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STRUCTURAL REQUIREMENTS FOR THE BINDING OF GLYCOPEPTIDES TO IMMOBILIZED *Vicia faba* (FAVA) LECTIN*

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ABSTRACT

The structural requirements for the binding of oligosaccharides and glycopeptides to immobilized *Vicia faba* agglutinin were investigated, and its carbohydrate specificity was compared with the specificities of the α-D-mannose-binding lectins concanavalin A, *Lens culinaris* agglutinin, and *Pisum sativum* agglutinin. Immobilized *Vicia faba* agglutinin can interact only with biantennary glycopeptides possessing an α-L-fucosyl group attached to the L-asparagine-linked 2-acetamido-2-deoxy-β-D-glucopyranosyl residue. An L-asparagine residue is essential for high affinity-binding to *Vicia faba* agglutinin–Sepharose. In addition, the enzymic exposure of a (nonreducing) terminal α-D-mannosyl group enhances the interaction of the glycopeptide with immobilized *Vicia faba* agglutinin. This sugar-binding specificity of immobilized *Vicia faba* agglutinin is similar to that of immobilized *Pisum sativum* agglutinin.

INTRODUCTION

The use of plant lectins in the structural and functional characterization of membrane glycoconjugates is widespread in many areas of biological research[1,2]. Immobilized lectins have been used for purification and fractionation of various glycoproteins and glycopeptides, and their elution profiles give valuable information on the carbohydrate structures[3]. The sugar-binding specificities have been investigated of several immobilized lectins, such as concanavalin A–Sepharose[4,5], *Lens culinaris* agglutinin–Sepharose[6,7], *Pisum sativum* agglutinin–Sepharose[6,7], wheat-germ agglutinin–Sepharose[8], pokeweed mitogen–Sepharose[9], and *Phaseolus vulgaris* agglutinin–Sepharose[10]. *Vicia faba* lectin purified from broad bean, concanavalin A, *Lens culinaris* agglutinin, and *Pisum sativum* agglutinin all have similar monosaccharide specificities, and may be classified as α-D-mannose-

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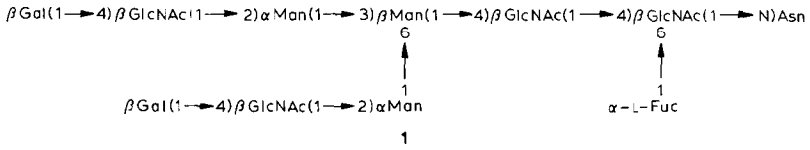
binding lectins. The interactions between the sugar chains of glycoproteins and these lectins, except for *Vicia faba* lectin, have been well investigated.

We now describe the structural requirements for the binding of the glycopeptides and oligosaccharides to immobilized *Vicia faba* agglutinin, and compare its specificity with the specificities of the α-D-mannose-binding lectins just mentioned.

EXPERIMENTAL

Preparation of lectin–Sepharose 4B. — Concanavalin A from jack-bean meal



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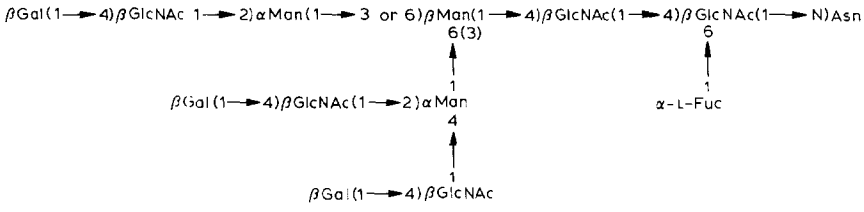

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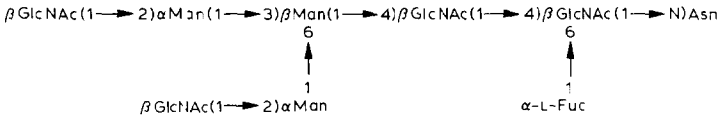

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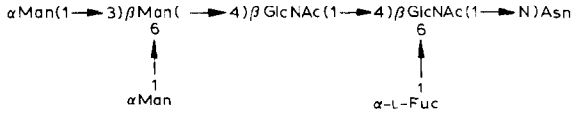

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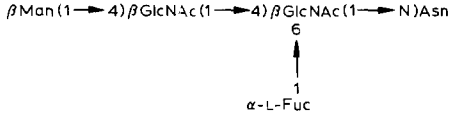

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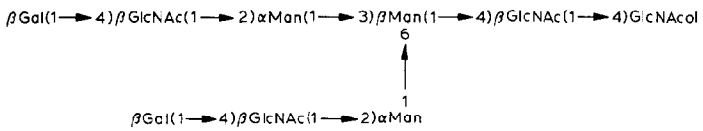

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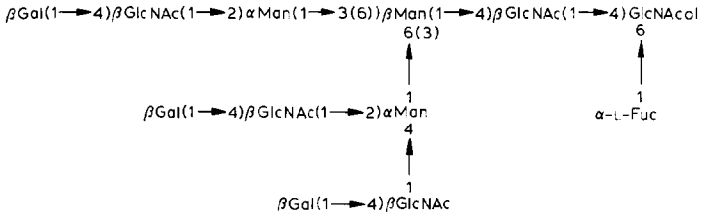

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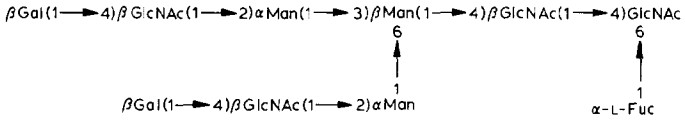

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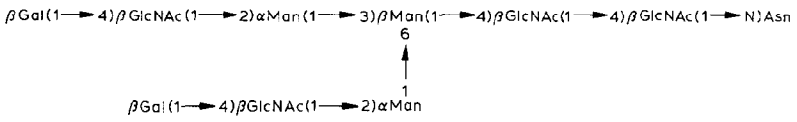

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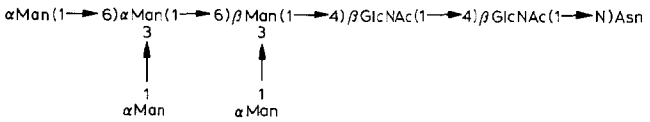

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(Sigma Chemical Co.) was purified according to the method of Agrawal and Goldstein[11]. *Lens culinaris* agglutinin was isolated by affinity chromatography as described by Toyoshima *et al.*[12]. *Pisum sativum* agglutinin was purified from common garden-pea seeds by affinity chromatography on Sephadex G-75 according to the method of Entlicher *et al.*[13]. These lectins were coupled to Sepharose 4B as described by Matsumoto *et al.*[14]. *Vicia faba* agglutinin was purified by affinity chromatography according to the method of Allen *et al.*[15] and Neuberger *et al.*[16]. *Vicia*

faba agglutinin–Sepharose 4B was prepared by using hydrazide–Sepharose 4B as described by Matsumoto *et al.*[17]. The amounts of lectins bound to Sepharose were estimated to be ~4.0 mg/mL of gel, by subtracting the amount of unbound protein in the supernatant liquor and washing solutions after the coupling reaction. Protein was determined by the method of Lowry *et al.*[18].

Oligosaccharides and glycopeptides. — Porcine thyroglobulin glycopeptides and oligosaccharides were prepared according to the method of Tsuji *et al.*[19] and Yamamoto *et al.*[20]. The structures of these standard glycopeptides and oligosaccharides, confirmed by a compositional analysis and methylation study to be as originally reported, are shown in formulas **1–10**.

Labeling of glycopeptides and oligosaccharides. — Glycopeptides were radioactively labeled by acetylation with [^{14}C]acetic anhydride (30 mCi/mmol; Amersham, England) according to the method of Tai *et al.*[21]. The labeled glycopeptides were purified by gel filtration on a column of Sephadex G-25. Oligosaccharides were labeled at the reducing-terminal residue by reduction with sodium borotritide (250 mCi/mmol, New England Nuclear, Boston, MA, U.S.A.) according to the method of Takasaki and Kobata[22].

Oligosaccharide **8** labeled at the (nonreducing) terminal D-galactosyl groups was prepared as follows. Glycopeptide **1** (1 mg) was incubated with D-galactose oxidase from *Dactylium denroides* (25 units; from Worthington, Freehold, N.J., U.S.A.) in 10mM sodium phosphate buffer, pH 7.2, containing 0.15M sodium chloride (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 purification of the labeled glycopeptide by gel filtration on a column of Sephadex G-25, hydrazinolysis was performed according to the method of Fukuda *et al.*[23]. The D-galactose-labeled oligosaccharide **8** released by hydrazinolysis was purified by gel filtration on a column of Sephadex G-25, and by affinity chromatography on a column of concanavalin A–Sepharose.

Modification of glycopeptides. — α-D-Mannosidase, β-D-galactosidase, and *N*-acetyl-β-D-hexosaminidase were purified from jack-bean meal (Sigma Chemical Co.) by the method of Li and Li[24]. Neuraminidase from *Arthrobacter ureafaciens* was purchased from Nakarai Chemicals Co. (Kyoto, Japan), and α-L-fucosidase (from *Charonia lampas)* from Seikagaku Kogyo Co. (Tokyo, Japan). *N*-[^{14}C]Acetylated glycopeptides (5000 c.p.m., 0.2 nmol) were incubated for 24–48 h at 37° with a glycosidase (50 munit) in an appropriate buffer. The products were isolated as described by Yamashita *et al.*[25]. Digestion with β-D-galactosidase, *N*-acetyl-β-D-hexosaminidase, and α-D-mannosidase was conducted in 50mM sodium acetate buffer, pH 4.0. For digestion with α-L-fucosidase, 0.1M sodium citrate–0.1M sodium phosphate buffer, pH 4.0, containing 0.5M sodium chloride, was used.

Affinity chromatography on columns of lectin–Sepharose 4B. — Radioactively labeled sample (2000–5000 c.p.m.; 10–20 μL, 0.1–0.25 nmol) was applied to a column (0.5 × 7.0 cm) of a lectin–Sepharose 4B that had been equilibrated with 10mM phosphate buffer, pH 7.2, containing 0.15M sodium chloride. After 1 h at

22°, the column was eluted with the same buffer, and then with several concentrations of methyl α-D-mannopyranoside in the same buffer, at a flow rate of 2 mL/h, 0.2-mL fractions being collected. Recovery of radioactivity was always >95%.

RESULTS AND DISCUSSION

Fig. 1. shows the elution profiles obtained from the *Vicia faba* agglutinin–Sepharose column chromatography of complex-type glycopeptides and oligosaccharides (**1**, **2**, **6**, and **7**) prepared from porcine thyroglobulin. Glycopeptide **1**, which is a biantennary, complex type of glycopeptide, was found to be significantly retarded on the column (see Fig. 1A). However, triantennary glycopeptide **2** did not interact with the column (see Fig. 1B). This indicates that immobilized *Vicia faba* agglutinin can distinguish the branching pattern of α-D-mannosyl residues, as can immobilized concanavalin A.

In order to elucidate the contribution to the interaction of sugar residues in the outer chain of the glycopeptides with a *Vicia faba* agglutinin–Sepharose column, a series of glycopeptides modified by treatments with various exo-glycosidases was tested. Fig. 2 shows the elution profiles of the modified glycopeptides from a *Vicia faba* agglutinin–Sepharose column. Removal of the β-D-galactopyranosyl groups from the branches of glycopeptide **1** enabled it to be retained on the column (see Fig. 2B). Further treatment of the glycopeptide with *N*-acetyl-

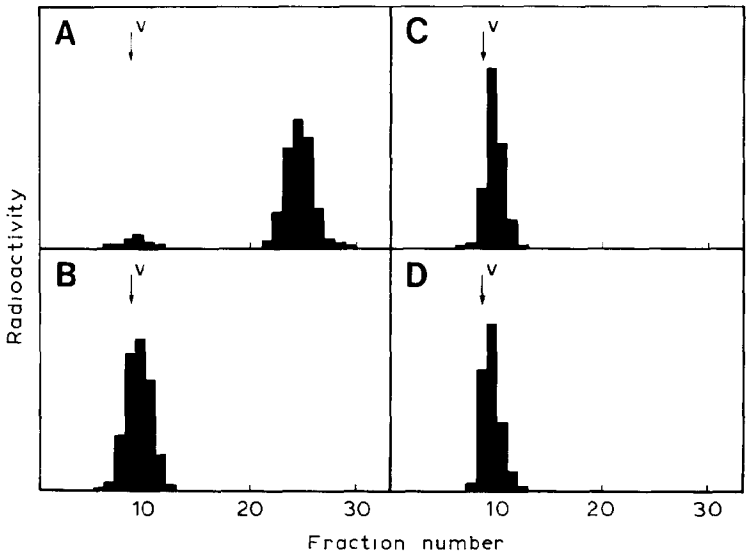

Fig. 1. Affinity chromatography of complex-type glycopeptides and oligosaccharide-alditols on a column of *Vicia faba* agglutinin–Sepharose 4B. [Experimental details are given in the text. Elution was performed with 10mM sodium phosphate buffer, pH 7.2, containing 0.15M sodium chloride. Arrow (V) indicates the void-volume fraction. Elution profiles of: (A) glycopeptide **1**, (B) glycopeptide **2**, (C) oligosaccharide-alditol **6**, and (D) oligosaccharide-alditol **7**].

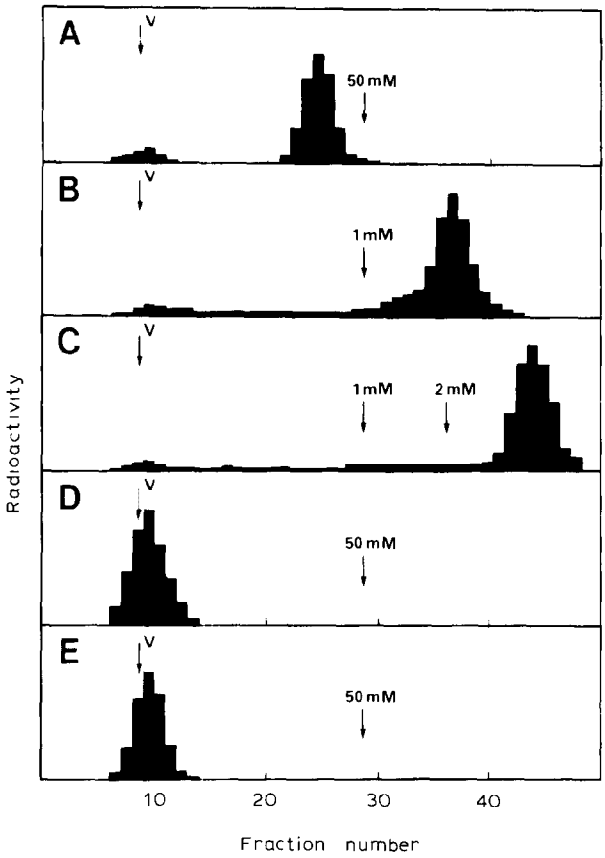

Fig. 2. Affinity chromatography of glycopeptide **1** and its glycosidase digests on a column of *Vicia faba* agglutinin–Sepharose 4B. [Experimental details are given in the text. Elution was performed with 10mM sodium phosphate buffer, pH 7.2, containing 0.15M sodium chloride, and then with portions of the same buffer containing several concentrations of methyl α-D-mannopyranoside. Arrow (V) indicates the void-volume fraction, and other arrows indicate the positions where the buffer was changed. Elution profiles of: (A) glycopeptide **1**, (B) glycopeptide **3**, (C) glycopeptide **4**, (D) glycopeptide **5**, and (E) glycopeptide **9**.]

β-D-hexosaminidase enhanced its affinity for *Vicia faba* agglutinin–Sepharose. The resulting glycopeptide **4** was also bound by the column, but was eluted when the concentration of methyl α-D-mannopyranoside in the buffer was increased to 2mM (see Fig. 2C). However, when this glycopeptide was treated with α-D-mannosidase, its reactivity with the column was abolished (see Fig. 2D). These results indicate that the enzymic exposure of a (nonreducing) terminal α-D-mannopyranosyl group enhances the interaction of the glycopeptide with *Vicia faba* agglutinin–Sepharose.

We previously reported that an L-asparagine residue is essential for the binding of a complex-type glycopeptide to immobilized *Pisum sativum* agglutinin, and

that an intact 2-acetamido-2-deoxy-β-D-glucopyranosyl residue at the potentially reducing terminal of a complex-type oligosaccharide was required for the binding to immobilized *Lens culinaris* agglutinin[7]. We attempted to clarify the structural requirements of the core portion of the glycopeptides for high-affinity binding to the column. As to the complex-type oligosaccharide-alditols, obtained by hydrazinolysis of the glycopeptides followed by reduction with sodium borotritide, neither biantennary nor triantennary oligosaccharide-alditols (**6** and **7**) were bound to the column (see Figs. 1C and 1D). This phenomenon was also observed in the case of immobilized *Lens culinaris* agglutinin and *Pisum sativum* agglutinin.

The contribution of the L-asparagine residue to the interaction was then examined. A radioactively labeled oligosaccharide possessing an intact 2-acetamido-2-deoxy-β-D-glucopyranosyl residue at the potentially reducing terminal was prepared from glycopeptide **1** by the D-galactose oxidase–NaB^3H_4 method, followed by treatment with hydrazine, and then subjected to affinity chromatography on a column of *Vicia faba* agglutinin–Sepharose. This oligosaccharide (**8**) was eluted, without retardation, from the column (data not shown). This indicates that an L-asparagine residue probably contributes to the binding of a complex-type glycopeptide to *Vicia faba* agglutinin–Sepharose, as in the case of *Pisum sativum* agglutinin–Sepharose.

Recently, Kornfeld *et al.* reported[6] that an α-L-fucosyl group attached to the L-asparagine-linked 2-acetamido-2-deoxy-β-D-glucopyranosyl residue is an important determinant in the binding of glycopeptides to immobilized *Lens culinaris* agglutinin and *Pisum sativum* agglutinin. To evaluate the contribution to the interaction with *Vicia faba* agglutinin–Sepharose of the α-L-fucosyl group in the complex-type glycopeptide, $Gal_2(GlcNAc)_2Man_3(GlcNAc)_2$-Asn (**9**), prepared from glycopeptide **1** by treatment with α-L-fucosidase, was applied to the column. As shown in Fig. 2E, this glycopeptide failed to interact with the column. Even after glycopeptide **9** had been treated with β-D-galactosidase, or with β-D-galactosidase and *N*-acetyl-β-D-hexosaminidase, the products did not bind to the column (data not shown). Therefore, an α-L-fucosyl group attached to the L-asparagine-linked 2-

TABLE I

INTERACTIONS BETWEEN VARIOUS GLYCOPEPTIDES AND OLIGOSACCHARIDES AND FOUR IMMOBILIZED α-D-MANNOSE-BINDING LECTINS

Immobilized lectins	*Glycopeptides and oligosaccharides examined*									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Concanavalin A	m[a]	—[a]	m	m	—	m	—	m	m	m
Lens culinaris	w[a]	—	s[a]	m	—	—	—	w	—	—
Pisum sativum	w	—	m	s	—	—	—	—	—	—
Vicia faba	w	—	m	s	—	—	—	—	—	—

[a]Key: m, moderate interaction; s, strong interaction; w, weak interaction; —, no interaction.

acetamido-2-deoxy-β-D-glucopyranosyl residue is an essential determinant in the binding of the glycopeptides to *Vicia faba* agglutinin–Sepharose. This conclusion was supported by the observation that high-D-mannose-type glycopeptides, $Man_{5-9}(GlcNAc)_2Asn$ (such as **10**), which are prepared from porcine thyroglobulin and do not contain the core L-fucosyl group, showed no interaction with a *Vicia faba* agglutinin–Sepharose column, although they have α-D-mannosyl groups on the nonreducing end (data not shown).

In Table I are summarized the interactions of the various glycopeptides and oligosaccharides (**1** to **10**) with *Vicia faba* agglutinin–, concanavalin A–, *Lens culinaris* agglutinin–, and *Pisum sativum* agglutinin–Sepharose. In this Table, the strength of interactions between glycopeptides or oligosaccharides and an immobilized lectin is given with reference to the individual lectin only, and is not defined across the four lectins. However, differences in the carbohydrate specificities among these immobilized lectins may be summed up as follows. (*1*) Concanavalin A, *Lens culinaris* agglutinin, *Pisum sativum* agglutinin, and *Vicia faba* agglutinin–Sepharose recognize biantennary oligosaccharides and glycopeptides. Triantennary oligosaccharides and glycopeptides that have α-D-linked outer D-mannopyranosyl residues substituted at O-2 and O-4 pass through these lectin columns. (Those having α-D-linked outer D-mannopyranosyl residues substituted at O-2 and O-6 were not tested in this study.) (*2*) An α-L-fucosyl group attached to the L-asparagine-linked 2-acetamido-2-deoxy-β-D-glucopyranosyl residue by a (1→6) linkage is an essential determinant in binding to *Lens culinaris* agglutinin, *Pisum sativum* agglutinin, and *Vicia faba* agglutinin, but not to concanavalin A. (*3*) An intact 2-acetamido-2-deoxy-β-D-glucopyranosyl residue at the reducing terminal of a complex-type oligosaccharide is essential for high-affinity binding to *Lens culinaris* agglutinin–Sepharose. An L-asparagine residue is also required for the binding of a complex-type glycopeptide to *Pisum sativum* agglutinin– and *Vicia faba* agglutinin–Sepharose. (*4*) The interaction of a complex-type oligosaccharide with *Lens culinaris* agglutinin–Sepharose is enhanced by the enzymic exposure of nonreducing-terminal 2-acetamido-2-deoxy-β-D-glucopyranosyl groups, whereas its interaction with *Pisum sativum* agglutinin– and *Vicia faba* agglutinin–Sepharose is enhanced only after the exposure of nonreducing-terminal α-D-mannopyranosyl residues.

Many studies have demonstrated the value of lectin-affinity chromatography for fractionation of glycopeptides and oligosaccharides. Lectins considered to be identical in terms of their monosaccharide specificities can often recognize subtle differences in the structures of glycopeptides and oligosaccharides. Therefore, the precise structural requirements of immobilized lectins for the binding of glycopeptides and oligosaccharides should be elucidated. The use of serial, lectin-affinity chromatography would provide an extremely simple and sensitive technique for the purification, and structural determination, of glycoconjugates.

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