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REQUIREMENT OF THE CORE STRUCTURE OF A COMPLEX-TYPE GLYCOPEPTIDE FOR THE BINDING TO IMMOBILIZED LENTIL- AND PEA-LECTINS

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ABSTRACT

Structural requirements for the binding of oligosaccharides and glycopeptides to immobilized lentil- and pea-lectins were investigated by use of radioactively-labeled glycopeptides and oligosaccharides. The results indicate that an intact 2-acetamido-2-deoxy-β-D-glucopyranosyl residue at the reducing end of a complex-type oligosaccharide is essential for high-affinity binding to lentil lectin–Sepharose but not to concanavalin A–Sepharose and that an asparagine residue is required for the binding of a complex-type glycopeptide to pea lectin–Sepharose. In addition, interaction of a complex-type oligosaccharide with lentil lectin–Sepharose was enhanced by exposure of nonreducing, terminal 2-acetamido-2-deoxy-β-D-glucopyranosyl groups, whereas interaction with pea lectin–Sepharose was enhanced only after exposure of nonreducing, terminal α-D-mannopyranosyl groups.

INTRODUCTION

The carbohydrate-binding specificities of both *Lens culinaris* (lentil) lectin[1–3] and concanavalin A[4,5] have been examined in detail by use of various glycopeptides as hapten inhibitors. Recently, Kornfeld *et al.*[6], and Debray *et al.*[7] reported that an α-L-fucosyl group attached to the asparagine-linked 2-acetamido-2-deoxy-β-D-glucopyranosyl residue through a (1→6) linkage is an important determinant in binding to lentil and *Pisum sativum* (pea) lectin, but not to concanavalin A. Contrary to our expectation, however, we did not observe binding to a column of lentil- or pea-lectin–Sepharose, of a sodium borohydride-reduced, complex-type oligosaccharide having an L-fucosyl group linked to a terminal 2-acetamido-2-deoxy-D-glucitol residue. In this paper, we describe the structural requirements for the binding of oligosaccharides and glycopeptides to immobilized lentil- and pea-lectin columns.

EXPERIMENTAL

Lectins and lectin columns. — Concanavalin A was purified from jack-bean

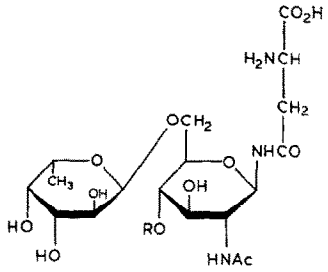
meal which was purchased from Sigma Chemical Co. (St. Louis, MO 14508) according to the method of Agrawal and Goldstein[8]. Lentil lectin used in this study was isolated by affinity chromatography as described by Toyoshima *et al.*[9]. Pea lectin was purified from common garden-pea seeds by affinity chromatography on Sephadex G-75 as described by Entlicher *et al.*[10]. These lectins were coupled to Sepharose 4B according to the method of Matsumoto *et al.*[11]. The amount of lectin bound to the Sepharose gel was estimated, in all three cases, to be ~4.5–5.0 mg/mL of gel by subtracting the amounts of the unbound lectin in the supernatant solution and in the wash after the coupling reaction from the amount of starting material.

Affinity chromatography on a lectin–Sepharose column. — The radioactively-labeled sample (2–5 × 10^3 c.p.m., 0.1–0.25 nmol) in a volume of 50 μL was applied to a lectin–Sepharose column (1.5 mL) equilibrated with an appropriate buffer and kept for 1 h at room temperature. The column was eluted with the same buffer, and then with 0.1M methyl α-D-mannopyranoside in the same buffer, at a flow rate of 3 mL/h, and fractions (1.5 mL) were collected. The buffer used for pea lectin–Sepharose and lentil lectin–Sepharose was 10mM sodium phosphate buffer, pH 7.3, containing 0.15M sodium chloride (PBS); and sodium acetate buffer, pH 6.0, containing 0.15M sodium chloride, mM manganese chloride, and mM calcium chloride was used for concanavalin A–Sepharose.

Glycopeptides (**1** *and* **2**) *and oligosaccharides* (**3** *and* **4**). — Glycopeptides **1** and **2**, and oligosaccharides **3** and **4** were prepared from porcine thyroglobulin as described previously[12,13]. The structures of these standard glycopeptides and oligosaccharides were confirmed to be as reported in the original papers by a compositional analysis and methylation study.

Labeling of glycopeptides and oligosaccharides. — The radioactive label was introduced into glycopeptides **1** and **2** by acetylation with [^{14}C]acetic anhydride (30.0 mCi/mmol, Radiochemical Centre, Amersham, England) by the method of Tai *et al.*[14]. The labeled glycopeptide was purified by gel filtration in a column of Sephadex G-25. Oligosaccharides **3** and **4** were labeled at the reducing terminal residue by reduction with sodium borotritide (250 mCi/mmol, New England Nuclear, Boston, MA 02118) by the method of Takasaki and Kobata[15] to give **5** and **6**, respectively. Nonreducing, terminal D-galactopyranosyl groups of glycopeptides were tritiated as follows: the glycopeptide (1 mg) was incubated with D-galactose oxidase from *Dactylium denroides* (25 units) in PBS (0.4 mL) for 18 h at 37°. Sodium borotritide (2.5 mCi) was added, and the mixture kept for 3 h at room temperature, followed by complete reduction with sodium borohydride (5 mg). After separation of the labeled glycopeptides by gel filtration in a column of Sephadex G-25 (1.5 × 100 cm), hydrazinolysis was performed according to the method of Fukuda *et al.*[16]. The D-galactose-labeled oligosaccharides released by hydrazinolysis were purified by gel filtration in a column of Sephadex G-25, and affinity chromatography in a column of concanavalin A–Sepharose.

Modification of glycopeptides and oligosaccharides. — α-D-Mannosidase, β-D-galactosidase, and *N*-acetyl-β-D-hexosaminidase were purified from jack-bean meal



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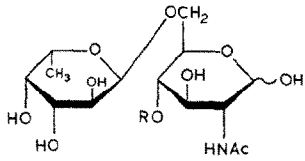

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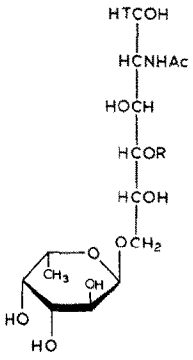

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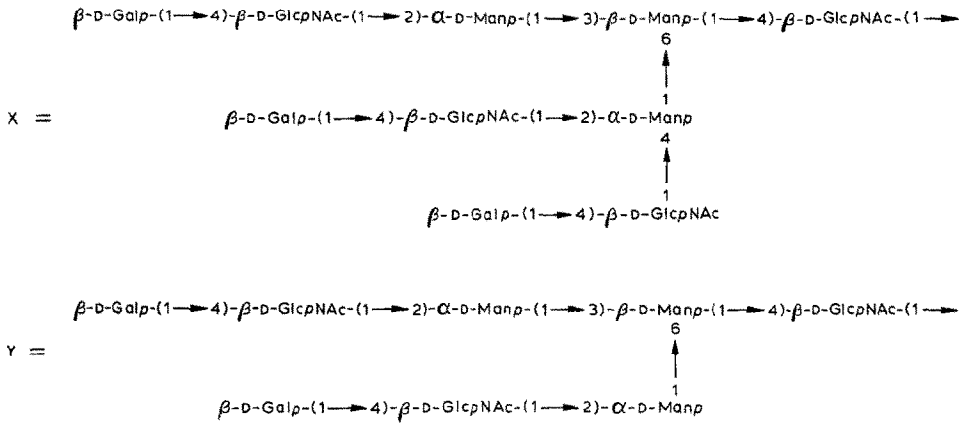

(Sigma Chemical Co.) by the method of Li and Li[17]. *N*-Acetylneuraminidase from *Arthrobacter ureafaciens* was purchased from Nakarai Chemical Co. (Kyoto, Japan). Glycopeptides and oligosaccharides (**1–4**) were digested for 24–48 h at 37° with a glycosidase (50 mu.) in an appropriate buffer (0.1 mL) under a toluene layer, followed

by heating for 3 min at 100° to stop the reaction. Then, each reaction mixture was passed through small columns of Dowex 50W-X8 (H^+, 0.8 mL) and Bio-Rad AG-3 (OH^-, 0.8 mL) ion-exchange resins. Recoveries of the modified glycopeptides were usually more than 90% of the theoretical values. Digestions with β-D-galactosidase, *N*-acetyl-β-D-hexosaminidase, and α-D-mannosidase were carried out in 50mM sodium acetate buffer (pH 4.0).

RESULTS AND DISCUSSION

The elution profiles from lentil- and pea-lectin–Sepharose columns of glycopeptide **2**, prepared from porcine thyroglobulin, are shown in Fig. 1. This glycopeptide bound to both lectin-Sepharose columns and could be eluted with 50mM methyl α-D-mannopyranoside. To remove the asparagine residue from **2**, the glycopeptide

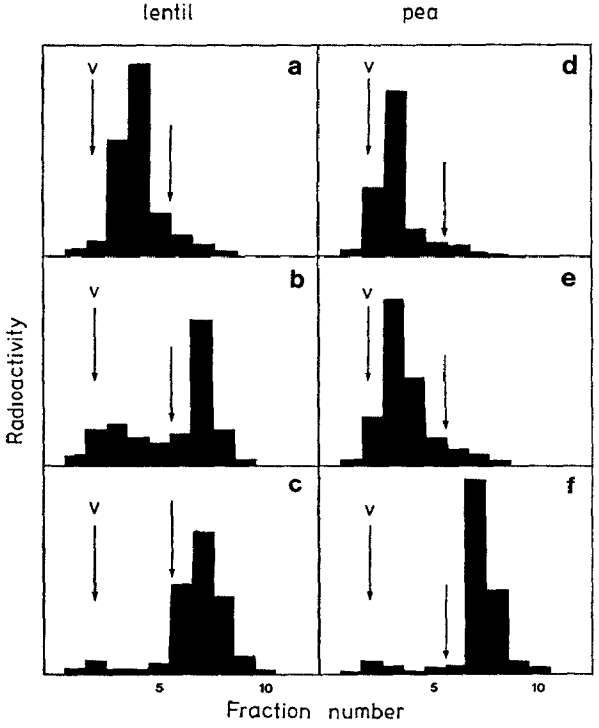

Fig. 1. Elution profiles of porcine thyroglobulin **2** glycopeptide, its D-galactose-labeled oligosaccharide (**4**), and its sodium borotritide-reduced alditol **6** on columns of lentil lectin–Sepharose and pea lectin–Sepharose. Experimental details are given in the text. Elution was performed with 10mM phosphate buffer, pH 7.3, containing 0.15M sodium chloride, and then with the same buffer containing 50mM methyl α-D-mannopyranoside. An arrow (V) indicates the void-volume fraction, and the other arrow indicates the position where the buffer was changed. Panels a, b, and c are the elution profiles for a lentil lectin–Sepharose column, and panels d, e, and f are the elution profiles for a pea lectin–Sepharose column: a and d, compound **6**; b and e, D-galactose-labeled oligosaccharide **4** from **2**; c and f, compound **2**.

was subjected to hydrazinolysis and the reducing 2-acetamido-2-deoxy-β-D-glucopyranose residue of the resulting oligosaccharide was labeled by reduction with sodium borotritide. The sodium borotritide-reduced oligosaccharide thus prepared bound to neither column (Fig. 1a and 1d), whereas it did bind to a column of concanavalin A–Sepharose. These results indicate that both lentil and pea lectin–Sepharose require an intact reducing 2-acetamido-2-deoxy-β-D-glucopyranosyl residue or an asparagine residue for high-affinity binding, whereas concanavalin A–Sepharose does not. In order to clarify the structural requirements for high-affinity binding of glycopeptides and oligosaccharides to these columns, a labeled oligosaccharide having tritiated D-galactose groups at the nonreducing terminals and a nonreduced 2-acetamido-2-deoxy-β-D-glucopyranosyl residue at the reducing terminal was prepared from glycopeptide **2** by hydrazinolysis, and applied to the lectin columns. Panels b and e of Fig. 1 show the elution profiles of the oligosaccharide. Most of the radioactivity was retained on the lentil lectin–Sepharose column, but it was not retained on the pea lectin–Sepharose column. When the reducing, terminal 2-acetamido-2-deoxy-β-D-glucopyranosyl residue of this oligosaccharide was reduced with sodium borohydride,

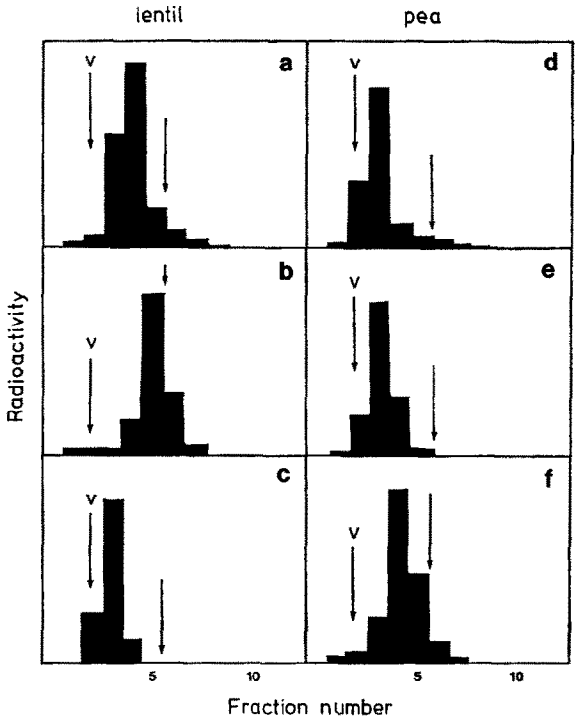

Fig. 2. Elution profiles of sodium borotritide-reduced alditol **6** from **2**, and of its exoglycosidase digests on columns of lentil lectin–Sepharose and pea lectin–Sepharose. Experimental details and symbols are the same as in the legend to Fig. 1. Panels a, b, and c are the elution profiles for a lentil lectin–Sepharose column, and panels d, e, and f are the elution profiles for a pea lectin–Sepharose column: a and d, compound **6**; b and e, compound **6** treated with β-D-galactosidase, c and f, compound **6** treated with a mixture of β-D-galactosidase and *N*-acetyl-β-D-hexosaminidase.

it showed the same elution profiles as shown in panels a and d of Fig. 1. However, a considerable proportion of the nonreduced oligosaccharide was not retained on the lentil-lectin column. The possible explanation for this observation may be that a part of the reducing, terminal 2-acetamido-2-deoxy-D-glucopyranosyl residues of the oligosaccharide preparation exists in the open-chain form or in the α-D-anomeric configuration. These results indicate that an intact 2-acetamido-2-deoxy-β-D-glucopyranosyl residue at the reducing terminal is essential for the high-affinity binding of a complex-type oligosaccharide to a lentil lectin–Sepharose column, and an asparagine residue possibly contributes to the binding of a complex-type glycopeptide to a pea lectin–Sepharose column. Furthermore, the tribranched, complex-type glycopeptide **1**, prepared from porcine thyroglobulin, was not retained by either lectin–Sepharose columns (data not shown) as it was on a concanavalin A–Sepharose column[13,20,21]. This result indicates that both lentil- and pea-lectin–Sepharose, like concanavalin A–Sepharose, may require two α-D-mannopyranosyl residues having free HO-3, -4, and -6 for tight binding.

To examine further the contribution of the outer chain sugar residues of oligosaccharides to the binding to lentil- and pea-lectins, a series of modified oligosaccharides was tested. Fig. 2 shows the elution profiles of these modified oligosaccharides from lentil- and pea-lectin–Sepharose columns. Removal of the β-D-galactopyranosyl groups from the branches of the oligosaccharide slowed its elution from the lentil lectin–Sepharose, but not from the pea lectin–Sepharose column. Further treatment of the oligosaccharide with *N*-acetyl-β-D-hexosaminidase decreased the interaction with lentil lectin–Sepharose, whereas this treatment resulted in enhanced retardation of the oligosaccharide on pea lectin–Sepharose (Fig. 2f). These results are quite reproducible and indicate that the presence of a nonreducing, terminal 2-acetamido-2-deoxy-β-D-glucopyranosyl group enhances the oligosaccharide interaction with a lentil lectin–Sepharose column, whereas the exposure of a nonreducing, terminal α-D-mannopyranosyl group enhances the interaction with a pea lectin–Sepharose column. These conclusions are in line with our previous observations[18,19] for hemagglutination–inhibition assays using synthetic oligosaccharides as inhibitors.

Lectins considered to be identical in terms of their specificity toward monosaccharides can often recognize subtle differences in the structures of oligosaccharides and glycopeptides. Therefore, after elucidation of the precise structural requirements of immobilized lectins for the binding of oligosaccharides and glycopeptides, those immobilized lectins will be useful tools for the fractionation and structural assessment of oligosaccharides and glycopeptides.

ACKNOWLEDGMENTS

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NOTE ADDED IN PROOF

A two-branched, complex-type glycopeptide having a 2-acetamido-2-deoxy-β-D-glucopyranosyl residue linked at O-4 of the β-D-mannopyranosyl residue {β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→3)-[β-D-GlcNAc-(1→4)]-[β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→6)]-β-D-Man*p*-(1→4)-β-D-Glc*p*NAc-(1→4)-[α-L-Fuc-(1→6)]-GlcNAc}, obtained from human glycophorin A, bound to both lentil- and pea-lectin-Sepharose columns.

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