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THE EFFECT OF LIPID COMPOSITION ON LIPOSOME-LECTIN INTERACTION

AVADHESHA SUROLIA AND B.K. BACHHAWAT
BIOCHEMICAL AND ENZYME ENGINEERING LABORATORIES
INDIAN INSTITUTE OF EXPERIMENTAL MEDICINE
JADAVPUR, CALCUTTA 700 032, INDIA.

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Summary :

Binding of a galactose-specific lectins from Ricinus communis (RCA_1) to liposome containing gangliosides, galactocerebroside and cytolipin H reveal that the lectin binds to glycolipids containing terminal nonreducing galactose residues. Lectin binding to galactocerebroside and cytolipin is strongly influenced by the chain length of fatty acid in phospholipids used for preparing liposomes and the concentration of cholesterol in liposomes.

INTRODUCTION :

The carbohydrate residues of cell surfaces have been implicated in many biological functions such as cell-cell interaction, as receptor for some neurotransmitters, toxins and viruses and as determinants of immunologic specificity (1-5). Cell-surface carbohydrates are contributed by membrane-associated glycoproteins and glycolipids. Receptor properties of glycoproteins have been demonstrated for some ligands (6), but a similar demonstration of the receptor properties of glycolipids has remained elusive possibly because they form micelles in aqueous solution. This problem may be obviated by incorporating the glycolipids in liposomes (7). As a preliminary step in this direction we have incorporated ganglio-

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Abbreviations : β-γ-dipalmitoyl-DL-α-lecithin (DPL), β-γ-dimyrsitoyl-DL-α-lecithin (DML); galactose-binding protein from Ricinus communis (RCA_1); ceramide-glucose-galactose-N-acetylgalactosamine-galactose (GM_1). N-acetylneuraminic acid

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sides and certain neutral glycolipids into liposomal membranes and have examined their recognition by a galactose-binding protein from Ricinus communis (RCA_1) (7,8,9). The liposome-containing glycolipids provide an interesting model system for the study of receptor-ligand interaction. This report provides a strong evidence that the recognition of the groups of a receptor is in part determined by its exposure on the membrane surface.

MATERIALS AND METHODS.

Preparation of liposomes - Unilamellar liposomes were prepared using β-γ-dipalmitoyl - DL-α-lecithin (DPL) or β-γ-dimyristoyl-DL-α-lecithin (DML) with varying concentrations of cholesterol (0-40 mol % of total lipids) containing galactocerebroside or cytolipin H (a ceramide dihexoside containing galactose as the terminal sugar) or purified GM_1, as described in our earlier publication (7). The amount of phospholipid, ganglioside, neutral glycolipid and cholesterol was calculated by estimating lipid phosphorus(11) N-acetylneuraminic acid(12) neutral sugar(13) and cholesterol (14) respectively.

Binding of RCA_1 to the liposomes - Binding was measured by adding varying concentration of lectin to different batches of liposomes in 0.05M Sodium phosphate buffer pH 7.0 containing 0.15M NaCl. The tubes were constantly shaken during incubation at the desired temperature for 3 hr. and then centrifuged at 105,000 g for 2 hr. the proteins remaining in the supernatant were estimated by Lowry's method (15) and subtracted from the amount of lectin added to obtain the amount bound.

RESULTS - No binding of RCA_1 was detected at 4°C with galactocerebroside liposomes prepared with DPL (Fig.1). At 45°C binding of RCA_1 to galactocerebroside was considerable and about 10% of the total galactocerebroside was estimated to be available to RCA_1. When DML was used for preparing liposomes a significant binding of RCA_1 to galactocerebroside at 4°C occured. From the saturation value of RCA_1 binding the proportion of galactocerebroside accessible to the lectin was calculated to be 23%. When 30% cholesterol was incorporated into these liposomes, the availability of galactocerebroside to lectin

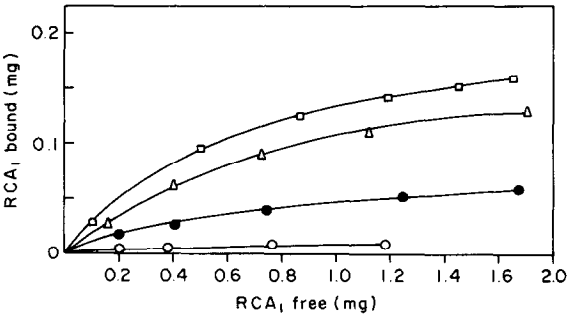

Fig. 1. Binding of RCA_1 to galactocerebroside liposomes. Binding studies were carried out as described in the text. Each batch of liposome contained 10 nmol of galactocerebroside and 500 nmol of phospholipid. Experiments with DPL liposomes at 4°C (○) and at 45°C (●), DML liposomes with 30% cholesterol (□) at 4°C and without cholesterol (△) at 4°C.

increased to 37%. Binding studies of RCA_1 carried out with DPL liposomes containing cytolipin indicate that without cholesterol less than 30% of the cytolipin is accessible for RCA_1 binding; the proportion that is accessible increases when 20% or more of cholesterol is present in the liposomes and at 40% cholesterol 60% of the cytolipin is recognized by RCA_1. When DML replaced DPL in liposomes the binding of RCA_1 to cytolipin was same (60%) at all the cholesterol concentration studied (0-40%).

The association constant, K_a (mol^{-1}) for the binding of incorporated glycosphingolipid to lectin was calculated according to equation (1) from the value of m at $\theta = 0.5$

$$Ka = \frac{\theta}{(1-\theta)m} \quad \text{----------} \quad (1)$$

where m = the concentration of RCA_1 free in solution and

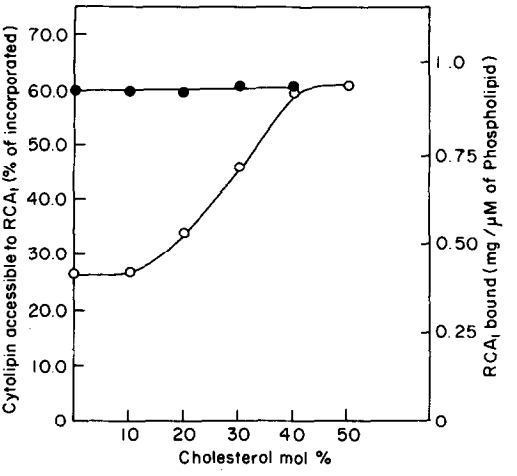

Fig. 2. Binding of RCA_1 to liposomes containing cytolipin. The liposomes containing cytolipin were prepared with DPL (○) or DML (●) varying in concentration of cholesterol. Each tube contained 7 nmol of cytolipin per 350 nmol of phospholipid. Temperature is 4°C. Experiments were performed as mentioned in the text.

θ = the fraction of glycolipids at the outer surface of liposome bound to RCA_1. K_a values of 0.23 and 0.36 x $10^6 M^{-1}$ were obtained for RCA_1 binding to galactocerebroside and cytolipin respectively. Binding of RCA_1 with liposomes containing GM_1 was similar whether DPL or DML was used to prepare the liposomes (Fig 3), also cholesterol did not effect the binding of RCA_1 to GM_1 in DPL or DML liposomes. K_a for RCA_1 binding to GM_1 was found to be 2.2 x $10^6 M^{-1}$.

Discussion :

Based on the results of our earlier studies we suggested that RCA_1 recognizes gangliosides through their terminal non-reducing galactose residues (7). Our initial findings (7) on the binding of lectins to liposomes containing glycolipids have been confirmed and extended by several recent reports

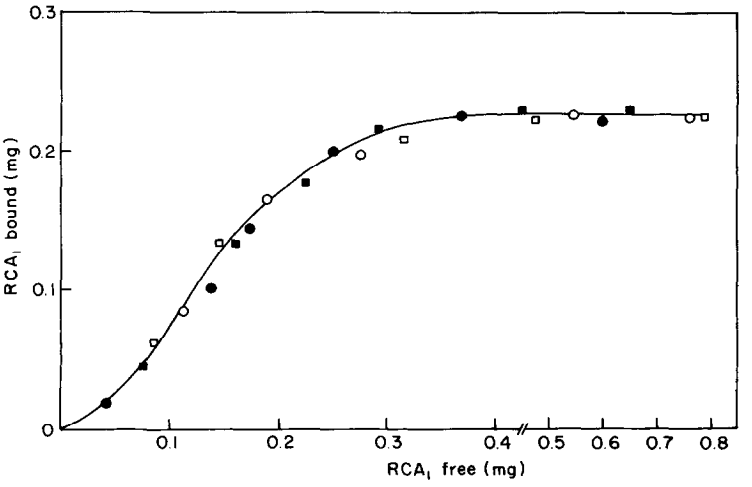

Fig. 3. Binding of RCA_1 to liposome containing GM_1. Experiments were carried out as described in the text. Each tube contained 6.5 nmol of GM_1 in DPL with 30% cholesterol (O) or without cholesterol (●) or in DML with 30% cholesterol (□) or without cholesterol (■).

(16, 17, 18), but no attempt was made to assess quantitatively the effects of chain lengths of the oligosaccharide and of the phospholipid used to generate liposomes nor the role of cholesterol in determining the availability of glycolipids to lectin. At 4°C we detected almost no binding of RCA_1 to galactocerebroside incorporated in DPL, but at 45°C binding of RCA_1 to these liposomes was observed. The poor binding of RCA_1 to galactocerebroside may be due to a restricted access of galactose residues of cerebroside by RCA_1 because in liposomes prepared with galactocerebroside (possessing only the single sugar) most of the galactose residues are embedded in the liposomal bilayer. This finding is consistent with the observations of Inoue et al (19) who have shown that a much greater incorporation of galactocerebroside into the lipid bilayer is required for complement-dependent damage of these liposomes,

as compared to liposomes containing Forssman antigen or globoside. We felt that the chain length of the phospholipid fatty acid may affect the accessibility of the single galactose residue of the galactocerebroside. Indeed binding of RCA_1 to galactocerebroside increased significantly when a phospholipid containing the shorter chain fatty acid (DML) replaced DPL in liposomes. Increasing the concentration of cholesterol had no effect on the recognition of GM_1 by RCA_1 but did result in a large increase in the availability of cerebroside and cytolipin to RCA_1. This is not surprising since gangliosides have a long carbohydrate chain freely accessible to the lectin. On the other hand our data with cytolipin H and galactocerebroside do suggest that cholesterol tends to increase the exposure of hydrophilic sugar residues on the surface of liposomes. This enhanced binding at higher cholesterol content may be due to a change in the membrane surface area or due to a subtle molecular effect on the local environment of receptor. Studies of interactions of lectins with liposomes containing glycolipids possessing short oligosaccharide chains should provide a useful tool to monitor the effects of cholesterol on the furface structure and function of membranes.

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