CLUSTERING OF GANGLIOSIDES IN PHOSPHOLIPID BILAYERS

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1. Introduction

Gangliosides have been implicated in a variety of cell surface functions as receptor candidates for toxins [1,2], hormones [3] and lectins [4]. Their structures contrast strongly with those of classical lipids, since the head groups are very large as compared to the hydrocarbon tails. Little is known about their organization in the lipid bilayers. A possible approach to this problem is to use model systems [5–8]. Here, we report evidence from electron spin resonance (ESR) measurements that gangliosides incorporated in lipid vesicles can interact and form clusters. Our results show that this clustering process is modulated by the lipid fluidity. Above the lipid phase transition of dipalmitoyl phosphatidyl choline, the model membrane can be viewed as a fluid system in which gangliosides are randomly distributed. Below the phase transition, the model membrane consists of a mosaic structure composed of ganglioside clusters embedded in the lipid matrix. Moreover, we demonstrated that the ganglioside organization modulates the permeability of liposomes to carboxyfluorescein. This result is briefly discussed in regard of the membrane action of biochemical ligands for which gangliosides are postulated as receptors.

2. Materials and methods

D,L-α-Dipalmitoyl phosphatidyl choline (DPPC) and gangliosides from bovine brain were purchased from Sigma. Carboxyfluorescein (CF) was an Eastman-Kodak product.

2.1. *Preparation of liposomes*

Lipid–ganglioside mixtures were dissolved in chloroform–methanol, 2/1 (v/v). The solvent was evaporated under nitrogen flow and the lipid–ganglioside film was dried overnight. Liposomes were obtained by mechanical stirring (Vortex mixer) of the film in 0.025 M Tris–HCl buffer (pH 7.2). The mean liposome diameter determined with a Coulter Nano-Sizer (Coulter Electronics, England) was 12 000 Å ± 1000 Å.

2.2. *ESR measurements*

The ESR measurements were performed on a Century Line Varian spectrometer (frequency modulation, 100 kHz; microwave power, 5 mW; modulation amplitude, 1 G). The temperature was regulated with a Varian variable temperature controller. Samples were sealed in 50 μl Drummond capillaries. The spectra were stimulated on a CDC 6600 computer with a FORTRAN IV interactive program as in [10].

The spin label gangliosides were synthetized as in [9]. The method allows the fixation of 3-carboxy-2,2,5,5-tetramethyl pyrrolidine-1-oxyl on the primary hydroxyl groups of the carbohydrate residues. Reaction yield was 48%. The gangliosides were incorporated in liposomes at various levels from 1–18 mol %, using the above procedure.

2.3. *Carboxyfluorescein release*

Self-quenching of carboxyfluorescein was used to demonstrate the release from liposomes [11]. Indeed, the fluorescence of carboxyfluorescein is quenched by ~96% at 100 mM in phosphatidylcholine vesicles [11]. The CF release induces a drastic fluorescence increase which can be followed without separation of vesicles from released solute [11]. We routinely excited the CF at 470 mm. Emission was followed at 520 nm with a Jobin-Yvon spectrofluorimeter. The temperature of the cuvettes was kept constant to within 0.5°C by circulating water bath.

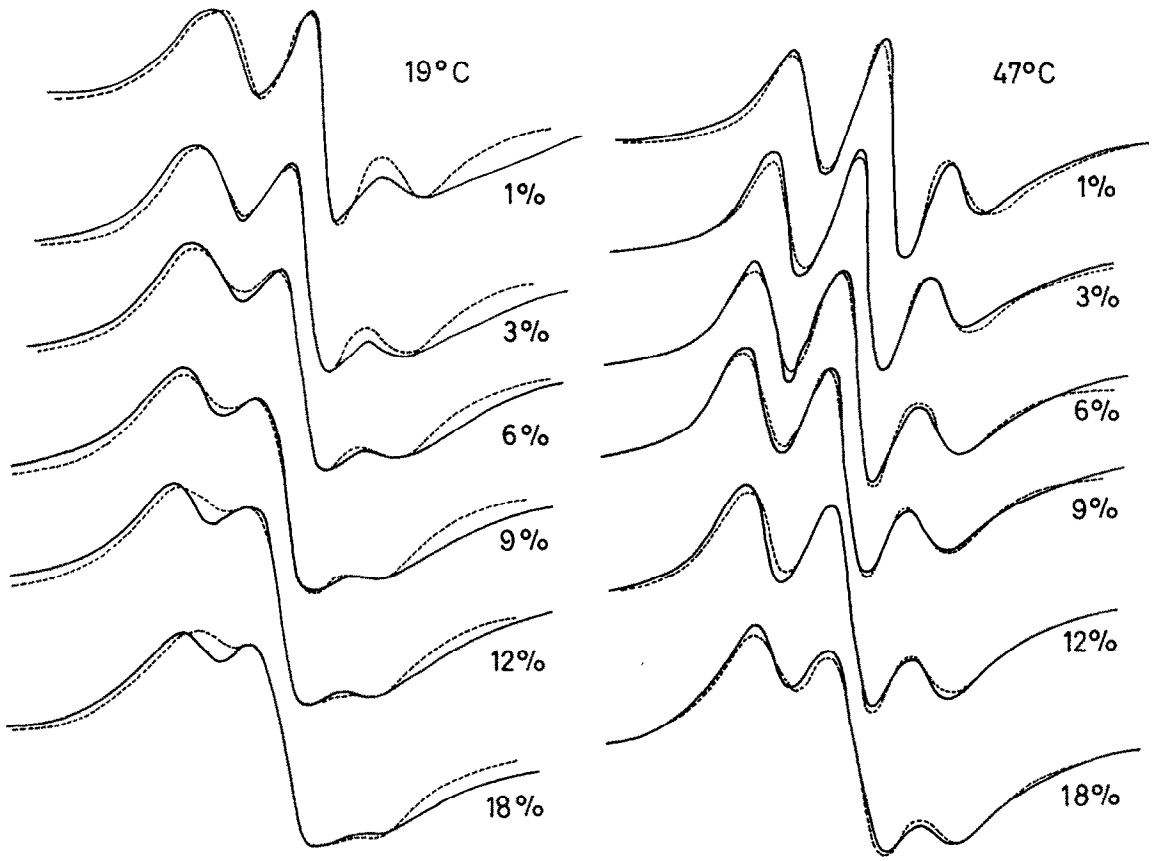

Fig.1. Comparison between measured (——) and simulated (- - -) ESR spectra at 19°C and 47°C for 6 ganglioside/lipid molar fractions. Simulation parameters: a_H (isotropic hyperfine coupling constant) = 14 G; linewidths H_j^0 = 5.5 G, 3.5 G, 7.0 G. At 19°C, the dipolar interactions are ΔH^d = 2 G (C = 0.01, C = 0.03) and ΔH^d = 2.5 G (C = 0.06, C = 0.09, C = 0.12, C = 0.18). At 47°C, ΔH^d = 2 G (C = 0.01, C = 0.03, C = 0.06) and ΔH^d = 2.5 G (C = 0.09, C = 0.12, C = 0.18). The corresponding values of W_{ex} are given in fig.2.

3. Results and discussion

Fig.1 shows the experimental ESR spectra (solid curves) observed at 19°C and 47°C with spin label gangliosides in DPPC liposomes. The total ganglioside concentration ranges from 1–18 mol %, corresponding to a label concentration of 0.48–8.6 mol %. The lines of the 1% spectrum at 47°C are broader than in the case of a conventional nitroxide spectrum at low concentration. A similar lineshape was observed when the spin-labeled gangliosides were dissolved in an organic solvent where no liposomal structure was present. A reasonable explanation for this effect is that more than one NO groups are fixed on some ganglioside carbohydrate residues, which produces intramolecular interaction.

Increasing the label concentration produces drastic modification of the spectra (fig.1). The individual lines become progressively broader, this effect being more pronounced at 19°C. These modifications have to be analysed in terms of intermolecular magnetic interaction occurring between the radicals. The experimental spectra can be satisfactorily simulated (fig.1, dashed lines) using the basis of a theoretical model [10,12] which implies two modes of interaction: the dipolar and the spin exchange. The exchange process measured by the parameter W_{ex} is the most important contribution. Moreover, the dependence of W_{ex} with respect to the label concentration gives some direct information about the molecular organization. Indeed, it can be shown [12] that in the case of a random distribution of the label:

$$W_{ex} = 4d_c D_{diff}(C/(1 + C))/3\lambda F$$

where d_c, λ and F are the critical interaction distances, the length of one diffusional jump and the area per molecule (A = 120 Å^2) [13]. D_{diff} is the lateral diffusion coefficient. By contrast, when the label organization ceases to be random, due to cluster formation:

$$W_{ex} = \overline{W_{ex}} \left[1 - (\pi d_c^2 n)^{1/2} \left[\frac{1+C}{C}\right]^{1/2}\right]$$

where n is the number of clusters per square centimeter and $\overline{W_{ex}}$ is the maximum exchange interactions within the cluster.

Fig.2 shows that at 47°C, W_{ex} is proportional to $C/(1 + C)$ indicating that the gangliosides are randomly distributed within the fluid DPPC lipid matrix. A ganglioside lateral diffusion coefficient (D_{diff}) was estimated to 7 X 10^{-8} cm^2/s which is in good agreement with the reported data [12]. At 19°C, W_{ex} is no more linearly related to $C/(1 + C)$ (fig.2) and the model suggests that the gangliosides are organized in clusters [10,12]. A more detailed analysis concerning the size of the clusters and the mean minimal distance between two clusters will be described elsewhere.

Fig.3 shows that this clustering process triggers the release of the CF which has been sequestered inside the liposomes. The fluorescence increase of CF as a function of time for DPPC liposomes at 19°C and 47°C is shown (left). A completely different pattern is observed when DPPC liposomes contain gangliosides (right). If at 47°C, the leakage is not significantly modified, a drastic change is observed at 19°C. The explanation which accounts for the ganglioside effect

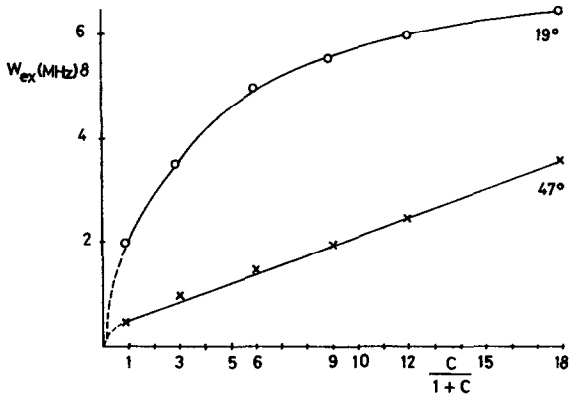

Fig.2. Relationship between W_{ex} and the ganglioside/lipid molar fraction $C/(1 + C)$ at 47°C and 19°C.

at 19°C and 47°C could be proposed in terms of the ganglioside clustering in the lipid matrix.

The ESR measurements demonstrate that above DPPC transition temperature, the two constituents are in the liquid crystalline state and the gangliosides diffuse freely in the lipid matrix. This picture is consistent with a recent theoretical evaluation [13]. No preferential large hydrophobic region can be formed and the perturbatory effects of isolated ganglioside molecules is minimized. On the other hand, ESR results show that, at 19°C, the gel state induces ganglioside clustering. In these conditions, gangliosides could form hydrophobic pores for carboxyfluorescein molecules and the increase of permeability would be a consequence of the electrostatic repulsions between the bulky negatively charged hydrophilic groups. This interpretation would be consistent with a recent work on ganglioside monolayers spread at the air–water interface, which demonstrates that the neuraminic acid residues caused an increase in the ganglioside limiting area [13]. Interestingly, it has been shown that the interaction of ganglioside or ganglioside-like receptors with glycoprotein hormones can modify the cell membrane permeability [14]. Generally, the scheme proposed to explain the hormone effects supposes that the interaction of the glycoprotein with the cell receptor induces a conformational change which allows its penetration within the membrane. This penetration leads to the observed permeability changes. Our results suggest that the permeability change may simply result from the clustering of the receptors induced by their specific biochemical ligands, without penetration of the hormone into the lipid bilayer.

The ESR approach offers a powerful tool for describing ganglioside receptors organization in model

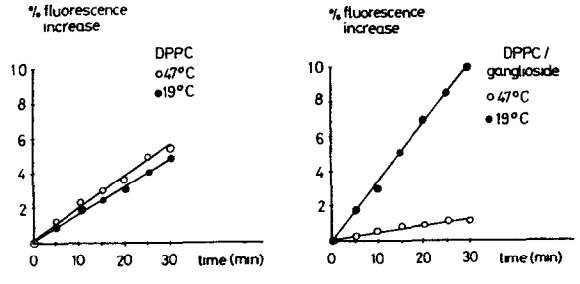

Fig.3. Carboxyfluorescein release. Fluorescence increase as a function of time for DPPC liposomes at 19°C and 47°C (left) and DPPC-gangliosides (3 mol %) liposomes at 19°C and 47°C (right).

membranes and for examining the role of the ganglioside lateral mobility in the recognition process.

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