Interaction of Glycosphingolipids and Glycoproteins: Thermotropic Properties of Model Membranes Containing G_{M1} Ganglioside and Glycophorin†

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ABSTRACT: High-sensitivity differential scanning calorimetry (DSC) was used to study the mutual interactions between a glycoprotein (human glycophorin, GPA) and a glycosphingolipid (G_{M1} ganglioside) embedded in large unilamellar vesicles composed of dimyristoylphosphatidylcholine (DMPC). The DSC thermograms exhibited by DMPC/G_{M1} vesicles, either in the presence or in the absence of GPA, are resolvable into two components. The relative contribution of the minor component, centered at higher temperature, to the total enthalpy and its transition temperature increase with the concentration of the glycolipid embedded in the vesicles. This minor peak, undetectable in the absence of ganglioside, is indicative of the occurrence of lateral phase separation and suggests that G_{M1} ganglioside-enriched domains are present within the bilayer. At a given concentration of G_{M1} embedded in the vesicles, the proportion of the phase-separated peak is higher in the presence of GPA, suggesting that the glycoprotein enhances the tendency of G_{M1} to segregate. Experiments investigating the thermotropic behavior of GPA show that the temperature of irreversible thermal unfolding of the glycoprotein inserted in DMPC vesicles, centered at 65.9 °C in the absence of G_{M1}, is shifted to 57.6 °C when G_{M1} is present in the bilayer. These results indicate that, at least in this experimental system, on the one hand, GPA enhances the tendency of the glycolipid to segregate within the membrane, and on the other hand, the glycolipid clusters affect the protein conformation and oligomerization in the membrane.

The fluid mosaic model, proposed almost 20 years ago (Singer & Nicholson, 1972), describes biological membranes as a mixture of lipids and proteins in a bilayer where the molecules are randomly distributed and can undergo free lateral motion without any long-range order. Successive investigations (Van Heerikhuizen et al., 1975; Klausner et al., 1980) showed the existence of membrane lipid domains, that is, of zones where the local composition is different from that of the surrounding environment (Metcalf et al., 1986; Simons & VanMeer, 1988; Wolf et al., 1990). As a consequence of lipid domain formation, important biochemical functions, for instance, the activity of intrinsic membrane proteins, enzymes, ion channels, and receptors, could be affected (Stubbs & Smith, 1984). Gangliosides, sialic acid containing glycosphingolipids present in the plasma membrane of vertebrate cells (Ledeen, 1978; Wiegandt, 1985), undergo lateral phase separation both in artificial membranes, (Thompson & Tillack, 1985; Masserini et al., 1990; Rock et al., 1991) and in cell membranes (Palestini et al., 1991). It has been shown that the ganglioside hydrophobic portion, ceramide, inserted in the lipidic core of the membrane, and the oligosaccharide hydrophilic moiety, protruding from the membrane and free to interact with external ligands, are factors able to generate and maintain this segregation (Masserini et al., 1989; Calappi et al., 1992). Integral membrane proteins, that in principle can interact with either ganglioside moiety, are able to affect the aggregation of gangliosides and of other lipids as well (Harvestick & Glaser, 1989; Wang et al., 1988; Sharom & Grant, 1978). On the other hand, glycolipids interacting with proteins can modulate their activity (Leon et al., 1981). It has been shown, for instance, that glycolipids and glycophorin, one of the major glycoproteins of the erythrocyte membrane (Marchesi et al., 1972), may interact with each other and modify their haptenic activity and reactivity, respectively, toward lectins (Endo et al., 1982).

In order to investigate the interactions between glycoproteins and glycolipids in a membrane, we investigated by high-sensitivity differential scanning calorimetry the thermotropic behavior of a membrane-mimicking system (large unilamellar vesicles, LUVs)[1] composed of dimyristoylphosphatidylcholine (DMPC), glycophorin (GPA), and G_{M1} ganglioside. In addition, we studied the irreversible thermal unfolding of GPA embedded in DMPC vesicles both in the presence and in the absence of G_{M1} ganglioside.

MATERIALS AND METHODS

Commercial chemicals were of analytical grade. Dimyristoylphosphatidylcholine and human glycophorin A (type MM) were purchased from Sigma Chemical Co. (St. Louis, MO). G_{M1} ganglioside was extracted and purified from beef brain according to the procedure described by Tettamanti et al. (1973). Its identification, structural analysis, and purity were assessed as previously described (Sonnino et al., 1978). The long-chain base composition was 48% (molar) C18:1 and 49% C20:1, the saturated species (C18:0 and C20:0) summing to about 3%. Stearic acid (18:0) was the preponderant fatty acid (95%).

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[1] Abbreviations: DMPC, dimyristoylphosphatidylcholine; DSC, differential scanning calorimetry; G_{M1}, II^{3}NeuAc-GgOse$_4$Cer,Galβ1–3GalNAcβ1–4(NeuAcα2–3)-Galβ1–4Glcβ1–1′Cer; GPA, glycophorin; LUVs, large unilamellar vesicles. Ganglioside nomenclature is in accordance with Svennerholm (1970) and the IUPAC–IUB Recommendations (1977, 1982).

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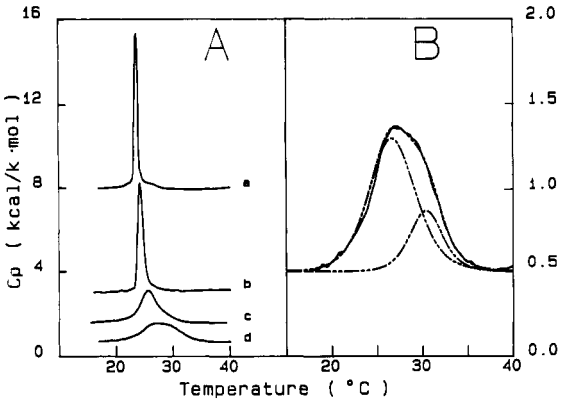

FIGURE 1: Heat capacity function vs temperature for DMPC LUVs containing up to 10% molar G_{M1} (panel A: a, 0% G_{M1}; b, 3.5% G_{M1}; c, 7.5% G_{M1}; d, 10% G_{M1}) and an illustration of the curve-fitting procedure (panel B) used to resolve the components of the DSC thermogram exhibited by DMPC LUVs containing 10% molar G_{M1} (solid line, experimental curve; dashed lines, theoretical curves).

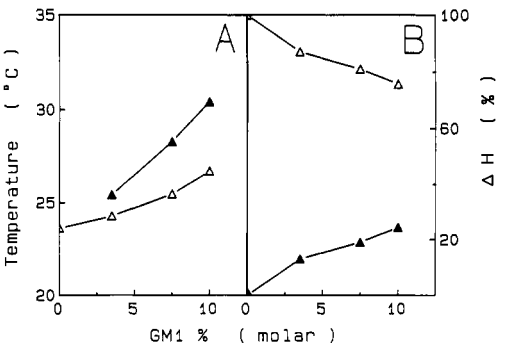

FIGURE 2: Effect of increasing amounts of G_{M1} on the T_m (panel A) and on the transition enthalpies (panel B) of the components of the DSC thermograms of DMPC LUVs resolved by the curve-fitting procedure: △ = lower temperature peak; ▲ = higher temperature peak.

Vesicle Preparation. Vesicles were prepared according to the method of MacDonald and MacDonald (1975), adequately modified. In brief, DMPC and G_{M1}, both dissolved in methanol–chloroform (3:1, v/v), were mixed in the established proportion. GPA (0.2% molar with respect to DMPC) was added from an aqueous solution. The mixture was dried under a gentle flow of N_2 and then lyophilized for 4 h in order to remove residual solvent. The film was swollen in 50 mM KCl containing 0.02% NaN_3 (2 mM final phospholipid concentration), and the suspension was sonicated for 1 min in a bath sonicator (G112SPIG, Laboratory Supplies, Hicksville, NY). A homogeneous population of large unilamellar vesicles (LUVs) was prepared by 10 successive extrusions of the aqueous dispersion of multilamellar vesicles through 1000-Å-pore-size filters (Nucleopore, Pleasanton, CA), using an N_2 pressure-operated extruder (Lipoprep, Ottawa, Ontario, Canada). The average size of the obtained vesicles was 109 ± 9 nm, as checked by laser-light scattering (Model BI-90, Brookhaven, Holtsville, NY). Vesicles were used within 1 day of preparation.

Differential Scanning Calorimetry. Calorimetric experiments were performed with a Microcal MC2D calorimeter (Microcal, Amherst, MA) interfaced to an IBM PC microcomputer for automatic data collection and analysis. Curve fitting was used to estimate the parameters (the transition temperature and the enthalpies) of the component peaks when the thermogram was the summation of overlapping peaks. The deconvolution was based on the assumption that the thermograms can be described in terms of a linear combination of two independent transitions, each approximating a two-state transition. Calorimetric scans were performed at a scan rate of 20 °C/h for the evaluation of the thermotropic properties of lipids and at 60 °C/h for the thermotropic properties of GPA. In the latter case, the reversibility of the thermal transition was checked by reheating the solution in the calorimeter cell immediately after it was cooled from the first scan.

RESULTS

As reported in Figure 1, panel A, the calorimetric scan of DMPC LUVs shows the presence of a gel–liquid crystalline transition characterized by an enthalpy change of 6.7 kcal/mol, a transition temperature of 23.6 °C, and a half-height width of 0.75 °C. Calorimetric scans of vesicles containing up to 10 mol % G_{M1} were performed. As a general trend, when the ganglioside content in the vesicle is increased, the main phospholipid gel to liquid-crystalline transition of DMPC is shifted toward higher temperatures and the peak becomes broader and asymmetric (Figure 1, panel A). The DSC thermograms can be fit by two components, which are resolved by the fitting procedure described above. The standard deviations between the theoretical and experimental curves were 0.018, 0.04, and 0.08 kcal K^{-1} mol^{-1} for vesicles containing 10%, 7.5%, and 3.5% G_{M1}, respectively. For example, the scan of vesicles containing 10 mol % G_{M1}, along with the theoretical curves of the deconvolution that best fits the experimental values, is reported in Figure 1, panel B. The experimental values (T_m and ΔH) of the deconvoluted peaks are plotted in Figure 2.

The amplitude of the main, lower temperature component decreases when the proportion of G_{M1} increases, while its position gradually shifts to higher temperature. On the other side, the minor peak, centered at higher temperature and undetectable in the absence of ganglioside, increases its amplitude in such a fashion that the total enthalpy change (the sum of the two peaks) remains constant. These results suggest that the lower temperature peak is relative to a domain mainly constituted of DMPC, while the higher temperature peak is relative to a phase-separated domain, implying that, in the presence of G_{M1}, some of the phospholipid molecules leave the main transition to assemble in a ganglioside richer, higher temperature melting domain. To ascertain the influence of GPA on the thermotropic properties of ganglioside-containing vesicles, a second set of experiments was performed on DMPC vesicles containing a fixed amount (0.2% molar) of GPA. In the presence of GPA (Figure 3, panel A) the total enthalpy change of DMPC decreases to 4.5 k/cal/mol, suggesting that the protein interacts with the phospholipid hydrophobic moiety, as already reported (Ruppel et al., 1982). In the presence of G_{M1} at increasing concentrations, the profile of the scans dramatically changes, broadening and showing the presence of a shoulder on the high-temperature side of the main transition. The total enthalpy change associated with the transition remains constant.

Deconvolution analysis shows that the heat capacity profile is well fit by two independent transitions, as reported in Figure 3, panel B, showing the experimental and the theoretical curves for vesicles containing 10% molar G_{M1}. The standard deviation between the theoretical and experimental curves was 0.016, 0.036, and 0.014 kcal K^{-1} mol^{-1} for vesicles containing 10%, 7.5%, and 3.5% G_{M1}, respectively.

In Figure 4 a plot of the ΔH and the T_m of the deconvoluted peaks as functions of the amount of ganglioside inserted in the vesicle is reported. As in the case of vesicles without GPA,

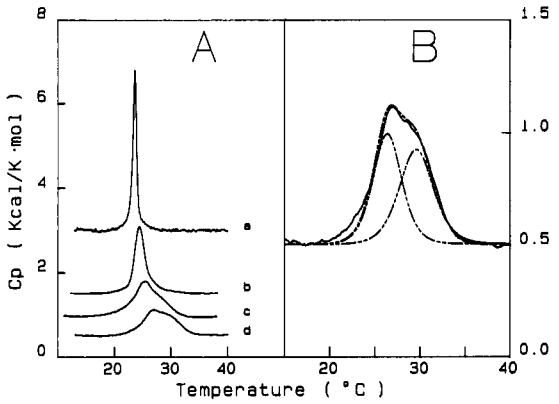

FIGURE 3: Heat capacity function vs temperature for DMPC LUVs containing 0.2% GPA and up to 10% molar G_{M1} (panel A: a, 0% G_{M1}; b, 3.5% G_{M1}; c, 7.5% G_{M1}; d, 10% G_{M1}) and an illustration of the curve-fitting procedure (panel B) used to resolve the components of the DSC thermogram exhibited by DMPC LUVs containing 0.2% GPA and 10% molar G_{M1} (solid line, experimental curve; dashed lines, theoretical curves).

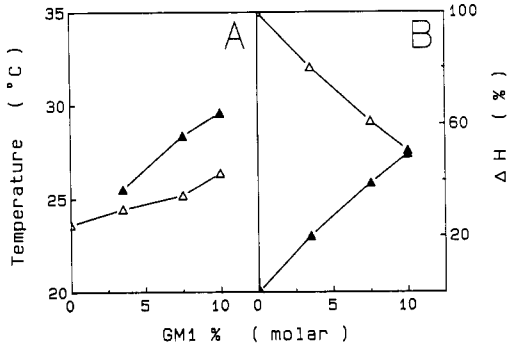

FIGURE 4: Effect of increasing amounts of G_{M1} on the T_m (panel A) and on the transition enthalpies (panel B) of the components of the DSC thermograms of DMPC LUVs containing 0.2% GPA resolved by the curve-fitting procedure: △ = lower temperature peak; ▲ = higher temperature peak.

the enthalpy change associated with the phase-separated peak, centered at higher temperature, is increasing along with the concentration of glycolipid. At a given G_{M1} concentration, the proportion of the high-temperature peak is remarkably higher in the presence than in the absence of GPA, while the thermodynamic parameter T_m remains substantially unmodified. These results suggest that the protein enhances the tendency of G_{M1} ganglioside toward phase separation.

In order to evaluate whether the presence of ganglioside in the bilayer affects the glycoprotein, the thermal behavior of GPA embedded in DMPC vesicles was studied either in the presence or in the absence of G_{M1}. In Figure 5 the calorimetric scan relative to GPA embedded in DMPC vesicles (0.2 mol % GPA) is reported, showing that the thermal denaturation of the protein, occurring at 65.9 °C, is irreversible, since no transition was observed in a second run of the samples. When G_{M1} (10 mol %) is also present, the temperature dramatically shifts to a lower value (57.6 °C). The ratio of the measured enthalpy of denaturation (ΔH_d) and the van't Hoff enthalpy of denaturation (ΔH_{vH}) is indicative of the number of molecules which associate in the cooperative process. (Engeseth & McMillin, 1986). For GPA embedded in DMPC LUVs the calculated $\Delta H_{vH}/\Delta H_d$ ratio is 5.8, consistent with the presence of the protein in the bilayer as an oligomer, and decreases to about 2.5 in the presence of G_{M1}. These data indicate that the glycoprotein is affected by the presence of gangliosides. As a consequence of this interaction, a change in the

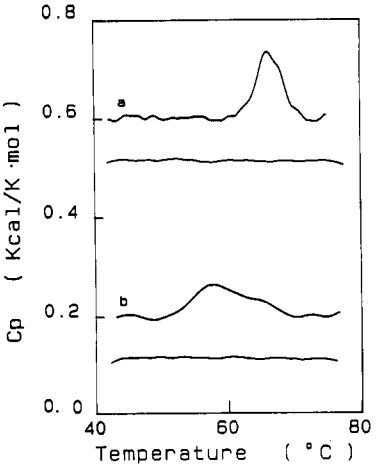

FIGURE 5: Heat capacity function vs temperature for GPA: trace a, 0.2% molar GPA reconstituted in DMPC LUVs; trace b, 0.2% molar GPA reconstituted in DMPC LUVs containing 10% molar G_{M1}. For each DSC thermogram, the rescan is also shown. The scanning rate for this experiment was 60 °C/h.

thermotropic properties of the protein is occurring, possibly due to a change in its degree of oligomerization.

DISCUSSION

When native G_{M1} is embedded in DMPC vesicles, remarkable effects are observed in the membranous system. The heat capacity profile can be deconvoluted in two partially overlapping peaks indicative of a ganglioside lateral phase separation. The lateral segregation displayed by G_{M1} in DMPC vesicles confirms data previously obtained with G_{M1} embedded in other phospholipids (Masserini & Freire, 1986) and strengthens the hypothesis that one of the factors leading to glycolipid membrane segregation is the difference in the lipid chain length between the ganglioside ceramide and the phospholipid fatty acyl moieties (Masserini & Freire, 1986; Masserini et al., 1989). Experiments to assess if this sort of phase separation is relevant also in the biological membrane have been reported (Palestini et al., 1991) and are currently in progress in our laboratories.

The presence of glycophorin in the vesicles markedly affects the bilayer structural organization, enhancing the tendency of G_{M1} to spontaneously form clusters. It has already been suggested that the presence of integral membrane proteins can influence and modify the membrane architecture (Rodgers & Glaser, 1991), giving rise to a nonrandom lipid distribution (Wang et al., 1988). The remarkable change of GPA thermal properties shown by the decrease, induced by the presence of the G_{M1}, both in the number of cooperative units and in the temperature of the irreversible unfolding, indicates that the glycoprotein is strongly interacting with the glycolipid. Therefore, it seems conceivable that GPA is enhancing G_{M1} segregation by attracting it, possibly by carbohydrate–carbohydrate interactions as previously suggested (Endo et al., 1982), rather than repelling it and leaving membrane regions enriched in GPA and depleted of ganglioside. Additional hypotheses, such a GPA-facilitated transfer of ganglioside molecules from one vesicle to another, deserve further attention and experimental work. We can speculate that in DMPC/GPA/G_{M1} vesicles the effect of the protein on the organization of the lipid bilayer is that of inducing the different components to organize in a supramolecular assembly due to this kind of interaction. A possible picture is that, above the vesicle surface, a lattice of GPA sugar residues is present under which the G_{M1} oligosaccharides are facilitated

to segregate. As a counterpart, the ganglioside clustering exerts an influence on GPA. It is likely that the glycolipid interacts through hydrogen bonding with specific sites of the glycoprotein, modifying its ability for specific conformations and its degree of oligomerization in the bilayer. The interaction with glycolipids could modify the exposure of the carbohydrate moieties of the protein and could be a mechanism for modulating or regulating the well-known receptor activity of glycoproteins (Allaway & Burness, 1986; Paul & Lee, 1987) on the cell membrane.

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