as described for panel A. (Panel C) Fluorescence anisotropy of cPnA in SOPC (filled symbols) and SOPC + 25 mol % glucocerebroside (open symbols). Data were obtained as described in panel A.

detects in pure SOPC. The data presented in Figure 3 are evidence that these novel GSL probes can detect solid-phase domains of neutral GSL in fluid-phase PC bilayers. This suggests that the GSL domains are devoid of PC. cPnA apparently partitions between the PC and GSL domains, giving an average anisotropy. Similar results were obtained when DEPC rather than SOPC was utilized (data not shown).

In mixtures of SOPC and 25 mol % ganglioside G_{M1}, the three probes detected a major gel–liquid-crystalline transition at a higher temperature; this temperature was approximately the same for all the probes (Figure 4). These results indicate that G_{M1} and SOPC are miscible at this ratio and that the

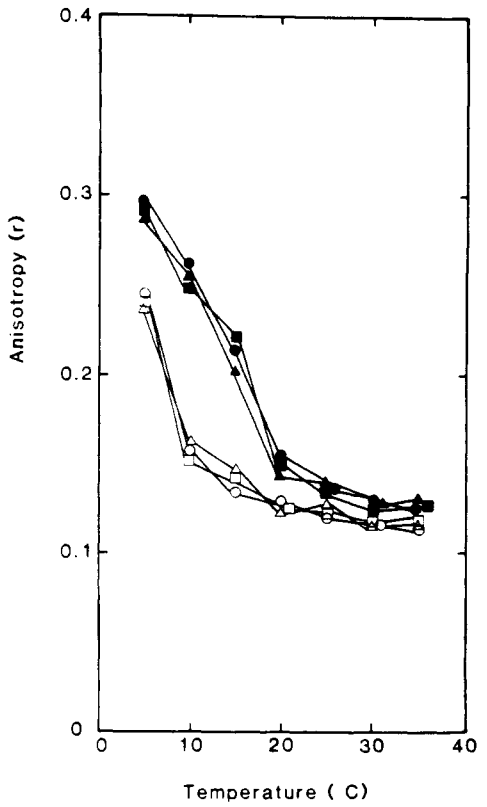

FIGURE 4: Fluorescence anisotropy of parinaroyl probes in SOPC and SOPC containing 25 mol % ganglioside G_{M1}. Data were obtained as described in the text; probe/lipid ratios were 1/200 in all cases. Open symbols = SOPC alone; filled symbols = SOPC + 25 mol % ganglioside G_{M1}: (●, ○) tPnA glucocerebroside probe; (▼, ▽) 1-palmitoyl-2-cPnA-PC probe; (■, □) cPnA probe.

ganglioside causes an increase in the transition temperature of the mixture above that of the SOPC alone.

DISCUSSION

The close resemblance (see Figure 1) between the *N-trans*-parinaroyl glycolipid probes and naturally occurring glycolipids (many of which are *N*-stearoyl species) suggests that these compounds could be useful probes for further investigation of the behavior of glycosphingolipids in model and biological membranes. These probes offer a significant advantage over other fluorescent molecules such as diphenylhexatriene (DPH) or cPnA, which can only report an average anisotropy for mixed-phase membranes. The parinaroyl GSL probes, although they exhibit similar partition coefficients to cPnA in a binary PC system, seem to be capable of detecting phase separation based on differences (either in the head group or the acyl backbone) between phospho- and sphingolipids. This is unlike the case for parinaroyl-PC or -PE probes, which seem to partition solely on the basis of acyl chain properties (Welti, 1982).

Previous reports (Bunow & Bunow, 1979; Sillerud et al., 1979; Uchida et al., 1979; Peters et al., 1984) have indicated that ganglioside G_{M1} and various PC species are miscible in the fluid lamellar phase at high PC/G_{M1} ratios. Other authors have observed that simple, neutral GSL such as glucosylcerebroside (Barenholz et al., 1983) and asialo-G_{M1} (Tillack et al., 1982) are not miscible in fluid PC lamellae, using differential scanning calorimetry and freeze-etch electron microscopy. Our data support these hypotheses. Fluorescence anisotropy of PnA probes in mixtures of SOPC and ganglioside G_{M1} (Figure 4) indicate that incorporation of ganglioside at these levels results in an increased transition temperature of

the mixture. These data agree quite well with the calorimetric measurements and conclusions of Bunow and Bunow (1979). All three probes detect a phase change at the same temperature. In contrast to these observations, the three PnA probes detect dissimilar phase changes in mixtures of SOPC and the neutral GSL (Figure 3). The parinaroyl GSL probe detects an increase in lipid order at a temperature above that of the SOPC, while the parinaroyl PC probe detects a transition corresponding to that of the SOPC alone. The free fatty acid probe detects a phase change at both temperatures, indicative of a phase separation. These results lend support to the notion that motion and distribution of neutral GSL in biological membranes might be very different from that of the gangliosides. This difference in membrane distribution between neutral and negatively charged GSL species may have profound implications for GSL function as receptors for various extracellular ligands, including hormones (Cuatrecasas, 1973), growth-regulatory substances (Kinders et al., 1982; Bremer et al., 1983), lymphokines (Liu et al., 1982), and immunotoxins directed toward GSL antigens (Weils et al., 1984).

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