Pure & Appl. Chem., Vol. 61, No. 7, pp. 1283–1292 1989.
Printed in Great Britain.
© 1989 IUPAC

Recent progress in the application of plant lectins to glycoprotein chemistry

Toshiaki Osawa

Division of Chemical Toxicology and Immunochemistry, Faculty of Pharmaceutical Sciences, University of Tokyo Bunkyo-ku, Tokyo 113, Japan

Abstract - Serial affinity chromatography by the use of immobilized lectin columns whose binding specificities have been precisely determined is a very sensitive and rapid method for the fractionation and structural assessment of oligosaccharides and glycopeptides. In this article, a possible scheme for separating a mixture of asparagine-linked glycopeptides is discussed with reference to the recent knowledge about the sugar-binding specificity of each lectin column.

INTRODUCTION

A plant hemagglutinin was first discovered in the seeds of Ricinus communis (castor beans) by Stillmark in 1888 (ref. 1). Afterwards a number of hemmagglutinins were found in various plant seeds, but they were generally considered to be nonspecific materials agglutinating animal erythrocytes irrespective of species and blood types. Boyd and Reguera (ref. 2) and Renkonen (ref. 3) undertook systematic screening of the extracts of hundreds kinds of plant seeds for blood group-specific agglutinating activity and discovered many blood group specific plant hemagglutinins. These discoveries led to the general concept that each plant hemagglutinin binds specifically to a certain structure on erythrocytes. Boyd gave the term "lectin" to these hemagglutinins (ref. 4). In 1952, Watkins and Morgan first reported that simple sugars are capable of inhibiting blood group specific lectins (ref. 5), and later, Mäkelä divided simple sugars into four groups based on the configuration of the hydroxyl groups at C-3 and C-4 of the simple sugars and indicated the lectins that could be inhibited by each group of sugars (ref. 6). Since then, terms such as D-galactose-binding lectins, D-mannose-binding lectins and L-fucose-binding lectins have been widely used. Typical lectins which are assigned to each group are listed in Table 1.

It has been almost 20 years since the importance of the studies on molecular mechanisms of various cell sociological phenomena began to be emphasized, and a number of recent studies suggested that glycoconjugates on the cell surface account for a great number of surface markers of the cells in various differentiation stages and with various functions. Furthermore, it has become apparent that these cell-surface markers control and determine cell-ligand and cell-cell interactions in many important cellular phenomena. In order to clarify the molecular mechanisms of these cellular phenomena and to study the changes in cell-surface characteristics during differentiation, elucidation of the structure of the sugar chains of these membrane glycoconjugates is indispensable. However, in most cases, membrane glycoconjugates are very difficult to isolate in sufficient quantities for structural studies by conventional methods. Moreover, these glycoconjugates usually exhibit microheterogeneity in the structure of their carbohydrate moieties.

Since each lectin binds specifically to a certain sugar structure, these lectins have been widely used for histochemical detection of sugars on the cell surface. In these studies, the results have often been correlated simply with the specificities of lectins disclosed by hapten inhibition assays with simple sugars used as inhibitors. However, the results of such assays cannot always be correlated with the structure of the binding sites on the cell surface.

We can divide the structure of the sugar chains of cell surface glycoproteins into two groups termed serine (or threonine)-linked sugar chains (mucin-type sugar chains) and asparagine-linked sugar chains. The latter group is further subdivided into three groups termed the high-mannose-type, complex-type and hybrid-type. Typical examples of these sugar chains are shown in Fig. 1.

From the results of hemagglutination-inhibition assays using various simple sugars and oligosaccharides as hapten inhibitors (refs. 7-10), we divided the lectins into three groups based on the type of sugar chains to which they preferentially bind as shown in Table 2.

TABLE 1. Classification of lectins based on simple inhibitory sugars

L-Fucose-binding lectins	Eel serum	Anti-H(O)
	Lotus tetragonolobus	
	Ulex europeus I	
	Aleuria aurantia	Blood group nonspecific
D-Galactose-binding lectins (including GalNAc-binding lectins)	Arachis hypogaea (peanut)	
	Ricinus communis (castor bean)	
	Sophora japonica	
	Bauhinia purpurea	
	Glycine mas (soybean)	
	Phaseolus vulgaris (red kidney bean)	
	Wistaria floribunda	
D-Mannose-binding lectins	Concanavalin A	
	Lens culinaris (lentil)	
	Pisum sativum (pea)	
	Vicia fava (fava bean)	
Di-N-acetylchitobiose-binding lectins	Cytisus sessilifolius	Anti-H(O)
	Laburnum alpinum	
	Ulex europeus II	
	Solanum tuberosum (potato)	Blood group nonspecific
	Wheat germ	
	Phytolacca americana (pokeweed)	
	Tomato	
	Datura stramonium	

Even among the same group of lectins in Mäkelä's classification (ref. 6), differences can be seen in the type of sugar chains to which they preferentially bind. For example, in the case of galactose-binding lectins, Agaricus bisporus (mushroom) lectin, Arachis hypogaea (peanut) lectin and Bauhinia purpurea lectin bind primarily to mucin-type sugar chains, whereas Ricinus communis lectin binds primarily to complex-type or hybrid-type sugar chains.

Recent investigations on binding specificities of various lectins indicate that even the lectins in the same group in Table 2 often bind to different sugar sequences in the same sugar chain. Detailed information on the structure of the sugar sequence which a lectin preferentially recognizes can best be obtained by the use of affinity chromatography on a column on which the lectin is immobilized.

Furthermore, many attempts have recently been made to fractionate and purify oligosaccharides and glycoconjugates by affinity chromatography by the use of immobilized lectin columns. The use of a series of different lectin columns whose binding specificities have been precisely elucidated enables us to fractionate a very small amount of radiolabeled oligosaccharide or glycopeptide (ca 10 ng depending on the specific activity) into structurally distinct groups, and to obtain a general picture of their structure, which make the subsequent structural study much easier.

In this review, I summarize the recent knowledge about the sugar-binding specificities of various immobilized lectin columns and discuss the application of those immobilized lectin columns to the fractionation and the structural study of various oligosaccharides and glycopeptides.

LECTINS WHICH DISCRIMINATE THE TYPE OF SIALYLATED SEQUENCES ON GLYCOCONJUGATES

In many glycoconjugates sialic acid is a major terminal sugar, being bound to other sugars in a variety of linkages. The sialylated sequences most commonly found in glycoconjugates are: Siaα2-3Galβ1-4GlcNAc-, Siaα2-6Galβ1-4GlcNAc-, Siaα2-3Galβ1-3GlcNAc-, Siaα2-3Galβ1-3GalcNAc and Siaα2-6GalNAc-.

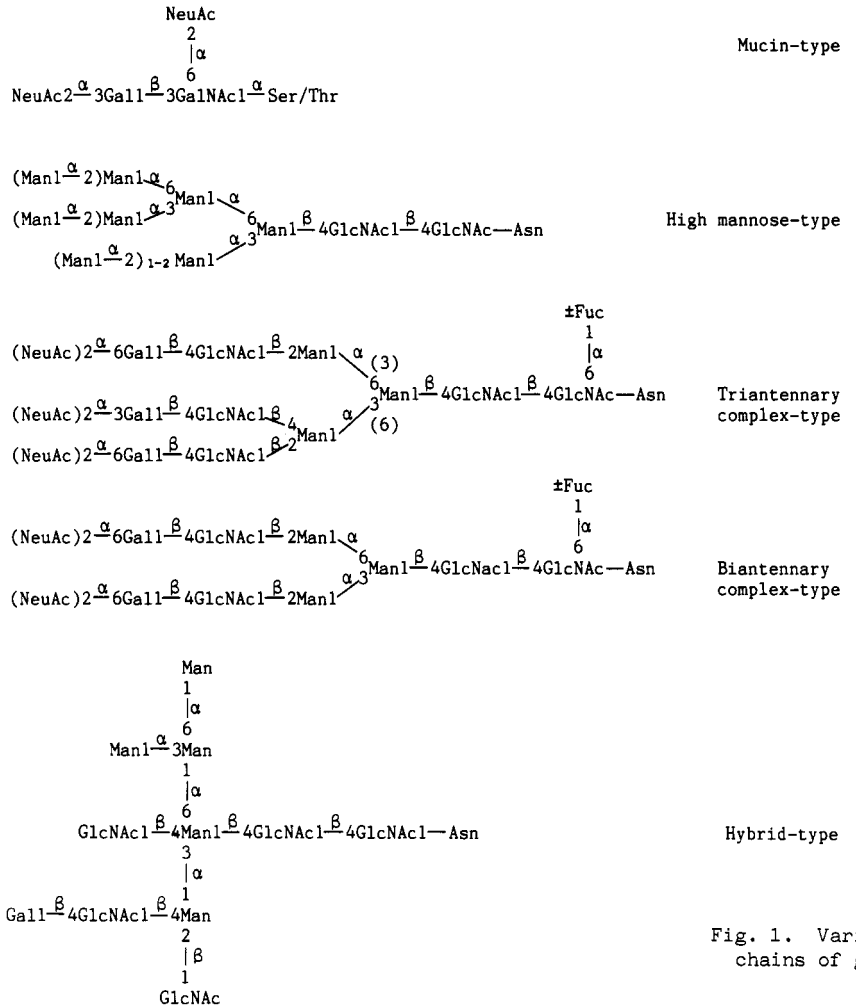

Fig. 1. Various types of sugar chains of glycoproteins

Sialic acid-binding lectins were found mainly in the hemolymph or sera of invertebrates such as the horseshoe crab Limulus polyphemus (ref. 11), the Indian horseshoe crab Carcinoscorpius rotunda cauda (refs. 12, 13), the slug Limax flavus (ref. 14), the snail Cepaea hortensis, (ref. 15), the marine crab Cancer antennarius (ref. 16) and the beetle Allomyrina dichotoma (ref. 17). Also among plant lectins, wheat germ agglutinin (refs. 18, 19), elderberry (Sambucus nigra L.) bark lectin (refs. 20, 21) and Maackia amurensis lectin (refs. 22, 23) are known to have strong affinity for sialic acid-containing sugar sequences.

However, most of them cannot discriminate among the types of sialylated sequences. Recently, affinity chromatography of two kinds of immobilized lectin columns was shown to be a very effective means of discriminating them. The immobilized lectin column prepared with elderberry bark hemagglutinin (SNA) binds oligosaccharides, glycopeptides or glycoproteins containing α2-6 linked sialic acid with high affinity, while those containing an α2-3-linked terminal sialic acid cannot bind to the column (ref. 21). Conversely, the lectin column prepared with the mitogenic Maackia amurensis leukoagglutinin (MAL) binds complex-type glycopeptides having an α2-3-linked terminal sialic acid residue, while the ones containing an α2-6-linked sialic acid cannot bind to the column (ref. 23). Although SNA binds O-linked (mucin-type) glycopeptides having the sialic acid α2-6Gal or GalNAc sugar sequence as well as complex-type glycopeptides with an α2-6-linked sialic acid, MAL cannot bind the sialylated O-linked glycopeptides even though they contain a sialic acid residue linked α2-3 to galactose.

As for the distinction between sialic acid-containing mucin-type and complex-type glycopeptides, an affinity column prepared with a lectin, allo-A, which was isolated from hemolymph of a beetle (Allomyrina dichotoma) (ref. 24) firmly binds oligosaccharides or glycopeptides carrying the sialylated Galβ1-4GlcNAc sugar sequence irrespective of whether the type of the sialylated sequence is α2-3 or α2-6, but this lectin column cannot bind mucin-type glycopeptides containing the sialylated Galβ1-3GalNAc sugar sequence. Among the β-galactose-binding lectins, Ricinus communis agglutinin I (RCA_1) has been most widely used for the separation of complex-type oligosaccharides or glycopeptides. However, RCA_1 can also bind to mucin-type oligosaccharides and glycopeptides (ref. 25). Therefore, allo-A is

TABLE 2. The types of sugar chains to which individual lectins preferentially binds

Type of sugar chian	Lectin	Simple sugar specificity
Ser/Thr-linked sugar chain (mucin-type chain)	Agaricus bisporus (mushroom)	Gal
	Arachis hypogaea (peanut)	Gal
	Bauhinia purpurea	Gal
	Iberis amara	Gal
	Maackia amurensis (hemagglutinin)	—
	Maclura pomifera	GalNAc
	Vicia graminae	Gal
	Vicia villosa	GalNAc
Asparagine-linked sugar chain	Concanavalin A	Man
	Datura stramonium	GlcNAc
	Lens culinaris (lentil)	Man
	Maackia amurensis (mitogen)	—
	Phaseolus vulgaris	GalNAc
	Phytolacca americana (pokeweed)	GlcNAc
	Pisum sativum (pea)	Man
	Ricinus communis	Gal
	Vicia faba (fava)	Man
	Wistaria floribunda (mitogen)	GalNAc
Either type of sugar chain	Sophora japonica	Gal
	Wheat germ	GlcNAc
	Wistaria floribunda (hemagglutinin)	GalNAc

a better tool for the separation of complex-type sugar chains, because most complex-type sugar chains are sialylated.

Phaseolus vulgaris (red kidney bean) erythroagglutinating and leukoagglutinating lectins are principally galactose-binding lectins for which the most essential structure for binding is the Galβ1-4GlcNAcβ1-2Man sugar sequence (ref. 9). However, Green and Baenziger (ref. 26) recently reported that both lectin columns retard oligosaccharides bearing α2→3- but not α2→6-linked sialic acid.

SEPARATION OF ASIALO ASPARAGINE-LINKED GLYCOPEPTIDES BY MEANS OF AFFINITY CHROMATOGRAPHY ON VARIOUS LECTIN COLUMNS

There are several types of asparagine-linked sugar chains as shown in Fig. 1, and in many cases a mixture of a variety of these sugar chains exists in the same glycoprotein molecule. Therefore, efficient methods for separating these asparagine-linked sugar chains are indispensable for elucidation of the structural and functional aspects of these sugar chains. In the following part of this article, a possible scheme for separating a mixture of asparagine-linked glycopeptides (Fig. 2) is discussed with reference to the detailed specificity of each lectin column to facilitate understanding the use of the lectin. In this scheme, all glycopeptides in Fig. 2 to be separated are N-[^{3}H]-acetylated asialo glycopeptides.

Lectins for step 1 - separation of biantennary complex-type, hybrid-type and high mannose-type glycopeptides from triantennary complex-type glycopeptides

The mixture of glycopeptides in Fig. 2 can be first subjected to affinity chromatography on a column of Con A-agarose. For the strong binding to the Con A-agarose column, at least two unsubstituted or 2-O-substituted α-mannopyranosyl residues are required (refs. 27-32). The presence of a bisecting GlcNAc group, β-linked 1-4 to the β-Man residue of the trimannosyl core, markedly weakens the binding to Con A-agarose. Thus, as shown in Fig. 2, the glycopeptides (IV-VII) possessing three peripheral Gal-GlcNAc branches linked to the core

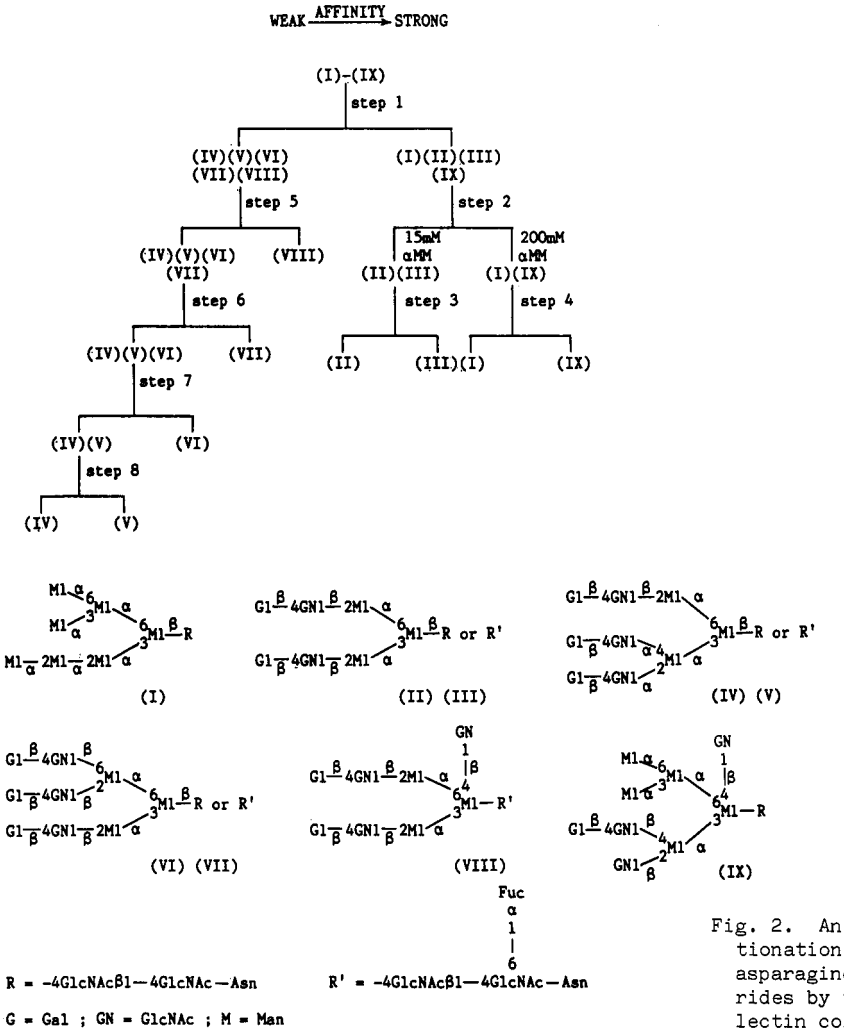

Fig. 2. An example of the fractionation of various types of asparagine-linked oligosaccharides by the use of immobilized lectin columns

(triantennary complex-type) pass through the column without any retardation, whereas the glycopeptides possessing two peripheral Gal-GlcNAc chains linked to C2 of outer mannose residues (II, III), and both the glycopeptides having the hybrid-type (IX) and the high-mannose-type (I) sugar chain are retained by the column. The glycopeptide (VIII) with a bisected biantennary complex-type sugar chain is in the passed-through fraction (ref. 30).

Step 2 - fractionation of glycopeptides based on the degree of binding to a Con A-agarose column

Among the glycopeptides bound to the column, biantennary glycopeptides (II, III) are usually removed by elution with 15 mM methyl α-D-mannopyranoside (αMM), while the high-mannose-type and the hybrid-type glycopeptides are obtained by elution with higher concentrations of αMM (200 mM). The separation of complex-type oligosaccharides from high-mannose-type oligosaccharides can also be carried out by affinity chromatography on a column of RCA-I-agarose, because this galactose-binding lectin column can interact with complex-type oligosaccharides having the Galβ1-4GlcNAc sequence even when it is sialylated.

Lectins for step 3 - separation of the biantennary glycopeptide with an α-fucosyl residue in the core

The immobilized lectin column best suited for step 3 is a column prepared from *Lens culinaris* (lentil), *Pisum sativum* (pea) or *Vicia faba* (fava) lectin. Con A, lentil, pea and fava lectins have quite similar specificity (refs. 33-35). The structural requirements for the binding of various glycopeptides and oligosaccharides to the immobilized lentil, pea and fava lectins are shown in Fig. 3 in comparison with those for binding to Con A-agarose. Basically, lentil, pea and fava lectins all require two unsubstituted or 2-O-substituted α-mannopyranosyl residues for tight binding. However, in contrast to Con A, lentil and pea lectins were found to bind triantennary complex-type glycopeptides in which one of the outer α-mannopyranosyl residues in the core is substituted at positions C-2 and

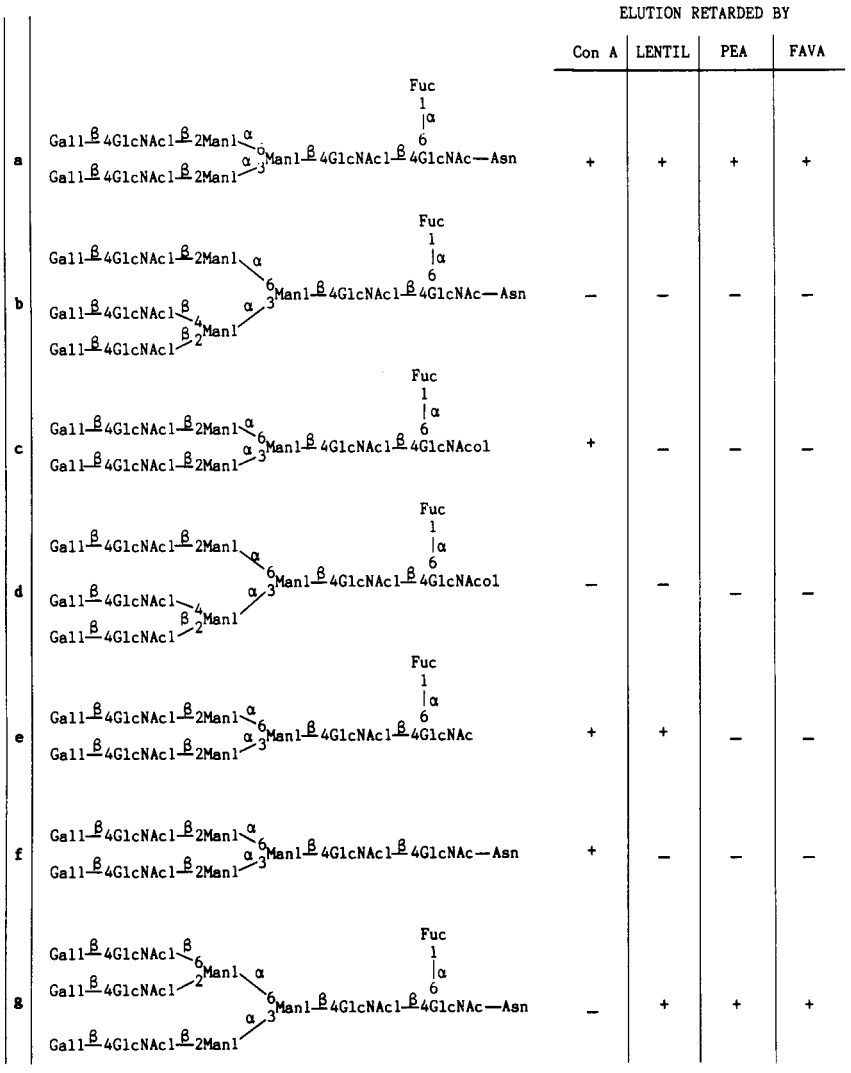

Fig. 3. Interaction of various oligosaccharides and glycopeptides with Con A-agarose, lentil lectin-agarose, pea lectin-agarose and fava lectin-agarose

C-6 as glycopeptide g in Fig. 3 or glycopeptide VI or VII in Fig. 2 (ref. 33). Furthermore, as shown in Fig. 3, the presence of an α1-6-linked fucose at the innermost GlcNAc residue in the core is essential for the tight binding of a glycopeptide to lentil, pea and fava lectin, but not for binding to Con A (refs. 33, 36). Subtle differences in binding specificity were found among lentil, pea and fava lectin columns (refs. 34, 35). Even an asparagine residue is required for high-affinity binding to immobilized pea and fava lectins, but not for binding to the immobilized lentil lectin which requires an intact GlcNAc residue at the reducing end for tight binding in the case of complex-type oligosaccharides.

Thus, when a mixture of glycopeptides II and III (Fig. 3) which are eluted from a Con A column with 15 mM αMM is applied to a column of immobilized lentil, pea or fava lectin, the glycopeptide III which has a fucose residue on the innermost GlcNAc residue in the core binds tightly to the column and is separated from glycopeptide II which does not have a fucose residue in the core. However, if radioactive oligosaccharides which are prepared by hydrazinolysis or digestion with a peptide:N-glycosidase of glycoproteins followed by reduction with NaB^3H_4 are to be separated, immobilized Aleuria aurantia lectin (AAL) which interacts with an α-fucosyl residue at the innermost GlcNAc residue (refs. 37, 38) should be used, since an intact innermost GlcNAc residue is required for tight binding to the lentil lectin-agarose and even an asparagine residue is necessary for tight binding to pea or fava lectin-agarose.

Lectins for step 4 - separation of a hybrid-type glycopeptide

Glycopeptides I and IX (Fig. 2) which are eluted from a Con A-agarose column with 200 mM αMM may be separated from each other by use of a wheat germ agglutinin (WGA)-agarose column. A hybrid-type glycopeptide (IX) is retained by the column and a high mannose-type glycopeptide (I) passes through the column.

WGA is classified under di-N-acetylchitobiose-binding lectins (Table 1), and WGA-agarose has been widely used for the purification of many glycoproteins and glycopeptides, but structural requirements for the binding to an immobilized WGA column were poorly understood for a long time. Most complex-type and high mannose-type glycopeptides are not bound by a WGA-agarose column in spite of the presence of a di-N-acetylchitobiose moiety in their core portion (ref. 39).

Yamamoto et al. (ref. 39) tested various glycopeptides and their partially degraded products for binding to a column of WGA-agarose and found that the GlcNAcβ1-4Manβ1-4GlcNAcβ1-4GlcNAc-Asn structure, in which the terminal GlcNAc residue originates from a bisecting GlcNAc residue, is essential for tight binding of glycopeptides to a WGA-agarose column. Therefore, most hybrid-type glycopeptides which have a bisecting GlcNAc residue are retained by or at least retarded on a WGA-agarose column. However, if the glycopeptide has an α-fucosyl residue at the innermost GlcNAc residue as in the case of a complex-type glycopeptide (glycopeptide VIII in Fig. 2) found in glycophorin A of human erythrocytes (ref. 40), the glycopeptide can be retained by the column only after removal of the α-fucosyl residue. Incidentally, most hybrid-type glycopeptides contain the bisecting GlcNAc residue, but do not have the α-fucosyl residue in the core. A WGA-agarose column is, therefore, suited for the separation of hybrid-type glycopeptides. E-PHA-agarose was also found to bind bisected complex-type glycopeptides (refs. 40, 41), but in this case, one of the outer Man residues which links α1-6 to the inner β-Man residue should not be substituted (ref. 42). Since most hybrid-type glycopeptides have a substitution on the Man α1-6 residue, these glycopeptides cannot interact with the E-PHA-agarose.

Recently, another important sugar-binding specificity of WGA-agarose was reported by Gallagher et al (ref. 43). They found that a WGA-agarose column binds poly(N-acetyllactosamine)-type glycans (Fig. 4). Since this type of sugar chain is in cell-surface glycoproteins of various types of mammalian cells, this finding may have revealed what is the major binding site for WGA on the surface of various mammalian cells. However, we recently found that the non-branched poly(N-acetyllactosamine)-type structure like cell surface antigen i and the poly(N-acetyllactosamine)-type oligosaccharides with a molecular weight of less than 4,000 cannot be retained by a WGA-agarose column. Among the other di-N-acetylchitobiose-binding lectins, blood group nonspecific Phytolacca americana (pokeweed), Datura stramonium (DSA) and Lycopersicon esculentum (tomato) lectins also bind poly(N-acetyllactosamine)-type glycans, and DSA can interact even with the i-antigen-type non-branched poly(N-acetyllactosamine) structure. On the other hand, immobilized lectin columns prepared from blood group specific, di-N-acetylchitobiose-binding lectins, such as Ulex II, Cytisus sessilifolius and Laburnum alpinum lectins cannot interact with poly(N-acetyllactosamine)-type oligosaccharides (H. Kawashima, S. Sueyoshi, Y. Konami, K. Yamamoto and T. Osawa, unpublished result). Although the poly(N-acetyllactosamine)-containing glycopeptides are not included in Fig. 2, these glycopeptides are easily separated by use of the immobilized lectin columns described here, and these lectins are also very useful for the detection of poly (N-acetyl-lactosamine)-type sugar chains on the cell surface (Y. Imai, H. Fukasawa, Y. Yamashita and T. Osawa, unpublished results).

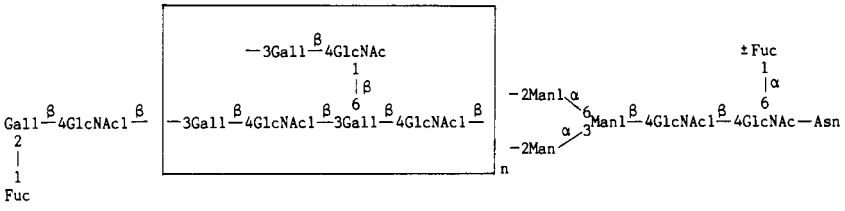

Fig. 4. Poly(N-acetyllactosamine)-type glycopeptide

Lectins for step 5 - separation of a triantennary complex-type glycopeptide with a bisecting GlcNAc

As already discussed, a bisecting GlcNAc residue that links β1-4 to the β-linked mannose residue in the core of an asparagine-linked glycopeptides is an important determinant for high-affinity binding to E-PHA agarose. A complex-type glycopeptide with a bisecting GlcNAc residue can be retained by or retarded on an E-PHA agarose column irrespective of the presence or absence of a fucose residue at the innermost GlcNAc residue in the core on condition that Galβ1-4GlcNAcβ1-2Manβ1-6 chain should not be substituted or modified (ref. 42). Therefore, the tetraantennary complex-type sugar chain with a bisecting GlcNAc residue does not interact with E-PHA-agarose, because the Manα1-6 residue is substituted by another outer chain. However, the bisected biantennary complex-type glycopeptide VIII in Fig. 2 can be separated by use of an E-PHA agarose column.

Lectins for step 6 - separation of the glycopeptide VII

Lentil lectin agarose tightly binds triantennary glycopeptides only when the glycopeptides bear α-fucosyl residue in the core and contain an outer mannose residue substituted at positions C-2 and C-6 (ref. 33). Therefore, glycopeptide VII can be separated in one step, by use of a lentil lectin-agarose column from a mixture of glycopeptides IV-VII in Fig. 2 which pass through the E-PHA agarose column.

Lectins for step 7 - separation of the triantennary glycopeptide VI

L-PHA agarose strongly interacts with triantennary and tetraantennary complex-type glycopeptides that have at least one of the α-linked outer mannose residues substituted at positions C-2 and C-6 with Galβ1-4GlcNAcβ1-sugar sequences irrespective of presence or the absence of an α-fucosyl residue in the core (refs. 41, 44). From the mixture of glycopeptides IV, V and VI in Fig. 2, glycopeptide VI, which is devoid of an α-fucosyl residue in the core and therefore cannot be retained by a lentil lectin-agarose column in step 7, is easily separated by use of a L-PHA-agarose column.

Lectins for step 8 - separation of the triantennary glycopeptides IV and V based on the presence or absence of an α-fucosyl residue in the core

In Fig. 2, glycopeptide V differs from glycopeptide IV in the existence of an α-fucosyl residue at the innermost GlcNAc residue in the core. A fucose-binding lectin, AAL, isolated from fruiting bodies of Aleuria aurantia (ref. 37) most strongly interacts with the complex-type oligosaccharides and glycopeptides with an α-fucosyl residue at the innermost GlcNAc residue irrespective of the presence or the number of outer chain moieties (ref. 38). Therefore glycopeptide V can be most easily separated from glycopeptide IV by the use of an AAL-agarose column. However, glycopeptide V cannot interact with a lentil lectin-agarose column because, in this triantennary glycopeptide, one of the outer mannose residues is substituted at positions C-2 and C-4 by βGlcNAc residues instead of at positions C-2 and C-6 which favors the binding to a lentil lectin-agarose column. Furthermore, an AAL-agarose column interacts even with an α-fucosyl residue in the peripheral portion of the complex-type sugar chain, and therefore, when this lectin column is used for the structural assessment of glycopeptides, considerable attention should be paid on the rather broad specificity of this lectin column in the interpretation of the results obtained. Actually, the oligosaccharides with Fucα1-2Galβ1-4GlcNAc or Galβ1-4(Fucα1-3)GlcNAc groups interacted with AAL-agarose, but less strongly than did complex-type oligosaccharides with a fucosylated core. Lacto-N-fucopentaitol II, which has a Galβ1-3(Fucα1-4)GlcNAc group, interacted less strongly than the above two groups. However, the oligosaccharides with Fucα1-2Galβ1-3GlcNAc or Galβ1-4GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAc groups showed almost no interaction with the immobilized lectin (ref. 38). Furthermore, the rather limited availability of AAL impedes the application of this lectin to glycoprotein chemistry, and I feel that other lectins which strongly interact with an α-fucosyl residue in the core portion of asparagine-linked glycopeptides should be explored.

SPECIFICITIES OF IMMOBILIZED LECTIN COLUMNS PREPARED FROM LECTINS WHICH PREFERENTIALLY BIND TO MUCIN-TYPE SUGAR CHAINS

Immobilized lectin columns prepared from lectins that preferentially bind to mucin-type sugar chains such as those listed in Table 2 have been less precisely analyzed for their sugar binding specificity. Sueyoshi et al. (ref. 45) quantitatively determined sugar binding specificities of various mucin-type sugar chain-binding lectins by use of a glycopeptide prepared from human erythrocyte glycophorin A (ref. 46) and a blood group A-active oligosaccharide and their enzymic degradation products (Table 3). On the basis of their results, they divided those lectins into two groups with respect to reactivity with typical mucin-type glycopeptides Galβ1-3GalNAcα1-Ser/Thr and GalNAcα1-Ser/Thr. One group, which consists of Agaricus bisporus (mushroom) lectin (ABA-1), Arachis hypogaea (peanut) lectin (PNA) and Bauhinia purpurea lectin (BPA), preferentially binds to Galβ1-3GalNAcα1-Ser/Thr, and the other, which consists of Glycine max (soybean) lectin (SBA) and Vicia villosa lectin ($VVA\text{-}B_4$), shows higher affinity for GalNAcα1-Ser/Thr than for Galβ1-3GalNAcα1-Ser/Thr. Among the immobilized lectin columns tested, only ABA-1-agarose retained a sialylated glycopeptide, which was prepared from human erythrocyte glycophorin A and contains three tetrasaccharide chains having the structure of NeuAcα2-3Galβ1-3(NeuAcα2-6)GalNAcα-. As shown in Table 3, the association constant of ABA-1-agarose for this oligosaccharide was $1.5 \times 10^5\ M^{-1}$. The other four lectin columns did not significantly interact with this sialylated glycopeptide and their association constants for the glycopeptide were below $5.0 \times 10^3\ M^{-1}$. However, after desialylation, the resulting glycopeptide bearing Galβ1-3GalNAcα1-Ser/Thr chains was retarded on the immobilized PNA and BPA columns with association constants of $8.6 \times 10^4\ M^{-1}$ and $2.4 \times 10^5\ M^{-1}$, respectively. On the other hand, the interactions of the desialylated glycopeptide with the immobilized SBA and $VVA\text{-}B_4$ were still very weak (Ka:$1.2 \times 10^4\ M^{-1}$ and $2.5 \times 10^3\ M^{-1}$, respectively). Further removal of galactose residues from the desialylated glycopeptide resulted in significant decreases in the association constants of the immobilized ABA-I and PNA, but the absence of

TABLE 3. Association constants of five lectins for glycopeptide CB-II (ref. 46) obtained from human erythrocyte glycophorin A, and a blood group A-active hexasaccharide and a blood group A-active heptasaccharide isolated from the feces of breast-fed infants (BioCarb Chemicals, Lund, Sweden)

Glycopeptide or Oligosaccharide	Lectins				
	ABA-1	PNA	BPA	SBA	VVA-B4
	Association constant (M^{-1})				
Leu/Ser-Ser(R)-Thr(R)-Thr(R)-Glu/Gly-Val-Ala-Hse					
R: NeuAc2→6(α)3Gal1→3(β)GalNAc→(α)	1.5×10^5	1.4×10^3	1.2×10^3	4.8×10^3	1.5×10^3
R: Gal1→3(β)GalNAc→(α)	4.1×10^6	8.6×10^4	2.4×10^5	1.2×10^4	2.5×10^3
R: GalNAc→(α)	6.9×10^3	1.5×10^3	2.0×10^4	9.4×10^4	2.5×10^5
GalNAc1→(α)3Gal1→(β)3GlcNAc1→(β)3Gal1→(β)4Glc (2→1α Fuc; 4→1α Fuc)	2.4×10^3	1.7×10^3	3.9×10^3	1.0×10^4	7.9×10^2
GalNAc1→(α)3Gal1→(β)3GlcNAc1→(β)3Gal1→(β)4Glc (2→1α Fuc)	1.5×10^3	1.2×10^3	2.3×10^3	1.1×10^4	1.5×10^3
GalNAc1→(α)3Gal1→(β)3GlcNAc1→(β)3Gal1→(β)4Glc	1.1×10^3	1.1×10^3	1.5×10^4	1.5×10^5	4.2×10^4
Gal1→(β)3GalNAcol	2.0×10^3	2.0×10^3	7.0×10^3	1.2×10^4	2.5×10^3

the β-galactosyl residue did not markedly affect the interaction with the immobilized BPA (Ka: 2.0×10^4 M^{-1}) and significantly enhanced the interaction with the immobilized SBA and VVA-B_4 (Ka: 9.4×10^4 M^{-1} and 2.5×10^5 M^{-1}, respectively). The difference between SBA-agarose and VVA-B_4-agarose was that the former preferentially interacted with the GalNAcα1-3Galβ1-3GalNAc-R sugar sequence, while the latter was highly specific for the GalNAcα1-Ser/Thr structure (Tn antigenic structure).

CONCLUSION

Serial lectin-agarose affinity chromatography for the separation and structural assessment of oligosaccharides and glycopeptides originally proposed by Cummings and Kornfeld (ref. 47) is very sensitive and rapid. For the final elucidation of the structures of oligosaccharides and glycopeptides, more conventional approaches such as sequential enzymic degradation, methylation, mass spectrometry and NMR spectroscopy should be carried out, but prior fractionation by use of immobilized lectin columns whose binding specificities have been precisely determined, make the structural study much easier.

A more detailed review on the sugar-binding specificities of immobilized lectin columns was recently published by us (ref. 48).

REFERENCES

1. H. Stillmark, Thesis, Univ. of Dorpat, Dorpat (1888).
2. W.C. Boyd and R.M. Reguera, J. Immunol. 62, 333-339 (1949).
3. K.O. Renkonen, Ann. Med. Exp. Fenn. 26, 66-72 (1948).
4. W.C. Boyd, In "The Proteins" (H. Neurath and K. Bailey, eds.), Vol. 2, Part 2, pp. 756-844. Academic Press, New York (1954).

5. W.M. Watkins and W.T.J. Morgan, Nature (London) 196, 825-826 (1952).
6. O. Mäkelä, Ann. Med. Exp. Biol. Fenn. 35, Suppl. 11 (1957).
7. T. Irimura, T. Kawaguchi, T. Terao and T. Osawa, Carbohyd. Res. 39, 317-327 (1975).
8. R. Kaifu, T. Osawa and R.W. Jenaloz, Carbohyd. Res. 40, 111-117 (1975).
9. R. Kaifu and T. Osawa, Carbohyd. Res. 52, 179-185 (1976).
10. K. Yokoyama, T. Terao and T. Osawa, Biochim. Biophys. Acta 538, 384-396 (1978).
11. A.-C. Roche and M. Monsigny, Biochim. Biophys. Acta 371, 242-254 (1974).
12. D.T. Dorai, B.K. Bachhawat and S. Bishayee, Anal. Biochem. 115, 130-137 (1981).
13. S. Mohan, D.T. Dorai, S. Srimal and B.K. Bachhawat, Biochem. J. 203, 253-262 (1982).
14. R.L. Miller, J.F. Collawan and W.W. Fish, J. Biol. Chem. 257, 7574-7580 (1982).
15. W.E. Holm, A.-M. Bergholm, B. Wagner and M. Wagner, J. Med. Microbiol. 19, 317-323 (1985).
16. M.H. Ravindranath, J.J. Higa, E.L. Cooper and J.C. Paulson, J. Biol. Chem. 260, 8850-8856 (1986).
17. S. Sueyoshi, K. Yamamoto and T. Osawa, J. Biochem. 103, 894-899 (1988).
18. V.P. Bhavanandan and A.W. Katlic, J. Biol. Chem. 254, 4000-4008 (1979).
19. B.P. Peters, S. Ebisu, I.J. Goldstein and M. Flashner, Biochemistry 18, 5505-5511 (1979).
20. N. Shibuya, I.J. Goldstein, W.F. Broekaert, M. Nsimba-Lubaki, B. Peters and W.J. Penmans, J. Biol. Chem. 262, 1596-1601 (1987).
21. N. Shibuya, I.J. Goldstein, W.F. Broekaert, M. Nsimba-Lubaki, B. Peters and W.J. Peumans, Arch. Biochem. Biophys. 254, 1-8 (1987).
22. T. Kawaguchi, I. Matsumoto and T. Osawa J. Biol. Chem. 249, 2786-2792 (1974).
23. W.-C. Wang and R.D. Cummings, J. Biol. Chem. 263, 4576-4585 (1988).
24. K. Umetsu, S. Kosaka and T. Suzuki, J. Biochem. 95, 239-245 (1984).
25. J.U. Baenziger and D. Fiete, J. Biol. Chem. 254, 9795-9799 (1979).
26. E.K. Green and J.U. Baenziger, J. Biol. Chem. 262, 12018-12029 (1987).
27. S. Ogata, T. Muramatsu and A. Kobata, J. Biochem. 78, 687-696 (1975).
28. T. Krusius, J. Finne and H. Rauvala, FEBS Lett. 71, 117-120 (1976).
29. S. Narashimhan, J.R. Wilson, E. Martin and H. Schachter, Can. J. Biochem. 57, 83-96 (1979).
30. S. Narashimhan, J.C. Freed and H. Schachter, Carbohyd. Res. 149, 65-83 (1986).
31. J.U. Baenziger and D. Fiete, J. Biol. Chem. 254, 2400-2407 (1979).
32. Y. Ohyama, K. Kasai, H. Nomoto and Y. Inoue, J. Biol. Chem. 260, 6882-6887 (1985).
33. K. Kornfeld, M.L. Reitman and R. Kornfeld, J. Biol. Chem. 256, 6633-6640 (1981).
34. K. Yamamoto, T. Tsuji and T. Osawa Carbohyd. Res. 110, 283-289 (1982).
35. Y. Katagiri, K. Yamamoto, T. Tsuji and T. Osawa, Carbohyd. Res. 129, 257-265 (1984).
36. H. Debray, D. Decout, G. Strecker, G. Spik and J. Montreuil, Eur. J. Biochem. 117, 41-55 (1981).
37. N. Kochibe and K. Furukawa, Biochemistry 19, 2841-2846 (1980).
38. K. Yamashita, N. Kochibe, T. Ohkura, I. Ueda and A. Kobata, J. Biol. Chem. 260, 4688-4693 (1985).
39. K. Yamamoto, T. Tsuji, I. Matsumoto and T. Osawa, Biochemistry 20, 5894-5899 (1981).
40. T. Irimura, T. Tsuji, S. Tagami, K. Yamamoto and T. Osawa, Biochemistry 20, 560-566 (1981).
41. R.D. Cummings and S. Kornfeld, J. Biol. Chem. 257, 11230-11234 (1982).
42. K. Yamashita, A. Hitoi and A. Kobata, J. Biol. Chem. 258, 14753-14755 (1983).
43. J.T. Gallagher, A. Morris and T. Dexter, Biochem. J. 231, 115-122 (1985).
44. S. Hammarström, M.-L. Hammarström, G. Sundblad, J. Arnarp and J. Lönngren, Proc. Natl. Acad. Sci. U.S.A. 79, 1611-1615 (1982).
45. S. Sueyoshi, T. Tsuji and T. Osawa, Carbohyd. Res. in press (1988).
46. R. Prohaska, T.A.W. Koerner, I.M. Armitage and H. Furthmayr, J. Biol. Chem. 256, 5781-5791 (1981).
47. R.D. Cummings and S. Kornfeld, J. Biol. Chem. 257, 11235-11240 (1982).
48. T. Osawa and T. Tsuji, Ann. Rev. Biochem. 56, 21-42 (1987).