Binding specificities of the lectins PNA, WGA and UEA I to polyvinylchloride-adsorbed glycosphingolipids

Kent Molin,.Pam Fredman and Lars Svennerholm

Department of Psychiatry and Neurochemistry, University of Gothenburg, St. Jörgen's Hospital, S-422 03 Hisings Backa, Sweden

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The binding specificities of the lectins PNA (peanut agglutinin), WGA (wheat germ agglutinin), and UEA I (*Ulex europeus* agglutinin I) against glycosphingolipids were investigated using an enzyme-linked immunosorbent assay (ELISA), utilizing the biotin-avidin system for detection of bound lectin. PNA showed the highest affinity to GA1, but also bound, though less strongly, to GM1 and GD1b. WGA bound to 3′-nLM1 and 6′-nLM1, the former twice as strongly as the latter, but not to any sialic acid containing glycolipid of the gangliotetraose series. UEA I showed a high affinity for the Le^a glycolipid which has an α1-4 linked fucose but not for the glycolipids with α1-3 or α1-2 linked fucose. Interestingly, 3′-nLM1 and nLA1, glycolipids lacking fucose, also bound UEA I. The results show that lectins should be used with caution for establishing terminal sugar sequences in glycosphingolipids.

Lectin *Ganglioside* *Glycosphingolipid* *ELISA*

1. INTRODUCTION

Lectins are carbohydrate-binding proteins of non-immunoglobulin nature, capable of binding to glycolipids and glycoproteins. They have widespread use as biochemical tools for glycoprotein fractionation, as they can be immobilized on various supports such as Sepharose column [2,3], and for studies of cell membrane components, where they are used to visualize carbohydrate moieties [4]. ^{125}I-labelled lectins have been used for detection of glycolipids on thin-layer chromatograms [5,6], and biotin-labelled lectins have been similarly used for detection of glycoproteins transferred to nitrocellulose sheets from electrophoretic gels [7].

Lectins exhibit different degrees of specificity in their interaction with carbohydrates. Some lectins bind preferentially to specific monosaccharides, others show higher affinities to complex sugar structures. It is important to note that a specific oligosaccharide structure involved in the binding of a lectin might be quite different from the best monosaccharide inhibitor [8]. Moreover, substituents that do not participate in binding may affect the specific interaction with the lectin. One of the most widely used methods for determining lectin specificity to date has been the hemagglutinating inhibition assay [8]. This is a semiquantitative method, where specificity is expressed as a titer of added carbohydrate ligands, and the information obtained refers to sugars in solution. In the present study, lipid-bound carbohydrate structures were

Abbreviations: PNA, peanut agglutinin; WGA, wheat germ agglutinin; UEA I, *Ulex europeus* agglutinin I. Ganglioside nomenclature according to Svennerholm [1.] Fuc-GM1 and Fuc-nLA1 are fucosylated GM1 (IV^2Fuc,II^3NeuAc-$GgOse_4Cer$) and nLA1 (III^3Fuc-$nLcOse_4Cer$), respectively. 3′-nLM1 and 6′-nLM1 are shorthand designations for IV^3NeuAc-$nLcOse_4Cer$ and IV^6NeuAc-$nLcOse_4Cer$, respectively. Le^a is the $Lewis^a$ blood group defining antigen; III^4Fuc-$LcOse_4Cer$ (see also table 1)

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immobilized on a plastic surface with lecithin and cholesterol, thereby probably altering the accessibility of the ligands in a way that can be compared with the situation for cell surface sugars.

2. EXPERIMENTAL

2.1. *Materials*

The biotin-labelled lectins PNA, WGA and UEA I and avidin-horseradish peroxidase (HRP) were obtained from EY Laboratories, San Mateo, CA. UEA I was also purchased from Sigma, St. Louis, MO, together with lecithin, cholesterol and *o*-phenylenediamine (Sigma grade). Titertec polyvinylchloride microtiter plates no. 77-172-05 were from Flow Laboratories, Irvine, Scotland, and bovine serum albumin (BSA) from Armour Pharmaceutical, Eastbourne, England.

All gangliosides and neutral lipids used for the binding assays (listed in table 1) were isolated at this department.

2.2. *Methods*

2.2.1. Lectin binding assay

Binding of the lectins PNA, WGA and UEA I to different glycosphingolipids was examined using ELISA [9]. The glycolipid ligands were immobilized using a modification of a method described by Hakomori et al. [10]. The wells of polyvinylchloride microtiter plates were coated with 20 μl of serial dilutions of glycosphingolipids in methanol containing lecithin (0.1 mg/ml) and cholesterol (0.06 mg/ml). The lipids were adsorbed by evaporating the solvent in vacuo at room temperature, after which the wells were incubated for 15 min with 200 μl of buffer A (0.05 M Tris-HCl, pH 7.5, containing 0.14 M NaCl, 1% BSA and 0.01% merthiolate) to block unspecific binding of the lectins. The buffer was aspirated, 50 μl of the same buffer containing the biotin-conjugated lectin at a concentration of 5 μg/ml was added to each well, and the plate, coated with parafilm, was incubated overnight at 4°C. After washing the wells 4 times with buffer B (0.01 M phosphate buffer, pH 7.4, containing 0.14 M NaCl), 50 μl of an avidin-HRP solution, 5 μg/ml in buffer A, was added. After 1 h at room temperature the plate was washed 4 times with buffer B, and 200 μl of a substrate solution (1.4 mM *o*-phenylenediamine in citrate buffer, pH 4.5, containing 0.01% H_2O_2) was added. The reaction was stopped after 5 min of incubation by adding 50 μl of 1 M H_2SO_4, and the absorbance read at 450 nm.

3. RESULTS AND DISCUSSION

Fig.1 shows the binding of the three biotin-conjugated lectins PNA, WGA and UEA I to serial dilutions of gangliosides and neutral glycolipids, as measured by ELISA. All serial dilutions started with 200 pmol of the respective glycosphingolipid. The limiting amount of glycosphingolipid needed for a detectable binding of lectin is shown in table 1.

In preliminary experiments the adsorption of the glycosphingolipids to the microtiter plates was performed without the addition of lecithin and cholesterol. With this procedure, however, reproducible results could not be obtained, but by adopting the method described by Hakomori et al. [10] the precision was improved. Still the slopes of the absorbance curves varied slightly when repetitive assays were performed, but the binding patterns remained constant.

PNA showed the highest affinity to GA1, and approx. 2 pmol of this glycolipid could be detected (fig.1). However, this lectin also bound to gangliosides Gm1 and GD1b, but approx. 10- and 25-times more substance was required for detection (table 1). These results are in accordance with earlier reports [11] indicating a primary affinity for the disaccharide Gal(β1-3)GalNAc, which is the terminal sequence of GA1, GM1 and GD1b. The weaker binding to GM1 has earlier been demonstrated by Momoi et al. [6]. In experiments with PNA-Sepharose affinity column chromatography they found that 40–50% of GA1 was retained on the column, while only 10% of total GM1 was bound. Using an ELISA method, the same authors found no binding of GM1 to the lectin. These results suggest that substitution by *N*-acetylneuraminic acid causes conformational changes decreasing binding ability. On the other hand, Månsson and Olofsson [12] using liposome-incorporated GA1 and GM1, found no difference in binding of these glycolipids to PNA-Sepharose. This demonstrates that the results might depend on the assay system used, and that comparisons have to be made for the same type of assays only. GD1b has

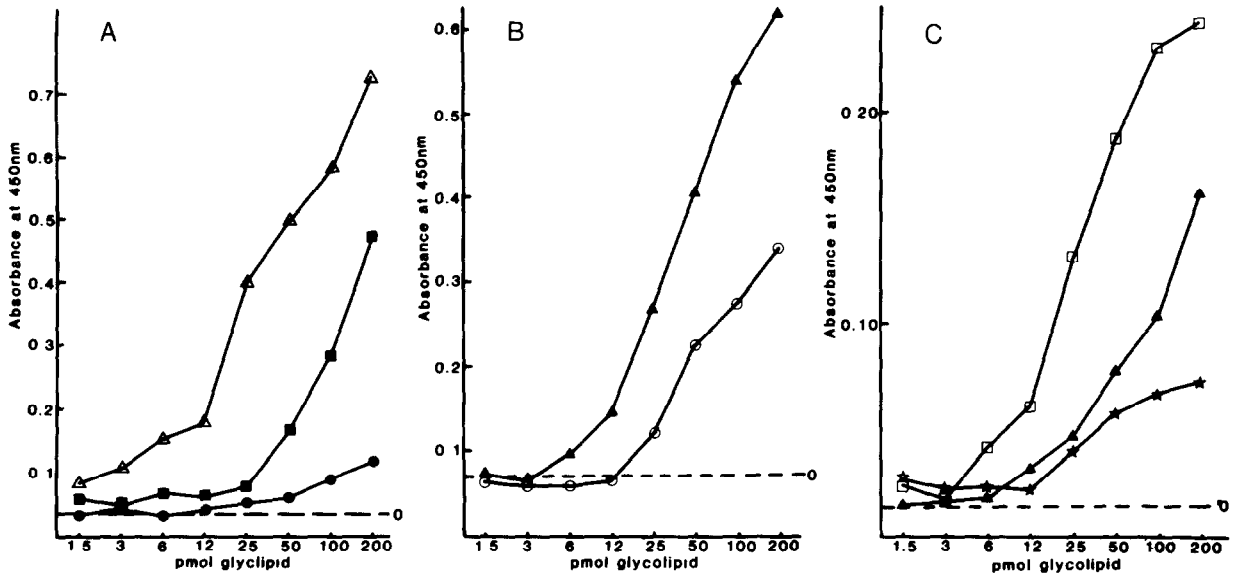

Fig.1. Binding of (A) PNA, (B) WGA and (C) UEA I to glycosphingolipids investigated by the ELISA method described in section 2.2. (△) GA1, (■) GM1, (●) GD1b, (▲) 3′-LM1, (○) 6′-LM1, (□) Le^a and (★) LA1. 0 denotes wells coated with lecithin and cholesterol only.

not, to our knowledge, been studied with regard to PNA-binding.

A weak binding to nLA1 was seen in some of the assays performed, but no reproducible detection limit could be determined. This neutral glycosphingolipid has the terminal structure

Table 1

Detection limits, specified as the ELISA titer where the absorbance curves reached twice the background level, in pmol for different glycosphingolipids when detected by the lectins PNA, WGA and UEA I using the ELISA method described in section 2.2

Glycosphingolipid		PNA	WGA	UEA I
		pmol glycosphingolipid		
Gangliotetraose series				
GM3	NeuAc(α2-3)Gal(β1-4)Glc(β1-1)Cer	>200	>200	>200
GM1	Gal(β1-3)GalNAc(β1-4)(NeuAcα2-3)Gal(β1-4)Glc(β1-1)Cer	25	>200	>200
Fuc-GM1	Fuc(α1-2)Gal(β1-3)GalNac(β1-4)(NeuAcα2-3)Gal(β1-4)Glc(β1-1)Cer	>200	>200	>200
GA1	Gal(β1-3)GalNAc(β1-4)Gal(β1-4)Glc(β1-1)Cer	2	>200	>200
GD1a	NeuAc(α2-3)Gal(β1-3)GalNAc(β1-4)(NeuAcα2-3)Gal(β1-4)Glc(β1-1)Cer	>200	>200	>200
GD1b	Gal(β1-3)GalNAc(β1-4)(NeuAcα2-8NeuAcα2-3)Gal(β1-4)Glc(β1-1)Cer	55	>200	>200
Lactoneotetraose series				
3′-nLM1	NeuAc(α2-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc(β1-1)Cer	>200	12	10
6′-nLM1	NeuAc(α2-6)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc(β1-1)Cer	>200	27	>200
nLA1	Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc(β1-1)Cer	>200	>200	15
Fuc-nLA1	Gal(β1-4)(Fucα1-3)GlcNAc(β1-3)Gal(β1-4)Glc(β1-1)Cer	>200	>200	>200
Lactotetraose series				
Le^a	Gal(β1-3)(Fucα1-4)GlcNAc(β1-3)Gal(β1-4)Glc(β1-1)Cer	>200	>200	4

Gal(β1-4)GlcNAc. The binding to this disaccharide has previously been reported by Pereira et al. [11], who in an inhibition assay found it 14-times less active than Gal(β1-3)GalNAc.

WGA showed binding to the gangliosides 3′-nLM1 and 6′-nLM1, the former being approximately twice as strongly bound (fig.1). The difference in affinity for the two isomers of nLM1 is in accordance with the findings of Kronis and Carver [13]. In a ^{1}H-NMR binding study these authors showed a preferential binding of the lectin to *N*-acetylneuraminyllactose with NeuAc attached in an α2-3 linkage as in 3′-nLM1.

No other NeuAc-containing structures were recognized by the lectin. This may be explained by the absence of *N*-acetylglucosamine (GlcNAc) in these structures, since it has been suggested that the combining site of WGA consists of three identical subsites capable of binding *N*-acetylglucosamine and *N*-acetylneuraminic acid but not *N*-acetylgalactosamine [14,15]. Hence it might be possible for ganglioside nLM1, which contains both *N*-acetylglucosamine and *N*-acetylneuraminic acid, to interact simultaneously with two subsites in WGA. This is supported by the fact that the neutral glycosphingolipid Fuc-nLA1, the fucose residue of which is linked to the subterminal GlcNAc, is not recognized under the conditions of this assay. nLA1 was weakly bound by the lectin in some of the assays performed, but as in the case of PNA no detection limit could be established.

UEA I, which has been reported to bind α-L-fucose specifically [8], bound only to one of the three fucose-containing structures tested, namely Le^a glycolipid. Additionally, two structures lacking fucose, 3′-nLM1 and nLA1, bound the lectin (fig.1). Le^a glycolipid has a fucose bound in an α1-4 linkage, while Fuc-GM1 and Fuc-nLA1, which were not recognized by the lectin, have an α1-2 and α1-3 linked fucose, respectively. From this it can be concluded that the lectin has a specificity for α1-4 linked fucose. In the case of Fuc-nLA1, which has the same carbohydrate backbone as 3′-nLM1 and nLA1 (lactoneotetraose backbone) the fucose bound to GlcNAc seems to have an inhibitory effect on the lectin binding. The high affinity for 3′-nLM1 and nLA1 suggests that the lectin has an extended combining site, interacting with Gal-GlcNAc-containing carbohydrate structures in addition to α1-4 linked fucose.

As indicated in section 2.1, UEA I lectins from two different sources were tested, both of them purified by affinity chromatography by the respective manufacturer. The Sigma lectin was biotinylated as described by Guesdon et al. [16]. No significant difference in the binding properties of the two preparations could be detected (not shown). However, the possibility cannot be excluded that these two commercial preparations are contaminated with UEA II, which is specific for *N*-acetylglucosamine.

This result illustrates the complexity of the binding of lectins to glycolipids. Different assays might give different results but the steric configuration of the oligosaccharide is also of importance. We conclude that lectins might be useful tools for group separations of glycolipids and for studying cell membrane carbohydrates but should be used with caution for establishing terminal sugars in glycolipids.

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