Lipid Domains in the Membrane: Thermotropic Properties of Sphingomyelin Vesicles Containing GM1 Ganglioside and Cholesterol†

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ABSTRACT: The thermotropic behavior of palmitoylsphingomyelin vesicles containing GM1 ganglioside and cholesterol has been investigated by high-sensitivity differential scanning calorimetry. The thermograms exhibited by binary palmitoylsphingomyelin/GM1 mixtures are resolvable into two components. The relative contribution of the minor component, undetectable in the absence of ganglioside, to the total enthalpy and its transition temperature (>40 °C) increase with the concentration of the glycolipid embedded in the vesicles. These data suggest the occurrence of lateral phase separation and that more ordered, higher melting GM1 ganglioside-enriched domains are present within the sphingomyelin bilayer. Studies on binary sphingomyelin/cholesterol mixtures confirmed the known tendency of the sterol to decrease the total enthalpy of sphingomyelin, forming cholesterol-enriched domains. The thermograms exhibited by ternary sphingomyelin/ganglioside/cholesterol mixtures in variable proportions (up to 20% molar GM1 or Chol) displayed, on increasing the content of either the sterol or the ganglioside, features addressable to sphingomyelin/cholesterol (peaks centered at temperature ≤40 °C, decrease of enthalpy) or to sphingomyelin/GM1 mixtures (peaks centered at a temperature >40 °C), respectively. This trend was confirmed by deconvolution analysis, showing that the thermograms are resolvable into components addressable to GM1-enriched and to cholesterol-enriched domains. Taken all together, the results show that the architectural features of sphingomyelin bilayers are strongly dependent on the presence of GM1 ganglioside and cholesterol, whose presence is leading to the formation of separate, GM1-enriched and cholesterol-enriched distinct domains. Ganglioside−sphingomyelin and sphingomyelin−cholesterol, together with mutual ganglioside-ganglioside, interactions could contribute to maintain a network of bonds extending to proteins, forming specialized membrane domains, such as caveolae, or others, whose experimental clues are the glycolipid-enriched detergent-insoluble fractions that can be isolated from cell membranes.

The presence and the biological role of lipid domains, that is of membrane areas having local lipid composition different from the surrounding environment, have been investigated either in artificial or in cellular models using a variety of techniques (Thompson & Tillack, 1985; Tocanne et al., 1989; Welti & Glaser, 1994; Masserini et al., 1989). An original approach to investigate this subject was introduced by a procedure for the isolation of membrane domains based upon their insolubility in cold nonionic detergents (Kurzchalia et al., 1995; Parton & Simons, 1995). It has been reported that membrane fractions prepared by this technique are peculiarly enriched in glycolipids, cholesterol, and sphingomyelin, and depleted of phosphatidylcholine (Brown & Rose, 1992; Sargiacomo et al., 1993; Cinek & Horeisì, 1992). These glycolipid-enriched detergent-insoluble fractions contain several proteins (Hope & Pike, 1996), including VIP21/caveolin, the marker of non-chlatrin-coated invaginations of the plasma membrane, caveolae, where in particular the enrichment of GM1 ganglioside has been proved by means of different techniques (Anderson, 1993; Parton, 1994; Fra et al., 1995a; Schnitzer et al., 1995; Rothberg et al. 1992). However, it has been reported that glycolipid-enriched detergent-insoluble fractions can be isolated also from cells lacking caveolae (Fra et al., 1995b), suggesting that different types of domains sharing common features of detergent insolubility and of glycosphingolipid enrichment are present and can coexist within the same cell (Schnitzer et al., 1995).

These observations suggest the possibility that interactions occurring between lipids lead to the formation of membrane domains (Simons & van Meer, 1988). This is also substantiated by the observation that cholesterol- and sphingolipid-rich liposomes are resistant to solubilization by detergents (Schroeder et al., 1994).

The aim of the present investigation is to assess, using high sensitivity differential scanning calorimetry (DSC),[1] the segregative properties of GM1 ganglioside within bilayers composed of sphingomyelin, in the presence of cholesterol. We chose liposomes as the study model, since they are a widely used membrane-mimicking system by which lipid−lipid interactions, far away from the complexity of a cellular membrane, can be more easily recognized. Moreover, the complexity of the model can be step by step increased by adding components one after the other, taking advantage of the information recorded on simpler mixtures and of the improvement of analytical techniques.

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[1] Abbreviations: SM, *N*-palmitoyl-D-sphingomyelin; ESM, egg yolk sphingomyelin; Chol, cholesterol; DSC, differential scanning calorimetry; T_m, gel to liquid-crystalline temperature transition; ΔH, enthalpy change.

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MATERIALS AND METHODS

Materials. *N*-Palmitoyl-D-sphingomyelin (SM) and sphingomyelin from chicken egg yolk (ESM) were purchased from Sigma (Milan, Italy). Monosialoganglioside GM1 was extracted and purified from bovine brain and characterized as described (Acquotti et al., 1990). The purity and integrity of gangliosides were checked by thin-layer chromatography (TLC) and nuclear magnetic resonance. Cholesterol (Chol) was from Fluka (Buchs, Switzerland). All other chemicals were of analytical grade, and doubly distilled water was used.

Fatty acid analysis was performed on ESM by GLC as described (Ghidoni et al., 1980). ESM contained 79% 16:0 fatty acid as the prevailing species, as reported (Calhoun & Shipley, 1979b).

Liposome Preparation. Lipids mixed in the prefixed proportion were dried under a stream of nitrogen from chloroform/methanol 2:1 (v:v) solutions, followed by high vacuum for at least 2 h. Multilamellar vesicles were subsequently produced by hydrating the dried lipid film with 50 mM KCl above the gel to liquid-crystalline transition temperature (T_m), assessed by differential scanning calorimetry (see below). A homogeneous population of large unilamellar vesicles was then obtained (Hope et al., 1986) by 10 successive extrusions through 2 stacked 100 nm polycarbonate Nucleopore filters (Costar, Milan, Italy) using an extruder (Lipex Biomembranes, Vancouver, Canada), thermostated at a temperature above the T_m. The final phospholipid concentration ranged between 2 and 4 μmol/mL. Vesicle size and homogeneity were assessed by quasi-elastic laser-light-scattering (BI-90 Brookhaven, Holtsville, NY, USA). Liposome size ranged from 95 to 110 nm for all samples and did not change after cycling through the phase transition. Dynamic laser-light-scattering measurements performed on the samples showed that a single, homogeneous population of vesicles was always present.

Phospholipid concentration was determined by the method of Bartlett (1959), cholesterol according to Siedel et al. (1983), and GM1 according to Svennerholm (1957).

Differential Scanning Calorimetry. Calorimetric analyses were performed with a Microcal MC-2D high-sensitivity differential scanning calorimeter (Microcal, Amherst, MA) interfaced to a personal computer for automatic data collection and analysis. The calorimetric scan was performed at a rate of 20 °C/h. Second scans performed on the same sample were essentially superimposable on the first one. Curve-fitting was used to estimate the T_m and the enthalpy change associated with the transition (ΔH) 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 more than one independent transition, each approximating a two-state transition (Terzaghi et al., 1993). The curve-fitting program (Microcal Deconv) was based on nonlinear least-squares minimization.

RESULTS

Binary Mixtures of SM and GM1. The c_p vs temperature plot of palmitoylsphingomyelin vesicles (Figure 1, panel A) exhibits the presence of an endothermic transition centered at 38.7 °C, with an associated ΔH of 6.1 kcal/mol. The corresponding figures obtained in the case of egg yolk sphingomyelin are 37.5 °C and 6.1 kcal/mol, respectively (data not shown). These data are comparable with previous results, obtained using SM multilamellar vesicles (Calhoun & Shipley, 1979a,b; Maulik & Shipley, 1996). The thermotropic behavior of SM mixed with increasing amounts of GM1, up to 20% molar, is reported in Figure 1. On increasing the GM1 proportion, the gel to liquid-crystalline transition of SM is shifted toward higher temperatures, and the peak becomes progressively broader and more asymmetric for the appearance of an evident shoulder on its high-temperature side, indicating the occurrence of lateral phase separation. The DSC thermogram 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 0.01, 0.02, and 0.04 kcal·K^{-1}·mol^{-1} for samples containing 5, 10, or 20% GM1, respectively. As an example, the theoretical curves that best fit the experimental data of vesicles containing 10% molar GM1 are reported in Figure 1, panel B. The data obtained by deconvolution analysis are summarized in Figure 2. On increasing the proportion of GM1 within the bilayer, the amplitude of the main, sharper, lower temperature melting component decreases, while its position gradually shifts to higher temperature (Figure 2). On the other side, the amplitude and the position of the minor, broader, higher temperature melting component, undetectable in the absence of GM1, increase. These results suggest that the lower temperature peak is relative to a domain mainly constituted of SM, while the higher temperature peak is relative to a phase-separated, ganglioside-rich domain. Similar results were obtained with ESM (data not shown).

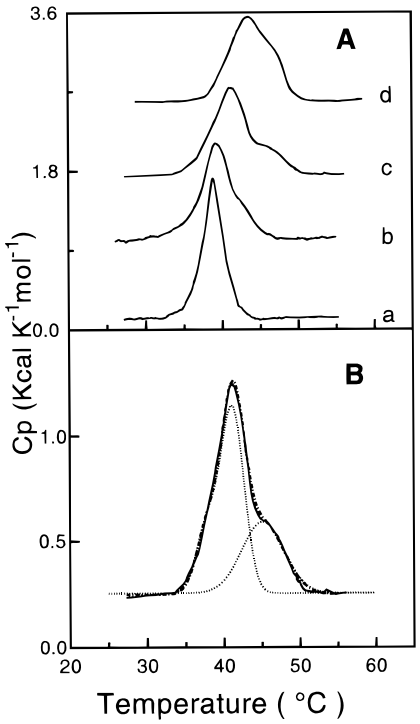

FIGURE 1: C_p vs temperature plot of palmitoylsphingomyelin vesicles containing different proportions of GM1 ganglioside. Panel A: a, 0% GM1; b, 5% GM1; c, 10% GM1; d, 20% GM1. Panel B: example of the curve-fitting procedure used to resolve the components of the thermogram exhibited by vesicles containing 10% GM1 (solid line, experimental curve; dotted lines, theoretical curves).

Binary SM/Chol and Ternary SM/GM1/Chol Mixtures. The thermotropic behavior of sphingomyelin/cholesterol vesicles has been previously investigated (Maulik & Shipley,

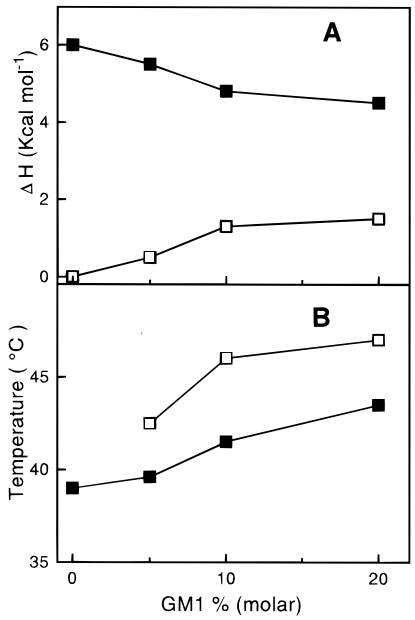

FIGURE 2: Effect of increasing proportions of GM1 ganglioside on the transition enthalpy, ΔH (panel A), and on the transition temperature (panel B) of the components of the DSC thermograms of palmitoylsphingomyelin/GM1 vesicles resolved by the curve-fitting procedure. ■ = lower temperature peak; □ = higher temperature peak.

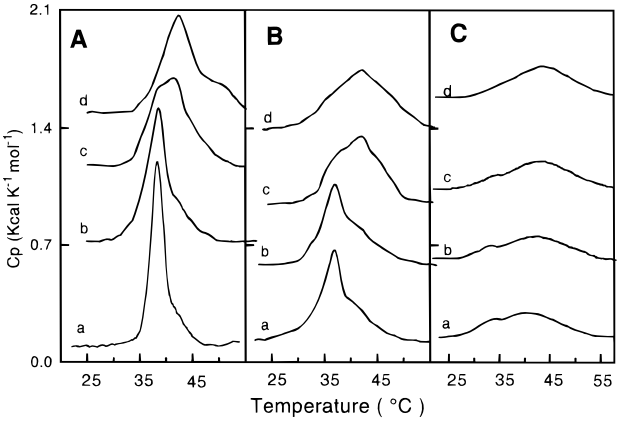

FIGURE 3: C_p vs temperature plot for vesicles composed of palmitoylsphingomyelin containing different proportions of GM1 ganglioside and cholesterol. Panel A: vesicles containing 5% cholesterol. Panel B: vesicles containing 10% cholesterol. Panel C: vesicles containing 20% cholesterol. Trace a, 0% GM1; trace b, 5% GM1; trace c, 10% GM1; trace d, 20% GM1.

1996; Estep et al., 1979; Calhoun & Shipley, 1979a). According to these data, in the presence of increasing Chol amounts the gel to liquid-crystalline transition peak of SM (ΔH 6.1 kcal/mol) progressively broadens while the enthalpy progressively decreases to 5, 4.1, and 2.1 kcal/mol at 5, 10, and 20% Chol, respectively (Figure 3, traces "a"; Figure 4).

In order to study the thermotropic behavior of SM/Chol/GM1 ternary mixtures, SM vesicles containing a fixed Chol amount and increasing GM1 proportions (5, 10, or 20% molar) were compared (Figure 3). As a general trend, on increasing the ganglioside content, the overall aspect of the thermograms progressively displayed features typical of the binary SM/GM1 ganglioside mixtures, that is, the appearance of high-temperature melting components in the scan. For instance, in the scans of the ternary SM/5% Chol/GM1 mixtures (Figure 3, panel A), on increasing the proportion

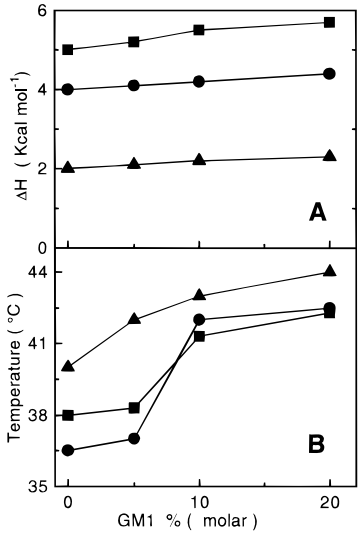

FIGURE 4: Effect of increasing proportions of GM1 ganglioside on the transition enthalpy, ΔH (panel A), and on the transition temperature (panel B) of ternary palmitoylsphingomyelin/cholesterol/GM1 ganglioside mixtures. Vesicles containing 5% (■), 10% (●), or 20% (▲) molar cholesterol.

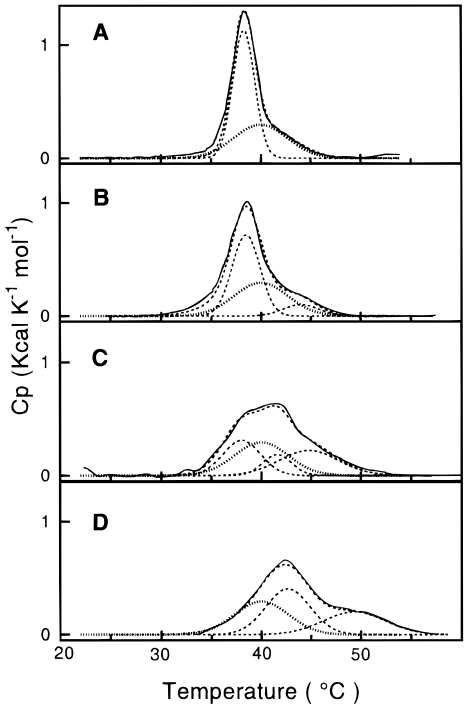

FIGURE 5: Illustration of the curve-fitting procedure used to resolve the components of the DSC thermogram exhibited by palmitoylsphingomyelin vesicles containing 5% cholesterol and increasing GM1 ganglioside proportions. Panel A, 0% GM1; panel B, 5% GM1; panel C, 10% GM1; panel D, 20% GM1. Solid line, experimental curves; dotted lines, theoretical curves.

of GM1 a peak centered at T_m >40 °C became progressively evident. At the same time, the peak centered at 38 °C in the scan of the binary SM/5% Chol mixture (trace a) progressively decreased. In the mixture containing 10% ganglioside (trace c), these peaks were strongly superimposed but well recognizable, indicating that at least two phases were coexisting. At 20% GM1 concentration, the peak centered at T_m >40 °C became predominant, and an additional shoulder was present in the thermogram at higher temperatures (trace d). This trend was also confirmed by the scans of ternary mixtures containing 10% Chol (Figure 3, panel B) and increasing ganglioside proportions and, even if in a

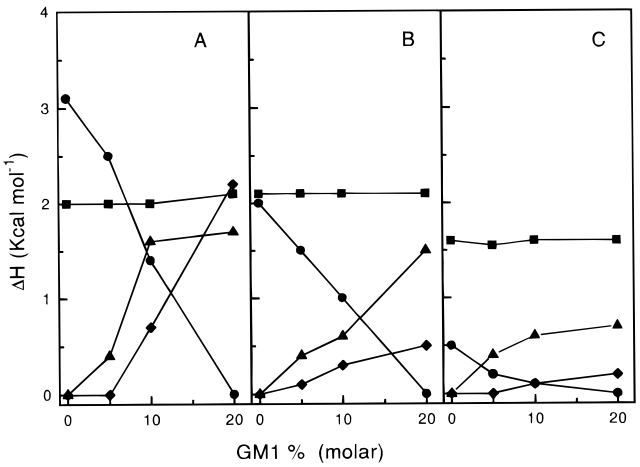

FIGURE 6: Effect of increasing amounts of GM1 ganglioside on the transition enthalpy of the components of the DSC thermograms of different sphingomyelin/cholesterol/GM1 mixtures resolved by the curve-fitting procedure. Panel A: vesicles containing 5% cholesterol. Panel B: vesicles containing 10% cholesterol. Panel C: vesicles containing 20% cholesterol. The symbols ●, ■, ▲, and ◆ are corresponding to the components obtained by the curve-fitting procedure, in order of increasing temperature. (●, ■) = peaks centered at temperature ≤40 °C. (▲, ◆) = peaks centered at temperature >40 °C.

much lesser degree owing to their broadness and low enthalpy, by the scans of ternary mixtures containing 20% Chol (Figure 3, panel C). Comparable results were obtained using ESM (data not shown).

At a given Chol proportion in the bilayer, the ΔH of ternary SM/Chol/GM1 mixtures was comparable with that of binary SM/Chol mixtures, and remained almost constant, or displayed a slight increase, on increasing the ganglioside content (Figure 4). This latter observation suggests that cholesterol undergoes formation of a Chol-rich phase in ternary mixtures as it undergoes in SM/Chol binary mixtures. This hypothesis was confirmed by deconvolution analysis of the thermograms, as will be described later.

An example, for SM/5% Chol vesicles containing increasing GM1 proportions is reported in Figure 5, where the theoretical curves that best fit the experimental data are plotted. As described (Estep et al., 1979; Calhoun & Shipley, 1979a), the calorimetric scan of the binary SM/Chol mixture can be fit by two components; one sharper, centered at a lower temperature; and a second broader, due to a Chol-poor and to a Chol-rich phase, respectively (Figure 5, panel A). On increasing the ganglioside concentration, additional, higher melting components (T_m >40 °C) appeared, and their amplitude increased at the expenses of the Chol-poor phase (at 38 °C), decreasing (Figures 5 and 6). Moreover, deconvolution of the thermograms showed the constant presence of a component corresponding, both in its position (40 °C) and in its ΔH (2kcal/mol), to the Chol-rich phase (Figures 5 and 6).

This trend was confirmed by submitting to deconvolution analysis also the scans of ternary mixtures containing 10 and 20% Chol. In general, at the highest cholesterol and GM1 proportion utilized, the overall number of relevant components was less. The data obtained by deconvolution analysis of all the mixtures examined are summarized in Figure 6. The standard deviation between the theoretical and experimental curves ranged between 0.008 and 0.04 $kcal \cdot K^{-1} \cdot mol^{-1}$.

DISCUSSION

While several studies on the thermotropic behavior and segregative properties of phospholipid bilayers embedding gangliosides have been extensively carried out (Sillerud, 1979; Bach & Sela, 1985; Pinnaduwage & Huang, 1988; Masserini et al., 1989; Terzaghi et al., 1993), the same issue has never been studied with sphingomyelin, one of the major components of the cell membrane. Moreover, this investigation is also relevant for the possible connection with glycolipid-enriched membrane domains and their biological role (Parton & Simons, 1995). The data herein obtained indicate that GM1 undergoes lateral phase separation with formation of domains in sphingomyelin bilayers. In fact, the thermograms of SM/GM1 mixtures are resolvable into two components by deconvolution analysis. 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 component, undetectable in the absence of ganglioside, is indicative of the occurrence of lateral phase separation and suggests that GM1 ganglioside-enriched domains are present within the bilayer, their proportion increasing with the proportion of glycolipid.

Upon comparison of these results, obtained with sphingomyelin, with those previously obtained with dimyristoylphosphatidylcholine (Terzaghi et al., 1993), it is possible to infer that the tendency of GM1 ganglioside to segregate within bilayers of either phospholipid is comparable. On the contrary, it is known that GM1 embedded in dipalmitoylphosphatidylcholine vesicles does not undergo lateral phase separation (Sillerud, 1979; Masserini et al., 1989). The different segregative properties of GM1 in dimyristoyl- or in dipalmitoylphosphatidylcholine vesicles can be explained on the basis that, as previously suggested (Masserini et al., 1989), the main driving force for ganglioside lateral phase separation in ganglioside/phospholipid bilayers is the difference in the lipid chain length of the glycolipid and of the phospholipid bilayer matrix. With respect to this hypothesis, it can be argued about the reasons for the different segregative behavior of GM1 in palmitoylsphingomyelin or dipalmitoylphosphatidylcholine, carrying lipid moieties of comparable length. To this purpose, it should be reminded that sphingolipids characteristically differ from glycerolipids at least in one feature, that is, the possibility of forming hydrogen bonds at the level of the lipid portion (Pascher, 1976). In fact, the presence of a hydroxyl and an amido group enables ceramide to act both as a hydrogen bond donor and as a hydrogen bond acceptor. This feature is not shared by glycerophospholipids which can act only as acceptors of hydrogen bonds at the level of the lipid portion. Therefore, it is possible that formation of intermolecular lateral hydrogen bonds between phosphatidylcholine (acceptor) and ganglioside (donor) remains restricted to a few molecules, failing to form domains in the case of dipalmitoylphosphatidylcholine/GM1 bilayers. On the contrary, in the case of sphingomyelin/GM1 bilayers, both ganglioside and sphingomyelin molecules are acting at the same time as hydrogen bond acceptors and donors. Therefore, a network of intermolecular lateral hydrogen bonds can be formed, involving a large number of molecules. This ability, coupled to the tendency of gangliosides to self-associate, possibly through carbohydrate−carbohydrate interaction (Thompson & Tillack, 1985; Terzaghi et al., 1993), can lead to the formation of a

ganglioside-enriched, mixed GM1/sphingomyelin domain. This event, that is the formation of an oligosaccharide hydrogen bond network without disruption of the interactions within the SM lipid bilayer, could also account for the increase of the transition temperature observed for SM/GM1 bilayers in comparison with pure SM.

Of course, it will also be interesting to investigate the behavior of ternary phosphatidylcholine/cholesterol/ganglioside or of quaternary phosphatidylcholine/sphingomyelin/cholesterol/GM1 mixtures, using also unsaturated phosphatidylcholine, in order to compare the segregative properties of these complex lipid mixtures, in a model closer to the cell membrane composition.

Further experiments were carried out in order to assess the influence of cholesterol on ganglioside domain formation within sphingomyelin bilayers. Before facing this problem, that is, before studying the behavior of ternary phospholipid/GM1/Chol bilayers, the behavior of phospholipid/Chol binary mixtures was investigated. The results confirmed previous data (Maulik & Shipley, 1996; Estep et al., 1979; Calhoun & Shipley, 1979a) indicating the occurrence of an interaction between SM and cholesterol, with formation of sterol-enriched domains within the bilayer.

As a final goal, ternary SM/Chol/GM1 mixtures were investigated. The results showed that, at any GM1 proportion, the amount of cholesterol in the bilayer controls the total enthalpy, decreasing with increasing sterol amount. Deconvolution analysis showed that, at a given Chol concentration and regardless of the proportion of GM1 in the bilayer, the Chol-rich domain originally detected in the binary SM/Chol mixtures was also present in the ternary mixtures. The relative contribution of this domain to the total enthalpy increases with the proportion of Chol, matching the behavior of binary SM/Chol mixtures, while the fraction of sphingomyelin not involved in the formation of Chol-rich domains decreases. This fraction of SM is available to assemble into GM1-enriched, more ordered, high-temperature melting domains, whose relative weight increases with the proportion of ganglioside in the bilayer. The data show that, under the experimental conditions, both cholesterol-rich and GM1-rich domains are coexisting.

Taken together, our results show that the architectural features of sphingomyelin bilayers are strongly dependent on the presence of GM1 ganglioside and cholesterol, leading to the formation of separate, GM1-rich and Chol-rich distinct domains. Our results suggest that ganglioside−sphingomyelin and sphingomyelin−cholesterol, together with mutual ganglioside−ganglioside (Thompson & Tillack, 1985; Terzaghi et al., 1993), interactions could contribute to form a network of bonds between the different lipid domains. The complexity of this network could increase, together with the possibility of participating in membrane-associated events, with the extension of the interaction to other membrane components, primarily proteins. For instance, it is reported that GM1 directly interacts with receptors (Mutoh et al., 1995) and that both GM1 ganglioside and cholesterol interact with caveolar proteins (Fra et al., 1995a; Murata et al., 1995). These interactions can actively recruit specific proteins from the membrane environment in a mixed lipid−protein domain. As a consequence, important membrane functions such as lipid and protein sorting and signal transmission could be affected (Simons & van Meer, 1988; Simons and Wandinger-Ness, 1990; Parton & Simons, 1995; Kutzchalia et al., 1995) by the formation of specialized membrane domains, such as caveolae, or in others, whose experimental clues are the glycolipid-enriched detergent-insoluble fractions isolated from cell membranes.

To assess whether or not the segregative phenomena reported in the present investigation are occurring in the plasma membrane represents an appealing working hypothesis that deserves further investigation.

REFERENCES

Acquotti, D., Poppe, L., Dabrowski, J., von der Lieth, C. W., Sonnino, S., & Tettamanti, G. (1990) *J. Am. Chem. Soc. 112*, 7772−7778.

Anderson, R. G. W. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 10909−10913.

Bach, D., & Sela, B. (1985) *Biochim. Biophys. Acta 819*, 225−230.

Bartlett, G. R. (1959) *J. Biol. Chem. 234*, 466−468.

Brown, D., and Rose, J. K. (1992) *Cell 68*, 533−544.

Calhoun, W. I., & Shipley, G. G. (1979a) *Biochemistry 18*, 1717−1722.

Calhoun, W. I., & Shipley, G. G. (1979b) *Biochim. Biophys. Acta 555*, 436−441.

Cinek, T., & Horeisì, V. A. (1992) *J. Immunol. 149*, 2262−2270.

Estep, T. N., Mountcastle, D. B., Barenholz, Y., Biltonen, R. L., & Thompson, T. E. (1979) *Biochemistry 18*, 2112−2117.

Fra, A. M., Masserini, M., Palestini, P., Sonnino, S., & Simons, K. (1995a) *FEBS Lett. 375*, 11−14.

Fra, A. M., Williamson, E., Simons, K., & Parton, R. G. (1995b) *J. Biol. Chem. 269*, 30745−30748.

Ghidoni, R., Sonnino, S., Tettamanti, G., Baumann, N., Reuter, G., & Schauer, R. (1980) *J. Biol. Chem. 255*, 6990−6995.

Hope, H. R., & Pike, L. J. (1996) *Mol. Biol. Cell 7*, 843−851.

Hope, M. J., Bally, M. B., Mayer, L. D., Janoff, A. S., & Cullis P. R. (1986) *Chem. Phys. Lipids 40*, 89−107.

Kurzchalia, T. V., Hartmann, E., & Dupree, P. (1995) *Trends Cell. Biol. 5*, 187−189.

Masserini, M., Palestini, P., & Freire, E. (1989) *Biochemistry 28*, 5029−5039.

Maulik, P. R., & Shipley, G. G. (1996) *Biochemistry 35*, 8025−8034.

Murata, M., Kurzchalia, T., Peranen, J., Schreiner, R., & Wieland, F. T. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 10339−10343.

Mutoh,T., Tokuda, A., Miyadai, T., Hamaguchi, M., & Fujiki, N. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 5087−5091.

Parton, R. G. (1994) *J. Histochem. Cytochem. 42*, 155−166.

Parton, R. G., & Simons, K. (1995) *Science 269*, 1398−1399.

Pascher, I. (1976) *Biochim. Biophys. Acta 455*, 433−451.

Pinnaduwage, P., & Huang, L. (1988) *Biochim. Biophys. Acta 939*, 375−382.

Rothberg, K. G., Heuser, J. E., Donzell, W. C., Ying, Y.-S., Glenney, J. R., & Anderson, R. G. W. (1992) *Cell 68*, 673−682.

Sargiacomo, M., Sudol, M., Tang, Z., & Lisanti, M. P. (1993) *J. Cell Biol. 122*, 789−807.

Schnitzer, J. E., McIntosh, D. P., Dvorak, A. M., Liu, J., & Oh, P. (1995) *Science 269*, 1435−1439.

Schroeder, R., London, E., & Brown, D. (1994) *Proc. Natl. Acad Sci. U.S.A. 91*, 12130−12134.

Siedel, J., Hägele, E. O., Ziegenhorn, J., & Wahlefeld, A. W. (1983) *Clin. Chem. 29*, 1075−1079.

Simons, K., and van Meer, G. (1988) *Biochemistry 27*, 6197−6202.

Simons, K., & Wandinger-Ness, A. (1990) *Cell 62*, 207−210.

Svennerholm, L. (1957) *Biochim. Biophys. Acta 24*, 604−611.

Terzaghi, A., Tettamanti, G., & Masserini, M. (1993) *Biochemistry 32*, 9722−9725.

Thompson & Tillack (1985) *Annu. Rev. Biophys. Chem. 14*, 361−386.

Tocanne, J. F., Dupou-Cezanne, L., Lopez, A., & Tournier, J. F. (1989) *FEBS Lett. 257*, 10−16.

Welti, R., & Glaser, M. (1994) *Chem. Phys. Lipids 73*, 121−137.

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