Ganglioside headgroups decrease lipid order in reconstituted phosphatidylcholine liposomes

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The effect of oligosaccharide carrying lipids on membrane fluidity has been investigated. Gangliosides GM1 and GQ1 were reconstituted into phosphatidylcholine bilayer membranes at low concentrations (< 5 mol%). A strong fluidizing effect was observed leading to a suppression of the phase transition temperature. This was most pronounced with highly sialylated gangliosides. Ca^{2+} reverses the effect due to phase separation phenomena. We assume a hydrophilic lipid-lipid interaction in accordance with previously studied glycoprotein-lipid interactions.

Membrane fluidity *Ganglioside* *Phase separation* *Hydrophilic interaction* *ESR*

1. INTRODUCTION

Gangliosides, acidic glycosphingolipids of the plasma membrane, incorporate with their hydrophobic ceramide lipid moiety into bilayer structures. The hydrophilic oligosaccharide part with at least one negatively charged *N*-acetylneuraminic acid (sialic acid) protrudes into the aqueous phase near the bilayer surface [1]. In vertebrates gangliosides are highly enriched in presynaptic nerve cell membranes [2] comprising up to 10 mol% of total lipid [3]. A distinct correlation between sialic acid content and the environmental temperature has been found in different animals [4]. A higher degree of sialylation has been determined at a lowered body temperature thus possibly facilitating the process of synaptic transmission. The question arises whether gangliosides may alter membrane fluidity by the interaction of its hydrophilic part with the lipid bilayer surface.

Very recently such a hydrophilic interaction between oligosaccharide chains and phosphatidylcholine membranes has been reported in reconstituted membranes containing the sialic acid bearing glycophorin [5]. Incorporation of about 0.1 mol% of the glycoprotein led to a reduction of the lipid order and to an increase in the dynamic properties of the membrane [6]. The lateral diffusion and the lipid fluidity were mainly influenced between the pre- and the main transition temperature of the uptaking lipid. The fluidizing effect could be visualized by electron microscopy.

Our aim was to determine whether the fluidizing effect of sialic acid containing glycoproteins could also be induced by glycolipids of similar structure. Therefore we incorporated gangliosides containing different amounts of sialic acids into phosphatidylcholine bilayer membranes and determined their phase transition temperature by EPR using the spin label technique. Monosialoganglioside GM1 at a molar fraction of $X_G = 2.5 \times 10^{-2}$ lowered the phase transition temperature by about 5°C. Tetrasialoganglioside (GQ1) induce a comparable effect at an even lower concentration around $X_G = 10^{-2}$. The effect could be reversed by addition of Ca^{2+} inducing a phase separation into complexed gangliosides and almost undisturbed phosphatidylcholine domains. Our results suggest a general hydrophilic interaction between sialic acid containing sugar residues and lipid surfaces leading to a subtle long-range disturbance of membrane order.

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2. MATERIALS AND METHODS

2.1. *Extraction of gangliosides from pig cerebral tissue*

Grey matter was homogenized in distilled water at 4°C in a Waring blendor and sonicated to further homogeneity with a Branson sonifier. Extraction of lipids was carried out according to [7] by 30 min incubation in chloroform/methanol/water (c/m/w, 1:2:0.55, by vol.) followed by phase partition in a solvent system adjusted to a c/m/w ratio of 1:2:1.4 (by vol.). The crude ganglioside extract was recovered from the hydrophilic phase by evaporation, resolubilization in distilled water, exhaustive dialyses against distilled water and lyophilization.

Different ganglioside species were separated by anion-exchange chromatography [8] on DEAE-Sepharose (Pharmacia, Uppsala) with a modified solvent system. Gangliosides were dissolved in a small volume of c/m/w (55:45:10, by vol.) and applied to the column. Phospholipids were first eluted with the same solvent, gangliosides were fractionated using a 3-chamber gradient of pure methanol and of methanol containing 0.2 or 0.5 M ammonium acetate. Fractions were analyzed for ganglioside and phospholipid content by thin layer chromatography (Silica gel 60, Merck, Darmstadt) [9] and sialic acid determination [10].

2.2. *Preparation of liposomes*

Pure gangliosides and DMPC (Fluka, Neu-Ulm) were dissolved in chloroform/methanol (1:1, v/v) and mixed in an appropriate lipid to glycolipid ratio. The mixture was dried to form a thin film in a 25 ml flask under vacuum and was kept in a dessicator for some hours.

The film was then swollen for 2 h in a wet atmosphere and then dispersed in water by rotation with small glass beads. Liposomes were sedimented at $12\,000 \times g$ and immediately applied to EPR spectroscopy.

Incorporation was controlled by analytical determination of phosphorus [11] and sialic acid [10] in the mixed liposomes. It varied between 50 and 70%.

3. RESULTS

Monosialo- (GM1) and tetrasialoganglioside (GQ1) (e.g., fig.1 for the headgroup structure) were reconstituted into dimyristoylphosphatidylcholine bilayer membranes. The lipid phase transition was determined by measuring the TEMPO partitioning between the aqueous and lipid phase [12]. The appearance of the EPR signal characterized by the height h_L is a measure for label dissolved in the fluid lipid phase and is therefore suited to measure thermotropic phase transitions as shown in fig.2. Pure DMPC exhibits a well-known phase transition at 23.5°C and a pretransition at about 15°C.

In the presence of low amounts of gangliosides (1.35 mol% GQ1 in fig.2) this phase transition is drastically broadened with an onset temperature of about 10°C. The midpoint of the melting curve at about 17°C is taken as phase transition temperature.

Addition of excess Ca^{2+} leads to an upward shift and to a sharpening of the phase transition. The midpoint is shifted to about 22°C which approaches the value the pure DMPC bilayer.

We investigated this effect at different ganglioside concentrations and with mono- as well as tetrasialylated ganglioside. The results are summarized in fig.3. Monosialoganglioside has only a minor effect on the DMPC phase transition up to about 2 mol%. However, in the range between 2

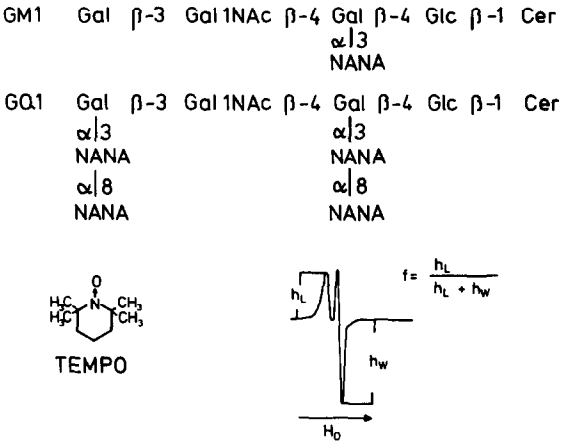

Fig.1. The structure of the oligosaccharide headgroup of ganglioside GM1 and GT1 is sketched together with the spin label 2,2,5,5-tetramethylpiperidine 1-oxide (TEMPO) and the corresponding EPR spectrum exhibiting both spin label dissolved in the lipid (h_L) and in the water phase (h_W).

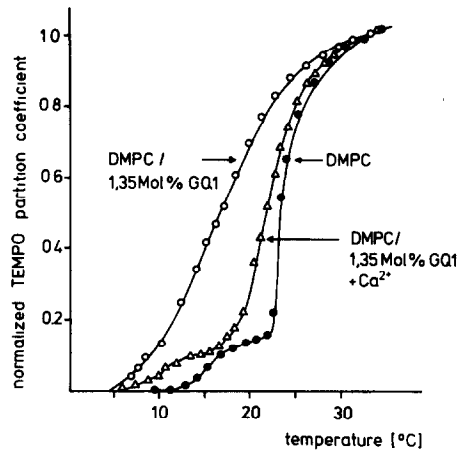

Fig.2. Phase transition curves of pure DMPC (●—●), mixed DMPC/GQ1 (1.35 mol%) membranes (○—○) and the latter in the presence of excess Ca^{2+} (△—△). The TEMPO partitioning parameter $f = h_L/(h_L + h_W)$ is normalized to compensate for dilution effects due to the Ca^{2+} addition. Typical values for f are $f = 0.5$ above the lipid phase transition and $f = 0.05$ below the lipid phase transition.

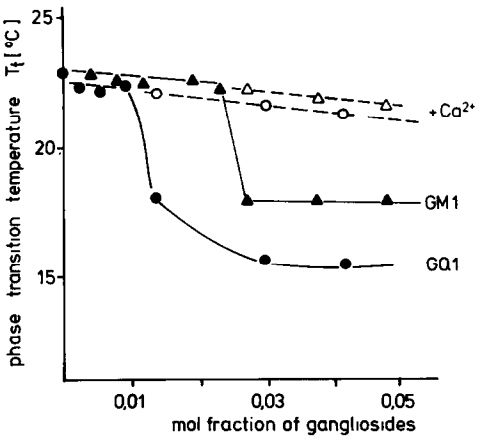

Fig.3. The phase transition of DMPC membranes is given as a function of ganglioside content for GM1 (▲—▲) and GQ1 (●—●) in the absence and presence of Ca^{2+} (△—△, GM1; ○—○, GQ1).

and 2.5 mol% the phase transition temperature is lowered to 17°C and then remains constant upon increasing the ganglioside content. Addition of Ca^{2+} in excess to the negative charges of the glycolipid elevates the phase transition midpoint temperature of the mixture almost to the value of pure DMPC. At concentrations below 2 mol% GM1, Ca^{2+} has no visible effect.

Tetrasialoganglioside (GQ1) induces a comparable effect, however, at an even lower concentration of about 1 mol% and the phase transition temperature is reduced to about 16°C. Again, this shift in the phase transition curve could be reversed by addition of Ca^{2+}.

4. DISCUSSION

Our results suggest a general hydrophilic interaction between sialic acid containing sugar residues and lipid bilayer surfaces leading to a fluidization of the bulk membrane. The covalent linkage to a hydrophobic membrane moiety is essential. Sialic acids or different sugars dissolved in the aqueous phase up to molar concentrations have no effect on the DMPC phase transition. Going from monosialo- to tetrasialoganglioside we observe a stronger fluidizing effect. Only one molecule of ganglioside per hundred phosphatidylcholine molecules is needed to decrease the lipid phase transition into the temperature range of the pretransition. Similar results have been found in glycophorin containing membranes [5,6]. Glycophorin, the integral protein of the erythrocyte membrane, carries 16 oligosaccharide chains which are terminated by sialic acids [13]. This protein with a large number of sugar residues and sialic acids was shown to induce membrane fluidity in DMPC bilayer membranes at a 10-fold lower concentration (0.1 mol%) compared to GQ1. These effects cannot be understood in terms of a pure hydrophobic lipid-protein or lipid-lipid interaction but by a substantial hydrophilic interaction between the bilayer surface and the sialic acid carrying sugar residues. An interaction between gangliosides has been observed [14]. The thermotropic properties of pure gangliosides measured by calorimetry were strongly influenced by the number and position of the negatively charged sialic acids in the headgroup region. Here we report such an interaction probably based on strong hydrogen bonding to phosphatidylcholine.

Gangliosides [15,16] as well as other negatively charged lipids [17] strongly bind divalent cations which triggers a phase separation of the charged complexed lipid [18]. In a recent calorimetric study authors in [19] could not detect a phase separation

induced by Ca^{2+}. However, they used large amounts of ganglioside (>25 mol%) and it is known that pure gangliosides show only very little binding of Ca^{2+} [20]. Phase separation in the presence of Ca^{2+} was observed by EPR spectroscopy with a probe located near the surface region of the lipid bilayer [21]. Our results clearly show the potency of Ca^{2+} in inducing a phase separation by segregating the sialic acid containing lipids in a phosphatidylcholine matrix.

It is important to note a possible partitioning of the glycolipid between the gel and the fluid lipid phase in the transition region. Further experiments to determine the boundaries of the two phases have to be performed to understand fully the phase behavior in the presence of complex mediating Ca^{2+}.

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