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SPECIFIC INTERACTION OF CONCANAVALIN A WITH GLYCOLIPID MONOLAYERS

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Summary

The effect of ^{131}I-labelled concanavalin A on the surface pressure and surface radioactivity of monolayers formed from phospholipids and from natural and synthetic glycolipids has been studied. The lectin binds to and penetrates dipalmitoyl phosphatidylcholine monolayers at a surface pressure of 15 dynes/cm and this interaction is inhibited by the presence of α-methyl mannose in the subphase. At surface pressures of 25 dynes/cm or higher, concanavalin A will interact with monoglucosyl diglyceride or diglucosyl diglyceride from *Acholeplasma laidlawii* and with synthetic glycolipids containing 2 or 3 α1 → 4-linked D-glucose residues in the headgroup, but not with phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, or with the ganglioside II^{3}NeuAc-GgOse$_4$-Cer. The binding to the glycolipid sugar group and penetration of the hydrocarbon region seem to occur simultaneously, as the time courses for the development of surface pressure and surface radioactivity coincide.

The initial interaction of concanavalin A with cell surfaces is thought to involve the recognition by the lectin of exposed sugar residues [1]. This is suggested by the inhibition of agglutination of cells by the presence of the appropriate 'hapten' sugars (D-glucose, D-mannose, α-D-glucosides or mannosides, or related structures), which are thought to compete for binding to this lectin [2—4]. There is evidence, however, that hydrophobic interactions also play a role in the action of concanavalin A. Agglutination of Ehrlich ascites carcinoma cells by concanavalin A could not be reversed by α-methyl

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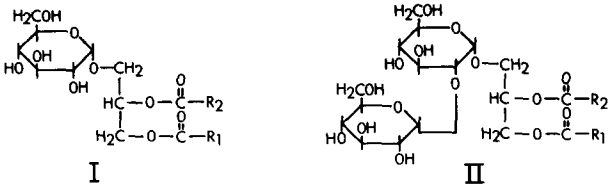
mannose after 10 min, nor could all of the membrane glycoprotein from these cells which bound to a concanavalin A-Sepharose affinity column be released without the use of detergent in addition to the hapten sugar [2]. Concanavalin A has recently been shown to bind to liposomes which contain no carbohydrate at all, and this binding was inhibited by hapten sugars [5,6]. Further support for the interaction of concanavalin A with the hydrophobic region of membranes came from the observation that the conductance of glycolipid-containing planar bilayer membranes increased dramatically upon addition of concanavalin A [7].

Although the lectin receptors in biomembranes have traditionally been considered to be glycoproteins [8], recent evidence indicates that glycolipids can also specifically bind lectins [6–7,9]. We have therefore investigated the association of ^{131}I-labelled concanavalin A with lipid and glycolipid monolayers by following changes in surface pressure and surface radioactivity, in order to evaluate the roles of sugar residue recognition and of hydrophobic interaction with the lipid hydrocarbon region in this binding.

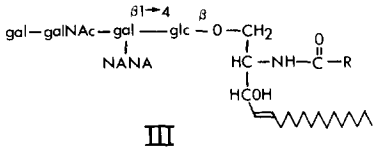
Glycolipids used as receptors were either synthetically prepared or isolated from natural sources. Their structural formulae are given in Fig. 1. Monoglucosyl diglyceride and diglucosyl diglyceride, isolated from *Acholeplasma laidlawii* B [10] were supplied by Dr. E. Bevers, and were purified by thin-layer chromatography on Silica G, using acetone/benzene/water (91:30:8, v/v) as a solvent [10]. The ganglioside II^{3}NeuAc-GgOse$_4$-Cer, isolated from human brain, was a gift from Dr. W. Ziegler. Egg phosphatidylcholine was isolated from hens' eggs according to established procedures and other phospholipids were synthesized as described previously [12]. Maltosyl-α1 → 4[1-deoxy-1-palmitamido-octadecylaminosorbitol] and maltotriosyl-α1 → 4[1-deoxy-1-palmitamido-octadecylaminosorbitol] were prepared from the corresponding oligosaccharides by reductive amination, followed by *N*-palmitoylation as employed by Wiegandt and Ziegler for the production of synthetic glycolipids [13]. Amylose was isolated from 600 g of starch by precipitation as a thymol complex [14]. This was partially hydrolyzed by refluxing 1 h in 6 l of 0.1 N H_2SO_4 and the mixture neutralized with $BaCO_3$. After removal of $BaSO_4$ by centrifugation, solvent was removed from the supernatant and the residue was taken up in 600 ml of water. Large oligosaccharides were precipitated by the addition of 1200 ml ethanol, followed by cooling on ice, centrifugation, and removal of solvent from the supernatant. This procedure was repeated once, followed by two further precipitations from half this volume of water by the addition of two volumes of acetone. The mixture of α1 → 4-linked glucose oligosaccharides thus obtained was fractionated by passage twice through a 5 cm diameter × 75 cm column of Sephadex G-10 and by descending paper chromatography for 4 days using a solvent of pyridine/ethyl acetate/acetic acid/water (5:5:1:3, v/v) to yield 230 mg of purified maltotetraose. Maltotriose (Aldrich) was purchased from Janssen Pharmaceutica, B2340 Beerse, Belgium.

Reductive amination of maltotriose and maltotetraose was carried out by refluxing for 6 h in methanol with 2.5 g of octadecylamine and 0.6 g of sodium cyanoborohydride per g of oligosaccharide, after addition of acetic acid to the solution to adjust the pH to 6.5. After removal of solvent, the



I

II



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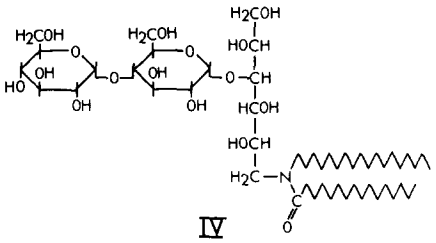

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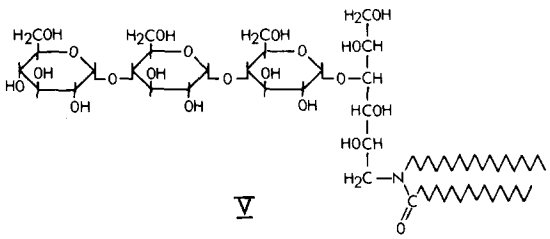

V

Fig. 1. Structures of glycolipids used: I, Monoglucosyl diglyceride; II, diglucosyl diglyceride; III, ganglioside II^{3}NeuAc-GgOse$_4$-Cer (NANA, *N*-acetylneuraminic acid); IV, maltosyl-α1 → 4[1-deoxy-1-palmitamido-octadecylaminosorbitol]; V, maltotriosyl-α1 → 4[1-deoxy-1-palmitamido-octadecyl-aminosorbitol].

reaction mixture was dissolved in *n*-propanol/water (7:3, v/v) and applied to a 4 cm diameter × 140 cm column of Sephadex LH-20, eluting with the same solvent. Fractions showing a positive anthrone test for carbohydrate were analyzed by thin-layer chromatography and those fractions containing pure product were retained and pooled.

Palmitic acid was activated for coupling by incubation at room temperature overnight in dry methylene chloride with a 1.5-fold molar excess of *p*-nitrophenol and of dicyclohexylcarbodiimide, followed by filtration to remove the urea. The reductaminated oligosaccharides were converted to their amide derivatives (compounds IV and V, Fig. 1) by incubation with a 10-fold molar excess of *p*-nitrophenylpalmitate in dry dimethylsulfoxide, with 2 drops of triethylamine added for 4 days at 37°C. After removal of solvent the products were purified by preparative thin-layer chromatography

on Silica G plates, using a solvent system of chloroform/methanol/water (65:35:6, v/v) (R_F = 0.4).

Concanavalin A, purchased from Pharmacia, was labelled with ^{131}I using the chloramine T method [15] and the iodinated lectin purified by affinity chromatography on Sephadex G-150. To investigate its interaction with monolayers, a cylindrical glass trough 8.5 cm in diameter and 2.5 cm deep was used. The rim was coated with paraffin to make it hydrophobic. ^{131}I-Labelled concanavalin A was injected beneath the film while stirring the subphase and the surface pressure and surface radioactivity were recorded automatically simultaneously as described previously [16]. Measurements of ^{131}I radioactivity made at different times were normalized to account for radioactive decay (half-life, 8.2 days).

Protein concentrations were measured by the method of Lowry et al. [17] and glycolipids were assayed by determining sugar content by the method of Dubois et al. [18]. Oligosaccharide mixtures or reductaminated sugars were analyzed by thin layer chromatography using *n*-butanol/acetic acid/water (2:1:1, v/v) as a solvent. Carbohydrate-containing components were detected on thin-layer plates by spraying with 0.5% α-naphthol in CH_3OH/H_2O (1:1, v/v), then with 30% aq. H_2SO_4 and heating briefly in a 140°C oven until purple spots appeared.

Fig. 2 shows the traces of surface radio-activity vs. time obtained when ^{131}I-labelled concanavalin A is injected into the subphase beneath films of

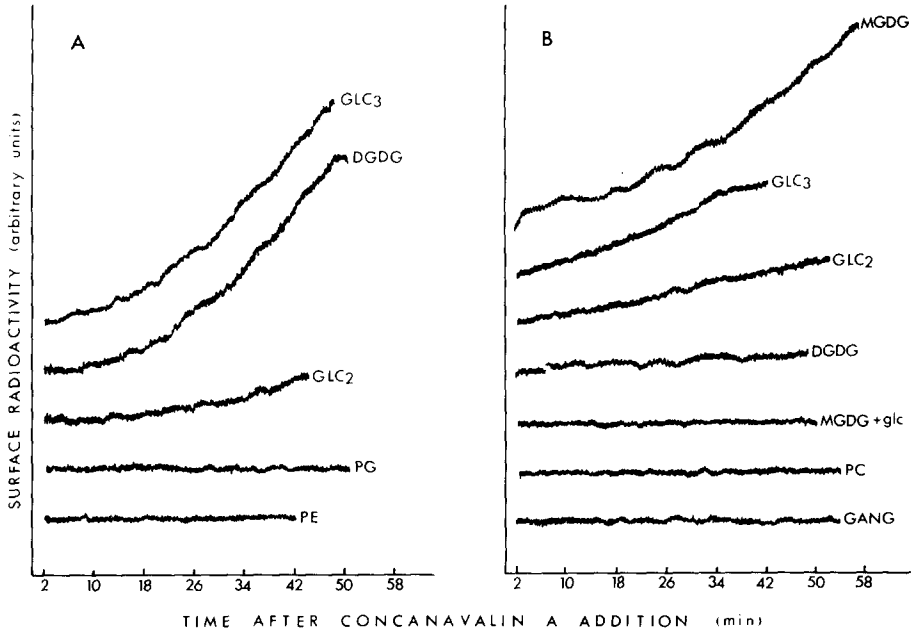

Fig. 2. Binding of concanavalin A to monolayers at initial surface pressures of 30 dynes/cm (A), or 35 dynes/cm (B). The surface radioactivity is continuously recorded after the addition of ^{131}I-labelled concanavalin A to the subphase: GLC_2, maltosyl-α1 → 4[1-deoxy-1-palmitamido-octadecylaminosorbitol]; GLC_3, maltotriosyl-α1 → 4[1-deoxy-1-palmitamido-octadecylaminosorbitol]; MGDG, monoglucosyl diglyceride; DGDG, diglucosyl diglyceride; PG, egg phosphatidylglycerol; PE, dielaidoyl phosphatidylethanolamine; PC, dipalmitoyl phosphatidylcholine; GANG, ganglioside II^3NeuAc-$GgOse_4$-Cer. In one case 200 mM D-glucose was present in the subphase (MGDG + glc).

various lipids at initial surface pressures of 30 dynes/cm or 35 dynes/cm. At both pressures all of the glycolipids containing a terminal glucose residue in an α linkage show some increase in surface radioactivity, indicating binding of concanavalin A to the surface. No binding to egg phosphatidylglycerol or dielaidoyl phosphatidylethanolamine is seen at 30 dynes/cm, nor to dipalmitoyl phosphatidylcholine or the ganglioside II^{3}NeuAc-GgOse$_4$-Cer at 35 dynes/cm. The presence of 200 mM glucose in the subphase completely inhibits the binding to monoglucosyl diglyceride, the lipid showing the strongest interaction with concanavalin A. At a lower surface pressure of 15 dynes/cm, concanavalin A binding to dipalmitoyl phosphatidylcholine monolayers is seen as well. This binding is inhibited by the presence of 200 mM α-methyl mannose in the subphase.

Fig. 3 shows the responses of surface pressure and surface radioactivity when varying amounts of ^{131}I-labelled concanavalin A are added to the subphases of films monoglucosyl diglyceride at initial surface pressures of 30 or 35 dynes/cm. The rapid initial increases in radioactivity are due to material in the subphase, rather than at the surface, and this background had stabilized within 2 min or less. As expected, the rate of absorption to the interface is dependent on the concentration of protein in the subphase. Higher initial surface pressures reduce both the rate of absorption and the total amount of protein absorbed. The time courses for surface pressure and surface radioac-

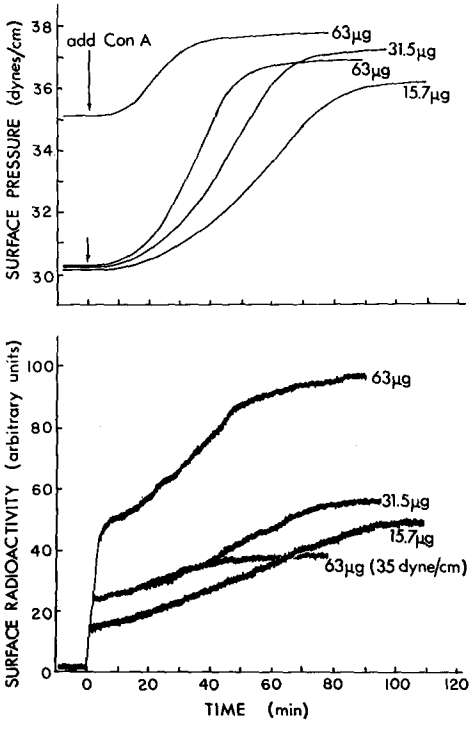

Fig. 3. Binding of concanavalin A (Con A) to monoglucosyl diglyceride monolayers. Various amounts of ^{131}I-labelled concanavalin A were injected into the subphase of monoglucosyl diglyceride films at initial surface pressures of 30 or 35 dynes/cm, and surface pressure and surface radioactivity were simultaneously recorded.

tivity increases are parallel, indicating that binding to sugar residues and penetration of the film occur coincidentally.

Inhibition by hapten sugars has previously been regarded as a diagnostic test for the specific binding of lectins to carbohydrate-containing receptors [19]. The inhibition of binding to non-sugar-containing low pressure films by α-methyl mannose seen in this study does not support that interpretation, and is in agreement with the observations reported by van der Bosch and McConnell [5], and by Boldt et al. [6] of hapten sugar-inhibitable binding of concanavalin A to phospholipid liposomes. The circular dichroism and magnetic circular dichroism results reported by Richardson and Behnke [20] suggest that hapten sugars can inhibit binding of concanavalin A to a membrane through conformational changes of the lectin; not only by competition for a carbohydrate binding site. At higher surface pressures, binding to and penetration of the film seems to require the recognition of specific receptor carbohydrates as well.

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