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A DIFFERENTIAL SCANNING CALORIMETRY STUDY OF THE INTERACTION OF GANGLIOSIDES WITH PEANUT LECTIN, SEROTONIN AND DAUNOMYCIN

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Summary

Thermotropic behaviour of human Tay-Sachs ganglioside and of mixed bovine brain gangliosides, before and after interaction with peanut lectin, serotonin and daunomycin, was investigated. Interaction of mixed brain gangliosides with peanut lectin or serotonin causes a decrease in the enthalpy of melting, whereas interaction of this lectin with Tay-Sachs ganglioside does not influence the enthalpy of melting. Serotonin causes a small increase in the enthalpy of melting of the Tay-Sachs ganglioside.

Introduction

Gangliosides have been recognized as important constituents of cell surfaces, since their role as receptors for numerous biologically-active agents, including hormones [1,2], cholera toxin [3], interferon [4] and lectins [5,6], has become apparent.

In an attempt to evaluate the role of the sugar-bearing lipids in the membrane structure and the mechanism of the interaction with various substances, several physicochemical studies were undertaken.

By employing fluorescence polarization [7] it was shown that lipid dispersions containing brain gangliosides have greater microviscosity than those of pure lipids. From EPR measurements it was also concluded that glycosphingolipids have the capacity to increase rigidity in fluid bilayer membranes [8] and that sugar chains of gangliosides have a tendency to undergo cooperative interactions among themselves, probably due to hydrogen bonding [9].

Interaction of lectins with cells results in their agglutination [10], and a

similar effect is exhibited by lectins on glycolipid-containing liposomes [5,6, 11,12]. Cholera toxin [13] and serotonin [14], interacting with ganglioside-containing liposomes, modify the properties of the lipid dispersions, leading to an increase in the permeability to glucose.

In spite of these studies little is known about the organization of gangliosides in membranes, and on how interaction with various exogenous ligands can influence this organization. Preliminary reports on the thermotropic behaviour of ox brain cerebrosides [15] and bovine brain gangliosides [16] have been described. In an attempt to clarify these questions, we have undertaken a study of the interaction of gangliosides (mixed bovine brain gangliosides and human Tay-Sachs-G_{M2}) with a galactose-specific lectin from peanut, a brain-active hormone-serotonin, and an anti-tumor drug-daunomycin, by employing differential scanning calorimetry. The results reported in this paper show that certain agents interacting with gangliosides can modify their structural integrity, as revealed by differential scanning calorimetry.

Materials and Methods

G_{M2} ganglioside was isolated from brains of Tay-Sachs patients by chloroform-methanol extraction and chromatography procedures previously described [17]. Mixed gangliosides were isolated from calf brain by chloroform-methanol extraction followed by alkaline methanolysis, phase partition, DEAE-Sephadex and silicic acid chromatography. Ganglioside preparations were dialyzed exhaustively against water to remove salts before being lyophilized and were found to be devoid of any impurities. The individual gangliosides (G_{T1}, GD_{1b}, GD_{1a}, GD_3, G_{M1} and G_{M2}) were identified by TLC. Ganglioside concentration was determined by the resorcinol method [18]. Peanut-lectin was prepared by affinity chromatography on Sepharose-ϵ-aminocaproyl-β-galactopyranosylamine as described [19]. Concentrated solutions of lectin (about 6 mg/ml) were prepared by dissolving the protein in a solution of $1.5 \cdot 10^{-1}$ M NaCl in 10^{-2} M Tris-HCl buffer, (pH 7.3). 5-Hydroxytryptamine (serotonin) oxalate salt was purchased from Sigma, (St. Louis, MO). Serotonin solution at a concentration of approx. 10^{-1} M was prepared by dissolving the material in $1.5 \cdot 10^{-1}$ M NaCl in 10^{-2} M Tris buffer and titrated with NaOH to pH 7.3. This solution was kept frozen and used within one day of the preparation. Daunomycin was purchased from Specia (Paris) and was used in its mannitol-free form dissolved in $1.5 \cdot 10^{-1}$ M NaCl in 10^{-2} M Tris-HCl buffer (pH 7.3). D-Galactose was obtained from Pfanstiehl, Illinois, U.S.A. It was dissolved in $1.5 \cdot 10^{-1}$ M NaCl + 10^{-2} M Tris-HCl buffer. 1.3—2.0 mg gangliosides were weighed directly into aluminum pans. Salt solution (about 10 mg) or salt + interacting substance were added, the pans were sealed hermetically and left for incubation at 37°C for 3 h.

The calorimetric measurements were performed on a Du Pont 990 differential scanning calorimeter with a cell base II. The calibrated mode was used, sensitivity of 0.02 mcal/s per inch and heating rate of 5°C/min.

Results

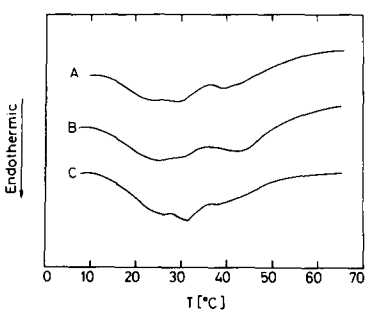
Fig. 1. presents the thermograms of G_{M2}-ganglioside alone and in the presence of either lectin or serotonin. As can be seen in Fig. 1 (1A, ganglioside alone), the transition is very broad and spans from about 10°C to about 55°C while the peak is composed of at least three peaks. The total enthalpy of melting is 3.0 mcal/mg. Addition of peanut agglutinin at ganglioside: agglutinin molar ratio of 700 : 1 (assuming a molecular weight of 50 000 for the sugar-binding site) had a very small effect on the shape of the thermogram (Fig. 1B) and did not influence the enthalpy of melting which is 3.1 mcal/mg. Ratios lower than 500 : 1 (ganglioside : lectin) could not be used due to limited solubility of the lectin.

Serotonin has some effect on the shape of the thermogram, the height of the peak appearing around 31°C increases due to interaction and the total enthalpy of melting increases by about 20% to 3.6 mcal/mg.

In Fig. 2 are presented thermograms of bovine brain gangliosides alone and interacting with peanut agglutinin and galactose + agglutinin. As seen from Fig. 2A, also in this case as for the ganglioside G_{M2} the range of melting is very wide (about 40°C), the melting peak is also a superposition of several peaks, but they are less distinguished as in the previous case. The enthalpy of melting is 2.5 mcal/mg.

Interaction with peanut agglutinin (presented at a molar ratio of 650 : 1) (Fig. 2B) causes a decrease in the enthalpy of melting by about 40%. The enthalpy of melting for the experiment, presented in the figure, is 1.4 mcal/mg. If the competitive sugar D-galactose is added to the reaction mixture (at a molar ratio of 1 : 2 ganglioside: D-galactose) before the agglutinin, no decrease in the enthalpy of melting is detected (Fig. 2C). The enthalpy is 2.3 mcal/mg.

The influence of serotonin on the thermograms of mixed bovine brain gangliosides was studied. The experiments were performed at molar ratios of ganglioside : serotonin from 4 : 1 to 1 : 2. Above the ratio of 1.5 : 1 a decrease



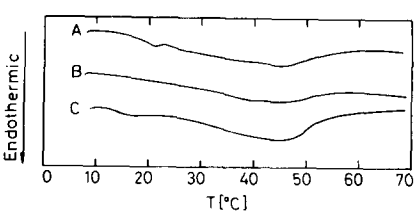


Fig. 1. The differential scanning calorimetry thermograms of ganglioside-G_{M2} alone and after interaction with peanut agglutinin lectin or serotonin. A, 1.5 mg G_{M2}; B, 1.8 mg G_{M2} + 70 μg agglutinin; C, 1.3 mg G_{M2} + 0.78 μmol serotonin. Heating rate 5° C/min; sensitivity 0.02 mcal/s per inch.

Fig. 2. The differential scanning calorimetry thermograms of mixed bovine brain gangliosides alone and after interaction with peanut lectin or galactose. A, 1.4 mg gangliosides; B, 1.4 mg gangliosides + 63 μg agglutinin; C, 1.6 mg gangliosides + 200 μg galactose + 63 μg agglutinin. Heating rate 5° C/min; sensitivity 0.02 mcal/s per inch.

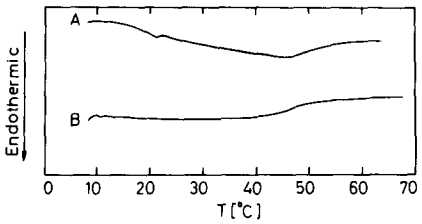


Fig. 3. The differential scanning calorimetry thermograms of mixed bovine brain gangliosides alone and after interaction with serotonin. A, 1.4 mg gangliosides; B, 1.1 mg gangliosides + 0.87 μmol serotonin. Heating rate 5°C/min; sensitivity 0.02 mcal/s per inch.

in the enthalpy of melting was found. The decrease amounted to 40—50% and did not change any further with the increase in the ratio of serotonin to gangliosides. A representative thermogram obtained at a ratio of ganglioside : serotonin 1 : 1.5 is presented in Fig. 3B.

We have previously shown [20] that daunomycin influences the thermograms of synthetic phospholipids, and it was of interest to see whether this drug has an effect on the melting properties of gangliosides. Mixed brain gangliosides were employed and the interaction was investigated at the ganglioside : drug molar ratios from 8 : 1 to 1 : 1, but even at a ratio of 1 : 1 no changes in the enthalpy of melting or the melting profile were detected.

Discussion

The thermotropic behaviour of gangliosides is very complex. Curatolo et al. [16] have shown that bovine brain gangliosides exhibit broad, reversible endothermic transition between 10 and 60°C with maxima at 15°C and 39°C. By employing very pure samples of brain gangliosides and a very sensitive instrument, we were able to obtain much bigger reversible endothermic transition in the range of 10—55°C. The enthalpy of melting of the mixed gangliosides was calculated and it is 2.4 mcal/mg. The maxima in the thermograms were much less pronounced than in the case of Curatolo et al. [16].

We have also investigated the thermotropic behaviour of a pure Tay-Sachs ganglioside (G_{M2}) of human origin. Here, as in the previous case, the thermogram is very broad, but at least three separate reversible maxima can be seen. The enthalpy of melting is 3.0 mcal/mg which is higher than in the case of mixed brain gangliosides.

Curatolo et al. [16] claimed that the phase transition in gangliosides is associated with a hydrocarbon chain rearrangement. The results presented here would suggest that oligosaccharide chains in gangliosides are also undergoing some reversible transformation (first and subsequent heating runs, as obtained by differential scanning calorimetry, are similar), and this process is also an endothermic one. Our conclusion is in keeping with the data of Sharom and Grant [9], who have shown that the ganglioside oligosaccharide chains undergo cooperative interaction among themselves.

However, at present, even in the case of the G_{M2} thermograms, we cannot assign the different regions of melting to any particular part of the molecule.

We have investigated the interaction of the gangliosides with different biologically-important reagents: the lectin PNA, the hormone serotonin, and the antitumor drug daunomycin. In these studies we have been using pure gangliosides rather than using them in mixtures with lipids as in liposomes, due to the possibility that gangliosides in membranes probably cluster in small 'microenvironments', and pure gangliosides can provide a model for such regions. Since the enthalpy of melting of the gangliosides is low, it would be impossible to study directly the influence of the interaction with the various ligands on the thermotropic behaviour of these glycosphingolipids in mixtures with lipids by analyzing their melting profiles.

In Fig. 1, we have presented the thermograms of G_{M2} interacting with peanut agglutinin, and with serotonin. No influence of peanut agglutinin on the enthalpy of melting of G_{M2} was found. This result suggests that as G_{M2} does not possess a terminal galactose residue which would be recognized by peanut agglutinin, no interaction occurs between the two components. Interaction with serotonin caused a small increase in the enthalpy of melting of the G_{M2} ganglioside. According to Woolley and Gommi [1], G_{M2} is not regarded as the receptor for serotonin. This hormone at physiological pH values is positively charged [21], and gangliosides bear negative charge, so nonspecific electrostatic interaction is likely to occur, decreasing the repulsion between the charged head groups, stabilizing the structure and causing a small increase in the enthalpy of melting.

The interaction with mixed bovine brain gangliosides gives different pictures (Figs. 2 and 3). The preparation of bovine mixed brain gangliosides used contains predominantly G_{M1} and GD_{1b}, both of which possess a terminal disaccharide residue (Gal(β1-3) GalNAc) that was shown to be a specific binding site for peanut agglutinin [19]. PNA interacting with the gangliosides causes a decrease in the enthalpy of melting. It was shown that hydrophobic polypeptides [22] or hydrophobic proteins [23] penetrate the hydrocarbon layers of phospholipids, fluidizing them, as expressed by a decrease in the enthalpy of melting. Peanut agglutinin is quite a hydrophilic protein [19] and its degree of penetration into the ganglioside hydrophobic layer is probably limited. The disorganizing effect may be due to the modification in the head group region but this interaction may also influence the cooperativity of interaction between the hydrocarbon chains.

Incubation with serotonin decreases the enthalpy of melting. The interaction seems to be specific, nonelectrostatic, in as much as another positively-charged substance investigated, daunomycin, has no effect on the thermotropic properties of these gangliosides. The interaction with serotonin probably involves only the head groups as it is less likely that this small charged hormone penetrates the hydrocarbon region. The interaction has a destabilizing effect, since the cooperativity of the gangliosides themselves decreases as indicated by the decrease in the enthalpy of melting. This destabilizing action of serotonin is in agreement with the report by Maggio et al. [14], who showed that interaction of serotonin with liposomes-containing gangliosides enhances the release of glucose.

In conclusion, we have shown that peanut lectin or serotonin modify the thermotropic properties of bovine brain gangliosides by a specific interaction as

the attachment occurs via specific recognition sites. Interation with serotonin can change the microenvironment and block binding of other materials or any processes requiring the more rigid structures as induced by the intact gangliosides.

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