Binding specificities of the lectins from *Helix pomatia*, soybean and peanut against different glycosphingolipids in liposome membranes

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The binding specificities of the lectins from *Helix pomatia*, soybean and peanut against glycosphingolipids containing β-linked terminal D-galactose and N-acetyl-D-galactosamine and the role of sialic acid as a modulator of the binding specificity were investigated. The test system used consisted of liposomes containing the glycosphingolipids and lectins coupled to gel columns. Of the investigated glycosphingolipids only ganglioside GM2 bound to soybean agglutinin while *Helix pomatia* agglutinin was found to bind only GA2. Peanut agglutinin showed good affinity both for ganglioside GM1 and its asialoderivative GA1.

Lectin *Ganglioside* *Glycosphingolipid* *Affinity chromatography* *Liposome membrane*

1. INTRODUCTION

Many lectins such as concanavalin A, lentil and wheat germ lectins bind sugar sequences present in most mammalian glycoproteins and are thus valuable tools for the isolation of membrane glycoproteins in general [1]. Another family of lectins contains several types of D-galactose and *N*-acetyl-D-galactosamine-binding proteins, such as *Helix pomatia*, soybean and peanut lectins. These lectins (each binding carbohydrate sequences only represented in restricted types of glycoconjugates [2]) constitute useful probes in studies of important cell surface markers of a carbohydrate nature.

Abbreviations: PNA, peanut agglutinin; HPA, *Helix pomatia* agglutinin; SBA, soybean agglutinin; WGA, wheat germ agglutinin; PC, phosphatidylcholine

Schematic structures of glycolipids used are given in fig.1

Murine mature and immature thymocytes may be distinguished by their different reactivities with PNA (main specificity D-Gal(β1–3)-D-GalNAc) [3] and B and T lymphocytes by their different binding to both HPA [4] and SBA [5] (main specificity *N*-acetyl-D-galactosamine), suggesting that the HPA binding structures may be different from those binding to SBA. Little is known about the cell surface carbohydrate structures involved in the binding to these 3 lectins [6,7]. To elucidate the possible role of sialic acid as a modulator of β-linked terminal, non-reducing D-galactose and *N*-acetyl-D-galactosamine, we have studied the SBA, PNA and HPA binding of the gangliosides GM2, GM1 and GD1a, and the corresponding desialylated glycosphingolipids GA2 and GA1.

2. MATERIALS AND METHODS

2.1. *Preparations of labelled glycolipids*

The neutral glycolipids GA1 and GA2 and the gangliosides GM1 and GM2 (fig.1) were isolated

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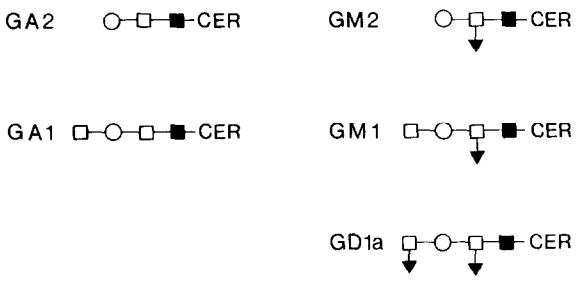

Fig.1. Chemical structure of the gangliosides used and their corresponding asialo derivatives. All sugars are of the D-configuration: (■) glucose; (□) galactose; (○) *N*-acetylgalactosamine; (▼) *N*-acetylneuraminic acid.

and radiolabelled by the galactose oxidase–sodium borotritride reaction as in [8]. The labelled glycolipids were diluted to a specific activity of 20000 dpm/nmol. Ganglioside GD1a was labelled in the ceramide moiety as in [9].

2.2. *Preparation of liposomes*

Phosphatidylcholine (purified from egg yolk), 500 nmol, was mixed with 10 nmol appropriate glycolipid. The organic solvents were evaporated to dryness under nitrogen at 40°C on a water bath. The lipids were suspended in 1.0 ml 0.1 M NaCl–0.1 M Tris–HCl buffer (pH 7.5) and were afterwards sonicated in an MSE ultrasonic disintegrator Mk2 with 9.5 mm probe at an amplitude of 22 μ under nitrogen at 0–4°C (ice water). The liposome suspensions were centrifuged at 10000 × *g* for 15 min and the clear supernatants were then used in the experiments. The recovery of glycolipids in the supernatants varied from 50–60%.

2.3. *Affinity chromatography*

The coupling of lectins to Affigel 10 (BioRad Labs.) and the chromatographic procedures have been reported [10]. Samples of 10 mg SBA (Pharmacia Fine Chemicals) and PNA (Boehringer-Mannheim) were coupled to 0.5 g (dry wt) of Affi-Gel 10 and reconstituted in 12.5 ml 0.1 M Tris–HCl buffer (pH 7.5). HPA–Sepharose and WGA–Sepharose were purchased from Pharmacia Fine Chemicals (Uppsala) and equilibrated in the same buffer. The glycolipid liposomes were analysed on gel-coupled lectins in minicolumns (inner diameter 6 mm, height 9 mm); 100 μl of each preparation was allowed to adsorb for 15 min at room temperature. The gels were washed with 10 bed vol. of 0.15 M NaCl–0.02 M Tris–HCl buffer (pH 7.5) and the bound material was eluted with appropriate sugars in the same buffer (HPA, SBA-0.005 M *N*-acetyl-D-galactosamine; PNA-0.05 M D-galactosamine, and WGA-0.05 M *N*-acetyl-D-glucosamine). The flow rates did not exceed 15 drops/min and 1.0 ml fractions were collected. All fractions were subjected to liquid scintillation counting, using Aqua-Luma plus (LKB-products, Stockholm) as a scintillation fluid.

3. RESULTS AND DISCUSSION

The optimal ratio of glycolipid to phosphatidyl choline in our test system was established by using GA1-liposomes and gel-bound PNA. A minium ratio of glycolipid to phosphatidylcholine of 1:200 was necessary for the binding but an increase of the proportion of glycolipid to 1:25 only slightly increased the binding capacity. For the final experiments a ratio of glycolipid to phosphatidylcholine of 1:50 was used. The following is a summary of the reasons for using liposomes as carrier for the glycolipids: In preliminary experiments we tried to use glycolipids solubilized in Triton X-100, a non-ionic detergent reported to be compatible with all 3 of the lectins being used [11]. Under these conditions, no glycolipids bound to the lectins. The phenomenon that non-ionic detergents may abolish lectin-binding has also been reported for the interactions between glycolipids and the *Ricinus communis* lectin [12]. Although GM1 and GA1 have been reported to bind to PNA in the presence of sodium deoxycholate [13], this detergent cannot be used for SBA and HPA due to strong interference with the binding activity of the lectin [11].

Both GM1 and GA1 liposomes reacted with PNA in a similar way (fig.2a). This lectin demonstrates a primary affinity for the terminal non-reducing-disaccharide D-Gal(β1–3)-D-GalNAc [2] and the sialic acid linked to the inner galactose obviously does not impair the binding to the lectin. This is in contrast to the findings [13] of a lower binding of GM1 than of GA1 to the PNA lectin of deoxycholate solubilized glycolipids. We also found that a sialic acid attached to the D-Gal-(β1–3)-D-GalNAc sequence as in GD1a completely abolished the binding to PNA (fig.3). The same

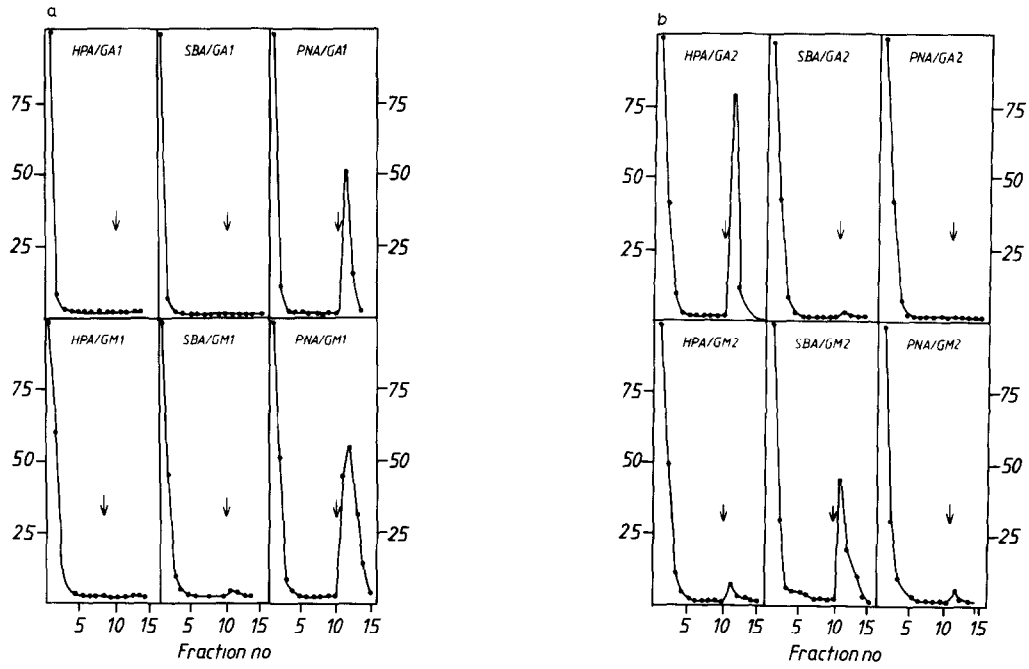

Fig.2. (a,b) Affinity chromatography on immobilized lectins of liposomes containing glycosphingolipids: (⟶) addition of eluting sugar (0.005 M *N*-acetyl-D-galactosamine–HPA,SBA; 0.05 M D-galactosamine–PNA). In each experiment 10000 cpm of glycosphingolipid label was used but to enable comparisons between the different experiments the cpm value of fraction 1 is set at 100 (see ordinate).

phenomenon has also been reported for blood groups MN-active substances [2]. We observed no significant binding of GM1 and GA1 to HPA and SBA.

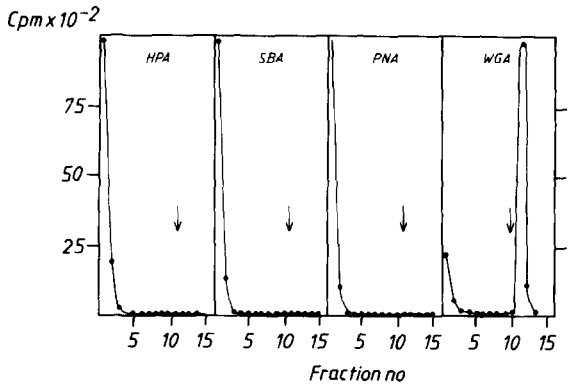

Fig.3. Affinity chromatography on immobilized lectins of ganglioside GD1a liposomes. Bound material was eluted with 0.005 M *N*-acetyl-D-galactosamine–HPA, SBA, 0.05 M D-galactosamine–PNA and 0.05 M *N*-acetyl-D-glucosamine–WGA as indicated by an arrow.

GA2 and GM2 show an interesting differential affinity for HPA and SBA since GA2 binds to HPA but GM2 does not, while the opposite pattern is evident for SBA (fig.2b). Both HPA and SBA are considered to have their main specificity for terminal non-reducing *N*-acetyl-D-galactosamine, although differences in their reactivities towards related structures such as D-galactose and *N*-acetyl-D-glucosamine have been reported [2,14]. It has also been suggested that the *N*-acetyl group at C_2 participates in the binding between HPA and *N*-acetyl-D-galactosamine [14]. In NMR studies of ganglioside GM1 [15] the interaction between the carbonyl oxygen of the acetamido group of *N*-acetyl-D-galactosamine and carbon atoms 2 and 3 of the *N*-acetylneuraminic acid was shown. This effect may explain our result, that GA2 but not GM2 binds to HPA. If the sialic acid of GM2 is fully accessible, this ganglioside should bind to WGA, which is a lectin with sialic acid binding properties. However, GM2 and GM1 did not bind to this lectin at all (fig.4), an observation which further strengthens the suggestion that the sialic

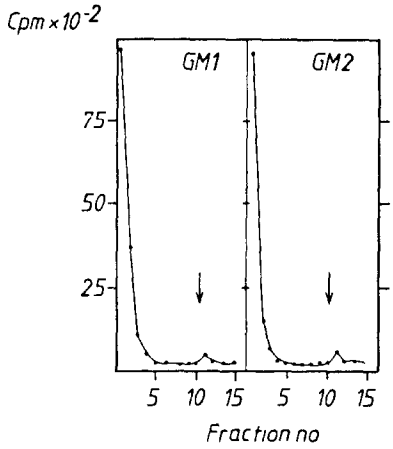

Fig.4. Affinity chromatography on immobilized wheat germ lectin of monosialoganglioside liposomes; eluent, 0.05 M *N*-acetyl-D-glucosamine (⟶).

acid residue of GM2 may interact with the *N*-acetyl-D-galactosamine residue. In contrast GD1a, with a terminal sialic acid, showed a prominent binding to WGA (fig.3).

We can offer no explanation as to why GA2 does not bind to SBA. However, our data indicate that the lectin-binding properties of a terminal non-reducing β-linked *N*-acetyl-D-galactosamine is modulated by the presence or absence of a sialic acid linked to a penultimate D-galactose.

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