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CALORIMETRIC STUDIES ON VARIOUS GANGLIOSIDES AND GANGLIOSIDE-LIPID INTERACTIONS

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Differential scanning calorimetry was used to investigate the thermotropic behaviour of various gangliosides differing in size and in the net negative charge. It was found that the number and the position of the negative charges in the headgroup region influence strongly the phase transition profiles. Interaction of G_{M1} ganglioside with egg phosphatidylcholine or cholesterol was also investigated. G_{M1} is completely miscible with egg phosphatidylcholine, giving only one transition peak at all ratios of the two components, implying that when gangliosides are in a more fluid lipid environment in biological membranes they will be randomly distributed. Interaction with cholesterol decreases the enthalpy of melting of the ganglioside. The decrease in enthalpy reaches a plateau at about 30 mol% cholesterol, suggesting a lower affinity of cholesterol for gangliosides than for sphingomyelin.

Introduction

Gangliosides are important constituents of cell surfaces acting as receptors for numerous biologically active agents: hormones [1,2], cholera toxin [3], interferon [4], and lectins [5,6]. Many biological studies involving gangliosides were performed, but only recently physico-chemical investigations of gangliosides and their interaction products with other lipids were undertaken. The aim of these studies was to evaluate the structure of these sphingoglycolipids and to learn about the mechanism of their action in the biological membranes.

It was shown that upon dispersion in aqueous solutions the gangliosides associate to form large micelles with very low critical micelle concentration [7,8]. From fluorescence polarization measurements it was inferred that lipid dispersions containing brain gangliosides have greater microviscosity than those of the pure lipids [9]. It was also shown that the number of sialic acid residues in the molecule of the ganglioside strongly influences the interactions in the hydrophobic region [10].

From measurements of gangliosides containing spin labels it was concluded that the gangliosides increase the rigidity of fluid membranes [11], and that the sugar chains have a tendency to undergo cooperative interactions among themselves probably due to hydrogen bonding [12]. Delmelle et al. [13] claim that the clustering property of the gangliosides is a function of the thermotropic proper-

Abbreviations: G_{M1}, galactosyl-*N*-acetylgalactosaminyl-(*N*-acetylneuraminyl)galactosylglucosylceramide; G_{D1a}, *N*-acetylneuraminylgalactosyl-*N*-acetylgalactosaminyl-(*N*-acetylneuraminyl)galactosylglucosylceramide; G_{D1b}, galactosyl-*N*-acetylgalactosaminyl-(*N*-acetylneuraminyl-*N*-acetylneuraminyl)galactosylglucosylceramide; G_{T1b}, *N*-acetylneuraminylgalactosyl-*N*-acetylgalactosaminyl-(*N*-acetylneuraminyl-*N*-acetylneuraminyl)galactosylglucosylceramide; G_{Q1b}, *N*-acetylneuraminyl-*N*-acetylneuraminylgalactosyl-*N*-acetylgalactosaminyl-(*N*-acetylneuraminyl-*N*-acetylneuraminyl)galactosylglucosylceramide.

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ties of the surrounding lipids. Gangliosides alone, or upon interaction with other lipids, were investigated by NMR spectroscopy [14,15], while the thermotropic properties of gangliosides and of their interaction products with synthetic phospholipids were studied by calorimetry [15–19]. It was shown that the gangliosides undergo phase transition in a wide range of temperatures [16–18] and their influence on the melting properties of synthetic lipids is very complex.

We have investigated previously the thermotropic behaviour of mixed bovine brain gangliosides and human Tay-Sachs ganglioside interacting with peanut lectin, serotonin or daunomycin [17].

The present studies were undertaken: (1) to investigate the thermotropic properties of various purified gangliosides in an attempt to learn how the increase in size and charge of the headgroup influences the phase behaviour, and (2) to investigate the interaction of natural lipids, such as cholesterol or egg phosphatidylcholine, with the purified gangliosides that might represent actual interactions occurring in biological membranes.

Materials and Methods

Mixed gangliosides were isolated from bovine brain by chloroform extraction, followed by alkaline methanolysis, phase partition, DEAE-Sephadex and silicic acid chromatography. The gangliosides were further fractionated into pure fractions, as described previously [20,21]. The individual fractions employed in the present study were: I, G_{M1}; II, G_{D1a} (65%) and G_{D1b} (35%); III, G_{T1b} (85%) and G_{D1b} (15%); and IV, G_{Q1b} (75%) and G_{T1b} (25%), as analyzed by TLC and densitometry (the numbers indicate weight percent in each fraction).

Egg phosphatidylcholine grade I (in chloroform/methanol (2:1, v.v) was purchased from Lipid Products, South Nutfield, U.K. Cholesterol was a Merck Product which was recrystallized from ethanol three times. The experiments for the calorimetric measurements were prepared by weighing the material directly into the aluminium pans. Usually 1–2 mg of the gangliosides or ganglioside-lipid mixtures were weighed and about 10 mg of salt solution were added. In an attempt to check the effects of gangliosides' concentration on the thermotropic properties, the experiments with the G_{M1} gangliosides were also performed at a concentration of 0.4 mg G_{M1} in 20 mg salt. For the experiments with cholesterol or egg phosphatidylcholine the gangliosides were dissolved in chloroform/methanol (1:1 v/v) and appropriate volumes of cholesterol or egg phosphatidylcholine solutions were added. The solutions were mixed, the solvents driven off by a stream of nitrogen and kept under high vacuum for 3 h. Subsequently, the material was weighed into the pans as above.

The salt solutions used were $1.5 \cdot 10^{-1}$ M NaCl in 10^{-2} M Tris-HCl buffer (pH 7.2). 4 M NaCl in 10^{-2} M Tris-HCl buffer or 0.5 M Tris-HCl buffer (pH 8.5). The measurements of gangliosides-egg phosphatidylcholine interactions were also performed in a mixture of ethylene glycol/salt (1:1, v/v), thus enabling to start the scanning at sub-zero temperatures. The calorimetric measurements were performed on a DuPont 990 differential scanning calorimeter with a cell base II. The calibrated mode was used, usually a sensitivity of 0.02 mcal/s per inch and a scan rate of 5 K/min were employed. For the G_{M1} experiments at the very low concentrations the sensitivity of 0.01 mcal/s per inch was employed. To evaluate the influence of scan rate the experiments with the G_{M1} gangliosides were also run at the scanning rates of 10 K/min, 5 K/min, 2 K/min, and at sensitivity of 0.04 mcal/s per inch.

In all the experiments the samples were rescanned several times, and after the first heating scan the profile and the enthalpy did not change any further. In the case of the interaction products the experiments were performed on the day of preparation and were repeated after storage of the pans for 48–72 h at 4°C.

Results and Discussion

Thermotropic behavior of individual gangliosides

Fig. 1 presents the schematic formulae of the various gangliosides. Fig. 2 shows the thermotropic profiles of the individual gangliosides. The material was dispersed in 0.15 M NaCl in 10^{-2} M Tris-HCl buffer, pH 7.4, and the experiments were repeated on preparations from different bovine brains. In some samples the peak appearing at lower temperature was less pronounced, but the enthalpy of

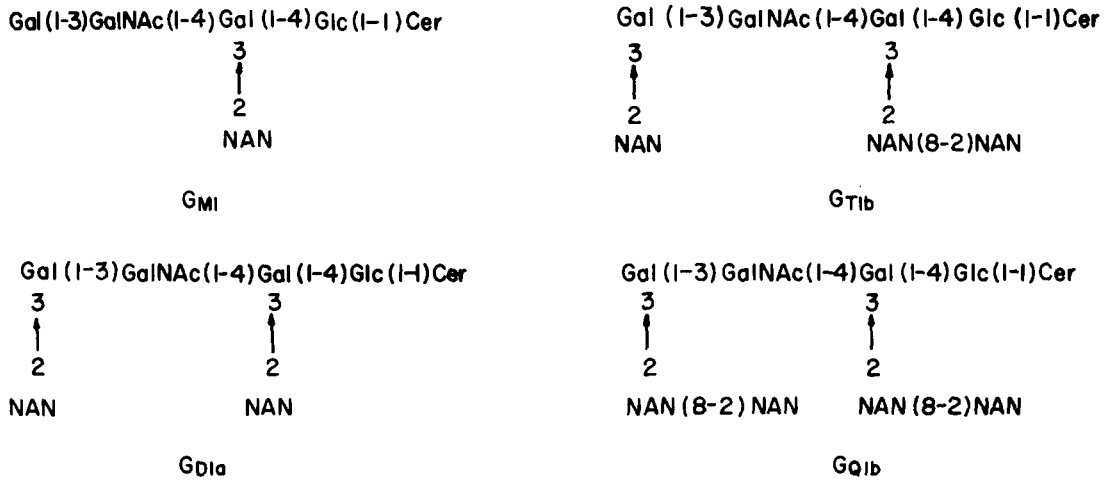

Fig. 1. Schematic formulae of different gangliosides.

melting was the same within experimental error.

The profile for the mixed bovine brain gangliosides (Fig. 2A) is similar to the one presented by us previously [17] and to that found by Bunow and Bunow [18]. However, Sillerud et al. [19] and recently Hinz et al. [15] did not detect phase transition in the gangliosides G_{M1} or G_{D1a} also by employing calorimetry, but did find phase transition in NMR studies [15]. The cause of this discrepancy is unknown inasmuch as that in our study we have detected phase transition of G_{M1} gangliosides at concentrations as low as 20 mg/ml, as compared to 15 mg/ml employed by Hinz et al. [15]. At the low concentration the peak profile of G_{M1} was less discernible but, nevertheless, distinct transition in the range of 10°C to 48°C was seen. The enthalpy of this transition was the same as the one obtained at higher concentrations. The influence of scan rate on the phase behaviour of the G_{M1} ganglioside was also investigated. It was found that the enthalpy of melting is practically the same at the scanning rates of 10 K/min, 5 K/min, and 2 K/min. The ΔH values, obtained in aqueous solutions when the scan was started at 0°C or at −2°C, are probably underestimated by about 10%, since the base lines and peak areas are drawn under the assumption that the heat flow at 5°C is zero when the output becomes stable. Yet, since we are interested in the relative effect of the number of sugar residues and charges per ganglioside molecule on the ΔH, such an underestimation is of no actual concern.

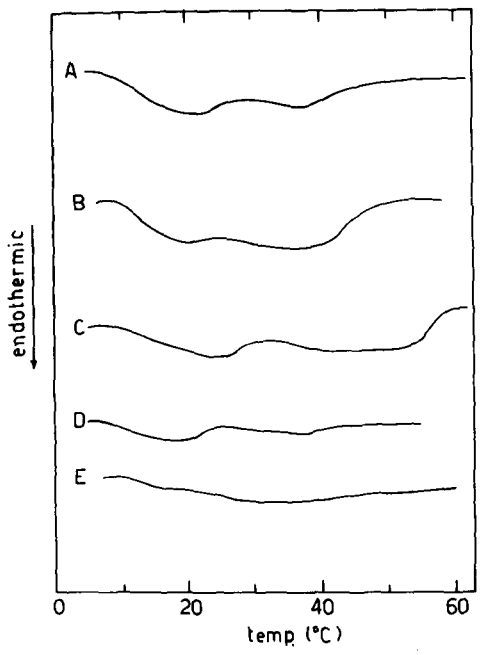

Fig. 2. The differential scanning calorimetry thermograms of different gangliosides. A, 1.9 mg mixed gangliosides; B, 2.1 mg G_{M1} ganglioside; C, 1.9 mg G_{D1a} ganglioside; D, 1.5 mg G_{T1b} (85%), G_{D1b} (15%); E, 1.5 mg G_{Q1b} (75%) and G_{T1b} (25%).

As seen from Fig. 2, the profiles of mixed bovine brain gangliosides and of the G_{M1} (A, B) are almost identical with enthalpy of melting of 2.7 mcal/mg ganglioside. The increase of the size of the headgroup and of the number of negative

charges (sialic acid) affects the profile. It becomes more shallow and finally a decrease of the enthalpy of melting is seen. The enthalpy of melting of G_{D1a} (Fig. 2C) is 2.8 mcal/mg. Further increase in the negative charge brings about flattening of the thermotropic profile and a concurrent decrease of the enthalpy of melting to about 1 mcal/mg for a fraction containing predominantly G_{T1b} (Fig. 2D) and that containing predominantly G_{Q1b} (Fig. 2E). To investigate the influence of the charge and of the electrostatic interactions on the thermotropic properties of the gangliosides, the experiments were repeated in two different salt media: 4 M NaCl in 10^{-2} M Tris-HCl buffer, pH 7.4 and 0.5 M Tris buffer, pH 8.5 (equivalent to approx. ~0.15 M salt). As the pK of sialic acid is 2.6–2.7 [22], and in micelles the pK should be much higher, even though at pH 7.4 and even more so at pH 8.5, complete ionization of the sialic acid should be obtained. Indeed, only small differences between the thermograms at pH 7.4 and 0.15 M NaCl and 0.5 M Tris, pH 8.5, were found, while the enthalpy of melting of the $G_{T1b} + G_{D1b}$ was only slightly decreased. Yet, 4 M NaCl caused a shift in the melting range by a few degrees to higher temperatures. This was accompanied by a shift of the temperature of the small peaks, and by a small increase in the enthalpy of melting, whereas the profile for the $G_{T1b} + G_{D1b}$ became less shallow.

As seen from Fig. 1, the main difference between the individual gangliosides is the number and the position of the sialic acid residues. The increase in the sialic acid is also accompanied by an increase in the C_{20}-sphingosine [7], whereas the fatty acid remains virtually the same stearic acid [23]. These two factors should have an opposite effect on the thermotropic properties of the gangliosides. Increase of the charge density causes a decrease of the melting temperature and of the enthalpy of melting as found for synthetic phosphatidylserine [24], or a small change in the transition temperatures as found for phosphatidic acid [25]. The charge effect is not only an electrostatic one leading to the formation of a lesser packed structure of the hydrocarbon chains, but the charge also influences the ability of the lipid to form hydrogen bonds and this depends on the type of the headgroups. These effects are of great importance in the case of gangliosides where an extensive hydrogen bonding of the network of sugars exists. However, the increase in C_{20}-sphingosines should cause an increase in the hydrophobic interactions leading to an increase of the enthalpy of melting. Actually some balance of such opposing effects is created as seen from the thermograms of Fig. 2 and this balance is especially pronounced in the case of G_{D1a} ganglioside.

Our data obtained by differential scanning calorimetry correlate nicely with the very recent data of Uchida et al. [10], who investigated the motion of the fluorescent probe diphenylhexatriene in gangliosides as a function of sialic acid content. The motion of diphenylhexatriene in the G_{M1} gangliosides was the most restricted one, becoming less restricted in G_{D1a} and G_{D1b} gangliosides. As the diphenylhexatriene probes the hydrophobic region of the molecule, their results show that changes in the headgroup region project onto the hydrocarbon region causing it to become less densely packed. Hinz et al. [15], by employing NMR spectroscopy, found that the midpoint temperature of the phase transition of G_{D1} ganglioside is lower than that of the G_{M1}. The charge density influences also the size of the ganglioside micelles [7].

Interaction with egg phosphatidylcholine

Calorimetry served also as a tool for the investigation of the interaction of gangliosides with phospholipids [15,18,19]. In all the cases phospholipids with transition temperatures above those of the starting temperature of gangliosides were employed. However, in biological membranes the gangliosides are most probably in an environment composed of lipids with low melting temperatures, so it was of interest to investigate the interaction of gangliosides with egg phosphatidylcholine. The experiments were performed in two ways: (i) the lipids were suspended in a mixture of salt/ethylene glycol (1 : 1, v/v), enabling also to investigate the region of melting in the sub-zero region of temperatures, and (ii) in salt solution.

Fig. 3 presents the thermograms of pure G_{M1} ganglioside, pure egg phosphatidylcholine and of the interaction products at different ratios of the interactants. The pure phosphatidylcholine melts in a wide range, the termination of its melting is at

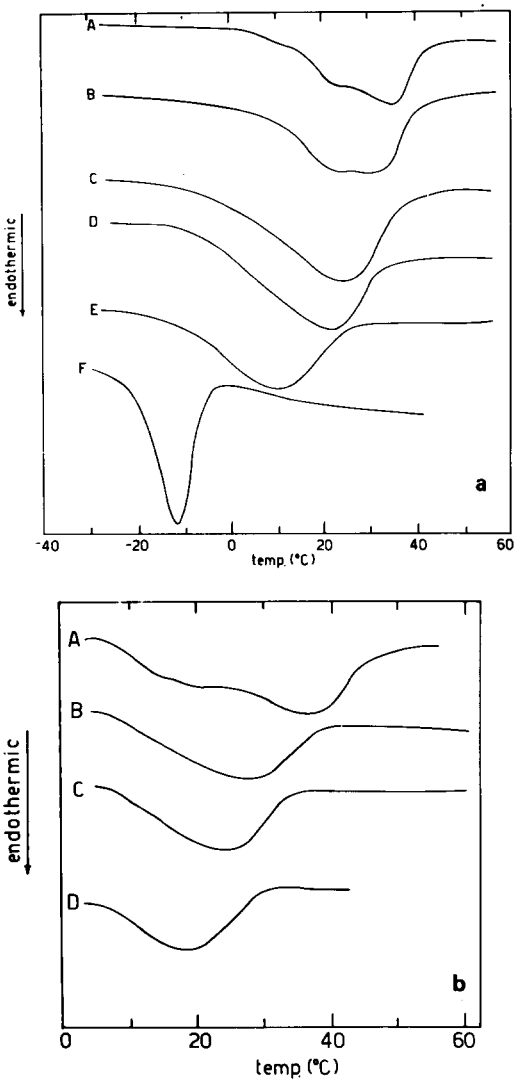

Fig. 3. (a) Thermograms of G_{M1} gangliosides, egg phosphatidylcholine and their interaction products. X_G, molar fraction of gangliosides. Medium ethylene glycol/salt (1 : 1, v/v). X_G: A, 1.0; B, 0.89; C, 0.71; D, 0.45; E, 0.30; F, 0 (egg phosphatidylcholine only). (b) Thermograms of G_{M1} gangliosides and their interaction products with egg phosphatidylcholine, medium salt. X_G: A, 1.0; B, 0.65; C, 0.45; D, 0.30.

−2.5°C, at a temperature where the gangliosides are still not undergoing melting. Due to interaction only one peak of melting is obtained at all the ratios investigated. The double peak of gangliosides merges into one, even at quite a high molar fraction of the glycolipid. This peak of the gangliosides shifts from approx. 37°C for pure compound to lower values and the range of melting becomes shorter with the progressive addition of the phospholipid. The same effect is seen when the

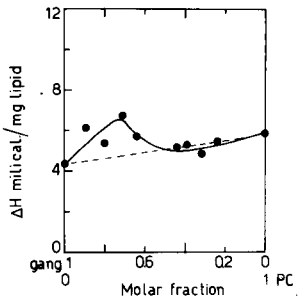

Fig. 4. The enthalpy of melting as function of molar fraction of the components. ●-●, experimental points; ---, theoretical line.

experiment was performed in salt solution, while the scanning was started at +1°C (Fig. 3B). From the thermograms of Fig. 3A, the enthalpy of melting vs. composition was drawn (Fig. 4). As seen from the figure, the enthalpy of melting of the pure gangliosides dispersed in ethylene glycol/salt is about 30% higher than that of gangliosides suspended in salt solution only. This discrepancy is due to two facts: (i) The water activity in solution of ethylene glycol/salt is lower than in pure water, causing stronger hydrogen bonding among the sugar heads of the gangliosides, probably resulting in an increase in the enthalpy of melting. An increase in the enthalpy of about 20% was detected when gangliosides dispersed in ethylene glycol/salt (1 : 1, v/v) were compared with gangliosides dispersed in salt only while in both cases the scan was started at 0°C. (ii) When the scan is started at 0°C (the instrument detects the deviation from base line at about 7°C), small part of the peak area is not taken into account while calculating the enthalpy of melting. In the figure, values of enthalpy are also presented (by broken lines) for each composition, assuming that each component in the mixture undergoes melting with the enthalpy of melting specific for the pure component. By comparing the experimental points of Fig. 4 with the theoretical line, it is apparent that the enthalpy is ideally additive for the two components, up to about 1 : 1 molar ratio, indicating an ideal mixing as implied already from Fig. 3A where

only one melting peak is discerned. At molar ratios of gangliosides/phosphatidylcholine higher than 1:1 the increase of the enthalpy of melting is higher than the theoretically predicted one, reaching a maximum at about 0.75 molar fraction of gangliosides. The increase in the enthalpy is much bigger than the experimental error. As the cross-sectional area of the headgroups in gangliosides is much bigger than that of the hydrocarbon chains, gangliosides will form micelles of quite loose structure [26]. The phosphatidylcholine associates with the hydrocarbon region of the gangliosides, and at a ratio of about three ganglioside molecules to one phosphatidylcholine molecule a structure is formed with more favourable ratio of the headgroup to those of the hydrocarbon tails, producing stronger cooperative interactions as reflected by the increase of the enthalpy of melting.

Cestaro et al. [27] investigated interaction of gangliosides with phosphatidylcholine by enzymic and non-penetrating reagent methods. They concluded that at low ratios of gangliosides the glycolipid was distributed symmetrically between the two layers of the liposomes, whereas at higher ratios the dispersions contained liposomes admixed with micelles of gangliosides and phospholipid. From our data we cannot know at what ratios there is the transition from lamellar to micellar structures. Only one transition peak is observed at all ratios of gangliosides to phospholipid. If there is a ratio where the micellar and lamellar structures coexist, they undergo phase transitions at the same or at closely overlapping temperatures. All we can conclude from our data is that around 3:1 ganglioside to phosphatidylcholine the molecular packing is probably the tightest rendering the highest values of ΔH.

Interaction of gangliosides with dipalmitoylphosphatidylcholine was investigated previously [15,19] by following calorimetrically the changes in the thermotropic behaviour of the phospholipid. Sillerud et al. [19] found a very complex thermotropic behaviour of the G_{M1}-dipalmitoyl mixtures. They concluded that up to molar fraction of 0.24 ganglioside is incorporated into the liposomal structure, but at higher concentrations mixed micelles are formed. Hinz et al. [15] have also found that interaction with gangliosides influences strongly the thermotropic behaviour of the synthetic lipid. When ganglioside G_{M1} was mixed with 1-stearoyl-2-oleoylphosphatidylcholine and examined by differential scanning calorimetry, complete miscibility was found only up to molar fraction of 0.3 [18]. These data differ from those presented by us for egg phosphatidylcholine where complete miscibility was found probably even at very high ganglioside mole fractions, indicating that the phase state of the pure phospholipid may influence very strongly the miscibility with the ganglioside.

Our data, showing that there is complete mixing between phosphatidylcholine and gangliosides, suggest that in biological membranes, whenever the gangliosides are in a more fluid lipid environment, they will be randomly distributed. This conclusion is in agreement with the data of Delmelle et al. [13], who found that gangliosides, when they are mixed with lipid above its phase transition temperature, will be randomly distributed and will form clusters only when the lipid is below its phase transition temperature.

Interaction with cholesterol

It is known that cholesterol fluidizes the phospholipid bilayer below the phase transition temperature of the lipid and renders it less fluid above the transition temperature [27]. As all mammalian plasma membranes where glycolipids are mainly found contain cholesterol, it was of interest to investigate the effect of cholesterol on the thermotropic properties of gangliosides. Fig. 5 presents the thermograms of G_{M1} ganglioside interacting with cholesterol. As seen from the figure, cholesterol above 10 mol% flattens the ganglioside melting profile, but even at 1:1 mole/mole ratio some fraction of the ganglioside still undergoes melting (Fig. 5E). This effect is more pronounced on Fig. 6, where the enthalpy of melting of the gangliosides interacting with cholesterol(ΔH) divided by the enthalpy of pure ganglioside (ΔH_0) is plotted as a function of mol% of cholesterol. At about 10 mol% cholesterol a steep decrease of the enthalpy of melting starts, but at about 30 mol% cholesterol it levels off reaching a value of about 50% of the initial enthalpy. This behaviour differs from that of the interaction products of cholesterol with sphingomyelin, which is related structurally to gangliosides. In sphingomyelin-cholesterol mix-

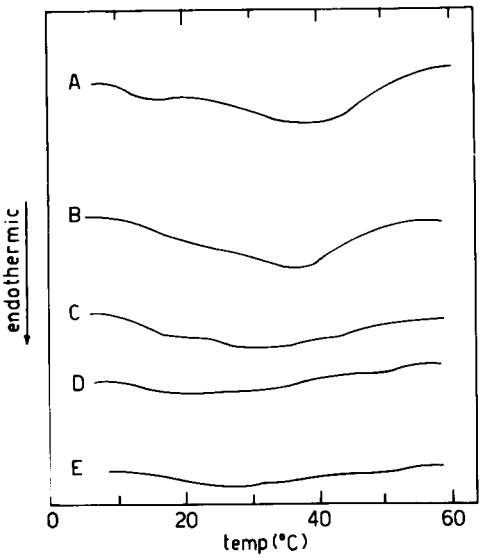

Fig. 5. Thermograms of G_{M1} ganglioside interacting with cholesterol. A, ganglioside only; B, 10 mol% cholesterol; C, 31 mol% cholesterol; D, 37 mol% cholesterol; E, 52 mol% cholesterol.

tures the enthalpy of melting vanishes completely at about 40 mol% cholesterol [27]. The lower affinity of gangliosides for cholesterol is probably due to the effect of the big headgroup region. In biological membranes, the interaction of cholesterol with the gangliosides will depend on the different types of lipids present.

The interaction of other gangliosides with egg phosphatidylcholine or cholesterol is currently being investigated.

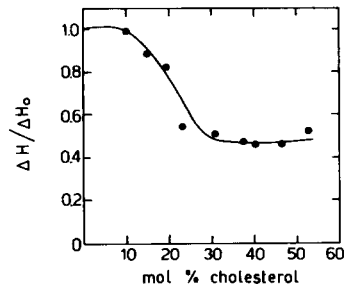

Fig. 6. The enthalpy of melting of the G_{M1} ganglioside in the presence of cholesterol (ΔH) divided by the enthalpy of melting of the pure ganglioside (ΔH_0) as a function of the mol% of cholesterol.

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