

Specificity of Twelve Lectins Towards Oligosaccharides and Glycopeptides Related to *N*-Glycosylproteins

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Glycopeptides and oligosaccharides of either the *N*-acetylglucosaminic or the oligomannosidic type derived from glycoproteins containing the *N*-glycosylamine linkage were used to define the specificity of different lectins (concanavalin A, *Lens culinaris* agglutinin, *Vicia faba* agglutinin, *Pisum sativum* agglutinin, *Ricinus communis* agglutinins, soybean agglutinin, wheat germ agglutinin, *Solanum tuberosum* agglutinin, *Datura stramonium* agglutinin, *Lotus tetragonolobus* agglutinin, *Ulex europeus* agglutinin) by studying the inhibition of human red blood cell agglutination by these structures. The results obtained show that lectins considered 'identical' in terms of monosaccharide specificity, possess the ability to recognize fine differences in more complex structures. In fact, different lectins are able to recognize different saccharidic sequences on the same glycan structure. As these sequences are likely to be common to numerous glycoproteins, including cell membrane glycoproteins, the results obtained with lectins in the study of cell surface carbohydrates have to be very carefully interpreted.

Moreover, our results confirm previous data on the spatial configuration of the glycan moiety of glycoproteins deduced from the construction of molecular models: the fact that oligosaccharides bearing an α -NeuAc-(2→6)-Gal unit are more powerful inhibitors than oligosaccharides bearing an α -NeuAc-(2→3)-Gal unit could be related to the high rotational freedom of α -2,6 linkage; the observation that glycoasparagines, glycopeptides and glycoproteins possess a higher affinity for lectins than the related oligosaccharides could be explained by the fact that the glycan–amino acid linkage leads to structures more rigid than those of the oligosaccharides themselves.

Lectins are now widely used as tools in the study of plasma membrane modifications in neoplasia. Numerous lectins have been purified and their properties well defined (for reviews see [1,2]). Lectins are able to bind sugars specifically, but it is well known that the monosaccharide residue in a terminal non-reducing position on a glycan is not the only carbohydrate moiety recognized. Indeed, Kornfeld et al. [3] have shown that the binding constant of the specific free sugar with a lectin may be several orders of magnitude lower than the binding constant of a glycoconjugate containing this sugar. Moreover, in the particular case of concanavalin A, the authors demonstrated that the most active part of a glycan structure of the *N*-acetylglucosaminic type are not the α -mannose residues in a terminal position but the disaccharide *N*-acetyl- β -D-glucosaminyl-(1→2)- α -D-mannoside.

In the present study, we have used numerous oligosaccharides and glycopeptides derived from *N*-glycosylproteins to define the specificities to twelve lectins (Table 1), by determining their ability to inhibit the agglutination of human red blood cells induced by these lectins. These results have been preliminarily reported in [4,5].

Abbreviations. NaCl/P_i, 0.01 M sodium phosphate, 0.14 M sodium chloride pH 7.2; Gal, D-galactose; NeuAc, *N*-acetylneuraminic acid; GlcNAc, *N*-acetyl-D-glucosamine; GalNAc, *N*-acetyl-D-galactosamine; Man, D-mannose; Fuc, L-fucose; Asn, L-asparagine.

Enzymes. β -D-Galactosidase (EC 3.2.1.23); *N*-acetyl- β -D-glucosaminidase (EC 3.2.1.30); α -L-fucosidase (EC 3.2.1.51); neuraminidase (EC 3.2.1.18).

Note. All sugars are of the D configuration unless otherwise stated.

MATERIALS AND METHODS

Materials

All the lectins used in this study (Table 1), except concanavalin A which was purchased from Pharmindustrie (Clichy, France), were purified by us by affinity chromatog-

Table 1. List of lectins used in this study and classified according to Goldstein and Hayes [2]

Specificity	Lectin
α -Mannose or α -glucose	Concanavalin A from <i>Canavalia ensiformis</i> (Jack bean lectin)
	<i>Lens culinaris</i> agglutinin
	<i>Vicia faba</i> agglutinin
	<i>Pisum sativum</i> agglutinin
β -Galactose and <i>N</i> -acetyl- β - galactosamine	<i>Ricinus communis</i> agglutinin I
	<i>Ricinus communis</i> agglutinin II
	Soybean agglutinin (<i>Glycin max</i> agglutinin)
<i>N</i> -Acetyl- β - glucosamine	Wheat germ agglutinin (<i>Triticum vulgare</i> agglutinin)
	<i>Solanum tuberosum</i> agglutinin (potato agglutinin)
	<i>Datura stramonium</i> agglutinin
α -L-Fucose	<i>Lotus tetragonolobus</i> agglutinin
	<i>Ulex europeus</i> agglutinin

Origin	Name	Abbreviation	Structure
Human milk	Neuraminyl ($\alpha 2 \rightarrow 3$) lactose	NL α -2,3	α -NeuAc-(2 $\rightarrow$ 3)- β -Gal-(1 $\rightarrow$ 4)-Glc
Human milk	Neuraminyl ($\alpha 2 \rightarrow 6$) lactose	NL α -2,6	α -NeuAc-(2 $\rightarrow$ 6)- β -Gal-(1 $\rightarrow$ 4)-Glc
Mannosidosis	Mannoside 1	M-1	α -Man-(1 $\rightarrow$ 3)- β -Man-(1 $\rightarrow$ 4)-GlcNAc
	Mannoside 2	M-2	α -Man-(1 $\rightarrow$ 2)- α -Man-(1 $\rightarrow$ 3)- β -Man-(1 $\rightarrow$ 4)-GlcNAc
	Mannoside 3	M-3	α -Man-(1 $\rightarrow$ 2)- α -Man-(1 $\rightarrow$ 2)- α -Man-(1 $\rightarrow$ 3)- β -Man-(1 $\rightarrow$ 4)-GlcNAc
Sialidosis	Sialoside 1	N-1	α -NeuAc-(2 $\rightarrow$ 3)- β -Gal-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 2)- α -Man-(1 $\rightarrow$ 3)- β -Man-(1 $\rightarrow$ 4)-GlcNAc
	Sialoside 2	N-2	α -NeuAc-(2 $\rightarrow$ 6)- β -Gal-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 2)- α -Man-(1 $\rightarrow$ 3)- β -Man-(1 $\rightarrow$ 4)-GlcNAc
	Sialoside 3	N-3	α -NeuAc-(2 $\rightarrow$ 6)- β -Gal-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 2)- α -Man-(1 $\rightarrow$ 3)- β -Man-(1 $\rightarrow$ 4)-GlcNAc
	Sialoside 4	N-4	α -NeuAc-(2 $\rightarrow$ 3)- β -Gal-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 2)- α -Man-(1 $\rightarrow$ 3)- β -Man-(1 $\rightarrow$ 4)-GlcNAc
	Sialoside 5	N-5	α -NeuAc-(2 $\rightarrow$ 6)- β -Gal-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 2)- α -Man-(1 $\rightarrow$ 3)- β -Man-(1 $\rightarrow$ 4)-GlcNAc
	Sialoside 6	N-6	α -NeuAc-(2 $\rightarrow$ 6)- β -Gal-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 2)- α -Man-(1 $\rightarrow$ 3)- β -Man-(1 $\rightarrow$ 4)-GlcNAc
	Sialoside 9	N-9	α -NeuAc-(2 $\rightarrow$ 6)- β -Gal-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 2)- α -Man-(1 $\rightarrow$ 3)- β -Man-(1 $\rightarrow$ 4)-GlcNAc
GM ₂ - Gangliosidosis variant O (Sandhoff's disease)	Oligosaccharide S-6a	S-6a	β -GlcNAc-(1 $\rightarrow$ 2)- α -Man-(1 $\rightarrow$ 3)- β -Man-(1 $\rightarrow$ 4)-GlcNAc
	Oligosaccharide S-5b	S-5b	β -GlcNAc-(1 $\rightarrow$ 2)- α -Man-(1 $\rightarrow$ 3)- β -GlcNAc-(1 $\rightarrow$ 4)- β -Man-(1 $\rightarrow$ 4)-GlcNAc
Fucosidosis	Fucoside 1	F-1	α -Fuc-(1 $\rightarrow$ 6)-GlcNAc
	Fucoside 2	F-2	β -Gal-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 2)- α -Man-(1 $\rightarrow$ 3)- β -Man-(1 $\rightarrow$ 4)-GlcNAc
	Glycopeptide I	GP-F-1	α -Fuc-(1 $\rightarrow$ 6)- β -GlcNAc-(1 $\rightarrow$)-Asn
	Glycopeptide II	GP-F-2	α -Man-(1 $\rightarrow$ 6)- β -Man-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$)-Asn
	Glycopeptide III	GP-F-3	$\left[\begin{array}{c} \beta\text{-Gal}-(1\rightarrow 4)-\beta\text{-GlcNAc} \\ \\ \alpha\text{-Fuc} \end{array} \right]_4 \begin{array}{l} (1\rightarrow 2) \\ (1\rightarrow 4) \\ (1\rightarrow 6) \end{array} \left[\begin{array}{c} \alpha\text{-Man}-(1\rightarrow 3) \\ \alpha\text{-Man}-(1\rightarrow 6) \end{array} \right] \beta\text{-Man}-(1\rightarrow 4)-\beta\text{-GlcNAc}-(1\rightarrow 4)-\beta\text{-GlcNAc}-(1\rightarrow)-\text{Asn}$

Fig.1. Structure of the oligosaccharides and glycopeptides isolated from the urines of patients with various lysosomal diseases [20] and used in the present study

raphy and were judged to be homogeneous by sodium dodecyl sulfate/polyacrylamide gel electrophoresis performed as described by Laemmli [6].

Lens culinaris Agglutinin. *Lens* agglutinin used in this study, was isolated by affinity chromatography as described by Toyoshima et al. [7]. This lentil lectin is a mixture of two proteins named LCH-A and LCH-B by Howard et al. [8], which can be further separated by ion-exchange chromatography on a carboxymethyl-cellulose column. Although these two isolectins can differ in their carbohydrate-binding specificity, the results presented in this study were obtained with the natural mixture as prepared by affinity chromatography.

Vicia faba Agglutinin. *Vicia* agglutinin was purified from *Vicia faba* seeds (broad bean) purchased from Vilmorin (France), by affinity chromatography on Sephadex G-150 as described [9].

Pisum sativum Agglutinin. *Pisum* agglutinin was purified from common garden-pea seeds by affinity chromatography on Sephadex G-75 as described by Entlicher et al. [10]. In fact, as for *Lens* agglutinin, this lectin is a mixture of two isolectins I and II, which are separable by DEAE-cellulose ion-exchange chromatography [10]. Although these two isolectins can differ in their carbohydrate specificity, the results presented in Table 2 were obtained with the natural mixture as prepared by affinity chromatography.

Ricinus communis Agglutinins I and II. Two distinct lectins are present in *Ricinus communis* seeds: RCA_I and RCA_{II}, according to the nomenclature of Nicolson et al. [11]. Lectin I is a hemagglutinin with a molecular weight of 120000; Lectin II is a toxin with a molecular weight of 60000.

In this study, *Ricinus* agglutinins I and II were prepared by combining affinity chromatography and gel filtration: first,

Origin	Abbreviation	Structure
A. Human serotransferrin	GP-hSTF	α -NeuAc-(2 $\rightarrow$ 6)- β -Gal-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 2)- α -Man-(1 $\rightarrow$ 3) β -Man-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$)-Asn
B. Human lactotransferrin	GP-hLTF-1	α -NeuAc-(2 $\rightarrow$ 6)- β -Gal-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 2)- α -Man-(1 $\rightarrow$ 3) β -Man-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$)-Asn α -NeuAc-(2 $\rightarrow$ 6)- β -Gal-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 2)- α -Man-(1 $\rightarrow$ 6) β -Man-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$)-Asn α -Fuc
C. Human lactotransferrin	GP-hLTF-2	α -NeuAc-(2 $\rightarrow$ 6)- β -Gal-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 2)- α -Man-(1 $\rightarrow$ 3) β -Gal-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 2)- α -Man-(1 $\rightarrow$ 6) α -Fuc β -Man-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$)-Asn α -Fuc
D. Hen ovotransferrin	GP-ovoTF	β -GlcNAc-(1 $\rightarrow$ 4) β -GlcNAc-(1 $\rightarrow$ 2)- α -Man-(1 $\rightarrow$ 3) β -GlcNAc-(1 $\rightarrow$ 4)- β -Man-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$)-Asn β -GlcNAc-(1 $\rightarrow$ 2)- α -Man-(1 $\rightarrow$ 6)
E. human Cohn's fraction IV	GP-F-IV	α -NeuAc-(2 $\rightarrow$ 3)- β -Gal-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 4) α -NeuAc-(2 $\rightarrow$ 6)- β -Gal-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 2)- α -Man-(1 $\rightarrow$ 3) β -Man-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$)-Asn α -NeuAc-(2 $\rightarrow$ 6)- β -Gal-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 2)- α -Man-(1 $\rightarrow$ 6)
F. Bovine lactotransferrin	GP-bLTF	α -Man-(1 $\rightarrow$ 2)- α -Man-(1 $\rightarrow$ 2)- α -Man-(1 $\rightarrow$ 3) β -Man-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$)-Asn α -Man-(1 $\rightarrow$ 3)- α -Man-(1 $\rightarrow$ 6) α -Man
G. Hen ovomucoid	GP- β ovo	β -Gal-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$?) β -GlcNAc-(1 $\rightarrow$ 4)- α -Man-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 4) β -GlcNAc-(1 $\rightarrow$ 2)- α -Man-(1 $\rightarrow$ 3) β -GlcNAc-(1 $\rightarrow$ 4)- β -Man-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$)-Asn β -GlcNAc-(1 $\rightarrow$ 2)- α -Man-(1 $\rightarrow$ 6) β -GlcNAc-(1 $\rightarrow$ 4)- α -Man-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 4)

Fig. 2. Structure of the glycopeptides isolated from human and bovine lactotransferrin, human serotransferrin, hen ovotransferrin, Cohn's fraction IV and hen ovomucoid, used in the present study

Ricinus seed extract was subjected to affinity chromatography on a Sepharose-*N*- ϵ -aminocaproyl- β -galactopyranosylamine column prepared according to Gordon et al. [12]. Both *Ricinus* agglutinins I and II were eluted with 0.1 M galactose and then separated on a Sephadex G-150 column equilibrated with NaCl/P_i.

Soybean Agglutinin. Soybean agglutinin was purified by affinity chromatography on a column of Sepharose-*N*- ϵ -aminocaproyl- β -galactosylamine according to Gordon et al. [12].

Wheat-Germ Agglutinin. Wheat-germ agglutinin was purified by affinity chromatography on a column of Sepharose-2-acetamido-*N*- ϵ -aminocaproyl-2-deoxy- β -glucopyranosylamine according to Lotan et al. [13].

Solanum tuberosum Agglutinin. *Solanum* agglutinin was purified according to the procedure of Delmotte et al. [14].

Datura stramonium Agglutinin. *Datura* agglutinin was purified from seeds of *Datura stramonium* by affinity chromatography on a Sepharose-ovomucoid column (unpublished results).

Lotus tetragonolobus Agglutinin. *Lotus* agglutinin was purified by affinity chromatography on a column of Sepharose-*N*- ϵ -aminocaproyl- β -L-fucopyranosylamine according to Blumberg et al. [15]. This lectin is in fact a mixture of three different proteins which can be further separated by ion-exchange chromatography on DEAE-cellulose [16] and which have been shown to possess the same specificity [17]. The

results presented were obtained with the natural mixture prepared by the affinity chromatography procedure described above.

Ulex europeus Agglutinin. *Ulex europeus* seeds contain two agglutinins with different sugar specificities as shown by Flory [18] and Matsumoto and Osawa [19]; the first has an affinity for α -L-fucose (lectin I) while the other seems to be specific for chitobiose (lectin II).

Ulex agglutinin I used in this study was purified by affinity chromatography on a column of Sepharose-*N*- ϵ -aminocaproyl- β -L-fucopyranosylamine prepared as described [15].

Oligosaccharides and glycopeptides shown in Fig. 1, were isolated by G. Strecker [20] from the urines of patients with various lysosomal diseases. Human transferrin and human lactotransferrin as well as glycopeptides prepared from them, glycopeptide isolated from ovotransferrin and glycopeptide isolated from Cohn's fraction IV (see structures in Fig. 2), were isolated by G. Spik.

The glycopeptide of the oligomannosidic type, isolated from bovine lactotransferrin (see structure in Fig. 2) was a gift from Dr A. Chéron.

All these structures derive from glycoproteins with the *N*-glycosidic linkage and belong to two types of glycan structures: the *N*-acetyllactosaminic type and the oligomannosidic type, according to Montreuil [21].

Some of these oligosaccharides or glycopeptides were enzymatically digested with neuraminidase from *Clostridium perfringens*, β -galactosidase, *N*-acetyl- β -D-glucosaminidase

from Jack-bean meal and α -L-fucosidase from rat kidney (Boehringer) as previously described [22].

Hemagglutination Tests

Agglutination of human red blood cells, group O⁺, in NaCl/P_i and inhibition of this hemagglutination by various oligosaccharides or glycopeptides was carried out in Linbro microtiter U-plates Titertek (Linbro Scientific Co, Hamden, USA) by a twofold serial dilution technique [19].

To each 0.05 ml of twofold serial dilution of sugar solution, in NaCl/P_i, was added an equal volume of lectin solution, carefully diluted to contain four minimum hemagglutinating doses.

After incubation for 1 h at room temperature, 0.05 ml of a 3% erythrocyte suspension in NaCl/P_i was added. The mixture was kept for 1 h at room temperature and then examined for agglutination.

Results were expressed as the minimum concentration (mM) required to completely inhibit one hemagglutinating dose; account was taken of the threefold dilution caused by the addition of lectin and erythrocytes.

RESULTS AND DISCUSSION

LECTINS WITH A SPECIFICITY FOR α -MANNOSE OR α -GLUCOSE

Concanavalin A

The results of the inhibition by various oligosaccharidic structures of hemagglutination by concanavalin A are presented in Table 2 and can be summarized as follows.

As shown on Fig. 3, concanavalin A presents a great affinity for the trimannosidic core substituted by two *N*-acetyl- β -glucosaminyl residues (oligosaccharide S-6a). The affinity is not decreased when this structure is modified by the addition of a β -(1 $\rightarrow$ 4)-linked *N*-acetylglucosamine on the β -linked mannose residue (oligosaccharide S-5b). On the contrary, the affinity is reduced when these *N*-acetylglucosamine residues are substituted by galactosyl residues (sialosides 5 and 6, and the asialoglycopeptide from human serotransferrin) or by sialogalactosyl residues (sialoside 9, glycopeptide from human serotransferrin). Moreover, the presence of an α -(1 $\rightarrow$ 6)-linked mannose residue in a terminal non-reducing position enhances the affinity as demonstrated by the fact that sialoside 3 (Fig. 1) is 66-fold more inhibitory than sialoside 2.

On the other hand, mannoside 1 (Fig. 1) which possesses an exposed α -(1 $\rightarrow$ 3)-mannose, but which lacks the core structure, is a poor inhibitor, even when this α -(1 $\rightarrow$ 3)-linked mannose is substituted by one or two α -(1 $\rightarrow$ 2)-linked mannose residues (mannosides 2 and 3). However, the glycopeptide isolated from bovine lactotransferrin which possesses the complete trimannosidic structure with the α -(1 $\rightarrow$ 3)-linked and α -(1 $\rightarrow$ 6)-linked mannose residues substituted by four additional α -linked mannose residues (oligomannosidic type structure) is a very good inhibitor of hemagglutination.

Substitution of the core α -(1 $\rightarrow$ 3)-linked mannose residue at position C-4, as in the glycopeptide from ovotransferrin, decreases the affinity of concanavalin A as compared to the asialoagalacto form of glycopeptide from serotransferrin (Fig. 3).

No difference can be found between the inhibitory power of a glycopeptide isolated from transferrin (Fig. 2A) and the oligosaccharidic structure derived from it (sialoside 9 in Fig. 1), suggesting that the *N*-acetylglucosamine-asparagine core

Table 2. Minimum concentration of various oligosaccharidic structures necessary to completely inhibit red cell agglutination by lectins specific for α -mannose

For structures see Fig. 1 and 2. n.d., not determined

Compounds	Minimum concentration for inhibition with			
	concanavalin A	<i>Lens</i> lectin	<i>Vicia</i> lectin	<i>Pisum</i> lectin
	mM			
L-Fucose	> 2.5	> 2.5	> 2.5	> 2.5
Glucose	5	5	2.5	1.25
Mannose	1.25	2.5	0.625	0.625
Fructose	1.25	5	5	2.5
α -Methylglucose	1.25	2.5	1.25	1.25
α -Methylmannose	0.312	1.25	0.312	0.312
Human transferrin	0.002	0.0165	0.008	0.008
Human lactotransferrin	0.002	0.0005	0.0005	0.0005
Glycopeptide from human serotransferrin				
complete form	0.026	0.003	0.013	0.013
asialo form	0.026	0.003	0.013	0.013
asialoagalacto form	0.015	> 0.83	> 0.83	> 0.83
Glycopeptide from human lactotransferrin				
form 1	0.026	0.0004	0.0008	0.0008
form 2	0.026	0.0004	0.0008	0.0008
Sialoside 9	0.026	0.415	0.104	0.415
Asialo form of sialoside 9	0.013	0.104	0.052	0.415
Glycopeptide from bovine lactotransferrin	0.0015	0.052	0.207	0.104
Mannoside 1	0.104	0.83	0.104	n.d.
Mannoside 2	0.026	0.210	0.105	n.d.
Mannoside 3	0.026	0.206	0.105	n.d.
Oligosaccharide S-6a	0.0003	0.157	0.31	n.d.
Oligosaccharide S-5b	0.0003	0.157	0.58	n.d.
Sialoside 1	> 0.83	> 0.83	0.83	n.d.
Sialoside 2	0.207	0.415	0.312	> 0.415
Asialo form of sialoside 2	0.207	0.207	0.207	> 0.415
Sialoside 3	0.003	0.207	0.104	n.d.
Sialoside 4	0.013	0.415	0.415	n.d.
Sialoside 5	0.026	0.087	0.044	n.d.
Sialoside 6	0.006	0.026	0.026	n.d.
Glycopeptide from ovotransferrin	0.052	0.415	0.052	n.d.

sequence does not influence the reactivity of the saccharide-binding site of concanavalin A. However, it can be noted that the native glycoproteins are more potent inhibitors than the glycopeptides obtained from them by exhaustive pronase digestion: the glycopeptide derived from lactotransferrin is 12-fold less inhibitory than the native glycoprotein, as are those derived from human transferrin when compared to the native glycoprotein.

Substitution of the *N*-acetylglucosamine residue involved in the *N*-glycosidic linkage by an α -(1 $\rightarrow$ 6)-linked fucose (Fig. 2B) does not modify the affinity of concanavalin A as compared to the non-fucosylated glycopeptide (Fig. 2A).

All the results described above are in good agreement with the study carried out by Kornfeld et al. [3], using glycopeptides isolated from human myeloma proteins and with that described more recently by Baenziger et al. [23], who used the Scatchard method to follow the interaction between various iodinated glycopeptides and concanavalin A, and to determine the association constants of these interactions.

Our own results, as expressed in Table 2, can be compared to these association constants and, from our values, the affinity of a given saccharidic structure for concanavalin A immobilized on Sepharose 4B can be predicted.

Lens culinaris Agglutinin

The results of the inhibition by various oligosaccharidic structures of hemagglutination by *Lens* agglutinin are given in Table 2.

As shown in Fig. 3, the best inhibitor for *Lens* agglutinin is a glycopeptide isolated from human lactotransferrin (Fig. 2B). Another glycopeptide with an additional fucose residue (Fig. 2C) possesses the same inhibitory power.

Removal of all the fucose residues from these glycopeptides with an α -L-fucosidase from rat kidney gives glycopeptides about 10-fold less inhibitory, as is the glycopeptide isolated from human serotransferrin (Fig. 2A).

These results show the importance of the α -(1 $\rightarrow$ 6)-linked fucose, as a major determinant of the binding. The α -(1 $\rightarrow$ 3)-linked fucose on the external *N*-acetylglucosamine residue, as found in glycopeptide 2 from human lactotransferrin, does not seem to play any role in this binding. On the other hand, the results confirm those found by Allen et al. [24] who discovered that 3-*O*-methyl-D-glucose and 3-*O*-benzyl-D-glucose are better inhibitors than glucose itself, suggesting the existence of a hydrophobic area in or near the lectin-binding site, which can interact with the methyl or the benzyl groups. The methyl group provided by the α -(1 $\rightarrow$ 6)-linked fucose would fit perfectly with this hydrophobic area.

In the case of concanavalin A, results had also shown the existence of a hydrophobic region close to the carbohydrate-binding site that could interact specifically with the phenyl groups of β -D-glucopyranosides [25], but our results show that the methyl group of L-fucose does not enhance the binding in this case. However, while the presence of an α -(1 $\rightarrow$ 6)-linked fucose near the *N*-glycosidic linkage seems to be a necessary condition for a maximal recognition of a saccharidic sequence by *Lens* agglutinin, it is not a sufficient one. In fact, glycopeptides possessing this α -(1 $\rightarrow$ 6)-linked fucose residue are not inhibitory (glycopeptides I and II of Fig. 1) or are very poorly inhibitory (glycopeptide III of Fig. 1). Moreover, the presence of terminal galactose residues seems to be necessary since in contrast to the asialo form, the asialo-galacto form of the glycopeptide of human serotransferrin is not inhibitory at all. In fact, removal of terminal sialic acid residues alone from the glycopeptide of human serotransferrin has no effect on the inhibitory activity as compared to the native glycopeptide. On the basis of these results, it can be concluded that *Lens* agglutinin recognizes bi-antennary *N*-acetyllactosaminic-type glycopeptide with galactose residues in a terminal non-reducing position, the binding being greatly enhanced by the presence of an α -(1 $\rightarrow$ 6)-linked fucose residue near the *N*-glycosidic linkage. These results can be compared with those published by Kornfeld et al. [26] who found that the most potent inhibitor for *Lens* agglutinin is a glycopeptide isolated from myeloma IgG which possesses the same glycan structure [3] as glycopeptide 1 of human lactotransferrin. These authors did not emphasize the importance of the presence of the α -(1 $\rightarrow$ 6)-linked fucose near the *N*-glycosidic linkage for a maximal recognition by the lectin.

The glycopeptide isolated from bovine lactotransferrin which possesses the classical trimannosidic core substituted by four additional α -linked mannose residues (oligomannosidic-type structure) is 140-fold less inhibitory than glyco-

Table 3. Accessibility to some lectins of saccharidic sequences with α -(2 $\rightarrow$ 3)-linked and α -(2 $\rightarrow$ 6)-linked sialic acid residues
n.d., not determined

Compounds	Minimum concentration for inhibition with			
	<i>Lens</i> lectin	<i>Vicia</i> lectin	<i>Ricinus</i> lectin	
			I	II
	mM			
Neuraminyl- α-(2→3)-lactose	> 1.66	> 1.66	0.625	n.d.
Neuraminyl- α-(2→6)-lactose	> 1.66	> 1.66	0.156	n.d.
Sialoside 1	> 0.83	0.83	> 0.83	> 0.83
Sialoside 2	0.415	0.31	0.83	0.415
Sialoside 4	0.415	0.415	0.415	0.104
Sialoside 5	0.087	0.044	0.087	0.051

peptide 1 from human lactotransferrin and 17-fold less inhibitory than the glycopeptide from human serotransferrin. *Lens* agglutinin also shows very little affinity for saccharides with mannose residues in a terminal non-reducing position (mannosides 1, 2 and 3).

Another point which appears from these results is the importance of the *N*-acetylglucosamine-asparagine sequence in the binding reaction. Sialoside 9 which possesses the same oligosaccharidic sequence as human transferrin glycopeptide except for the *N*-acetylglucosamine-asparagine sequence, is about 130-fold less inhibitory than the glycopeptide itself. In this respect, *Lens* agglutinin differs from concanavalin A for which a glycopeptide and the corresponding oligosaccharide possess the same inhibitory power.

All these results are also in favor of the concept of an extended carbohydrate-binding site for *Lens* agglutinin.

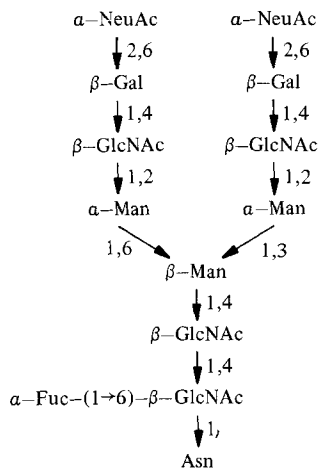
It can be noted that, in contrast to concanavalin A, with *Lens* agglutinin the native glycoproteins possess about the same inhibitory power as the glycopeptides derived from them by exhaustive pronase digestion.

Another important point, shown in Table 3, is that *Lens* agglutinin presents a greater affinity for sugar sequences substituted by sialic acid residues bound at the C-6 position of galactose rather than at the C-3 position. This observation can be related to the fact that an α -(2 $\rightarrow$ 6)-linkage possesses a higher rotational freedom than α -(2 $\rightarrow$ 3)-linkage [27,28]. Thus, the saccharidic sequences recognized by the lectin are, in the case of an α -(2 $\rightarrow$ 6)-linkage, more accessible to the lectin.

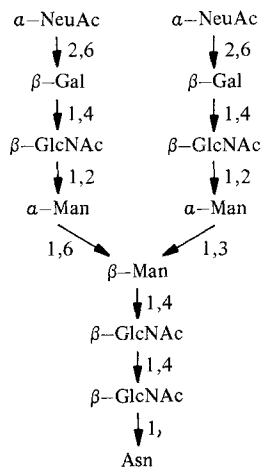
Vicia faba Agglutinin

The results concerning inhibition by various oligosaccharidic structures of hemagglutination by *Vicia* agglutinin, are presented in Table 2 and in Fig. 3. They are very similar to those we obtained with *Lens* agglutinin. In fact, the best inhibitor found for *Vicia* agglutinin is glycopeptide 1 from human lactotransferrin with an α -(1 $\rightarrow$ 6)-linked fucose on the *N*-acetylglucosamine residue involved in the *N*-glycosidic linkage, and removal of this L-fucose residue gives a glycopeptide (like that from human serotransferrin) 17-fold less inhibitory. The results of Allen et al. [24], suggesting the existence of a hydrophobic zone in or near the carbohydrate-binding site

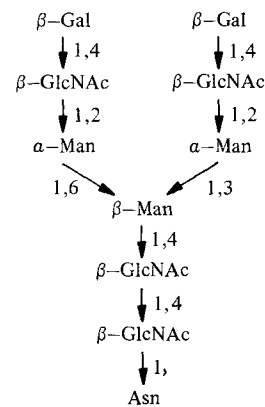
Glycopeptide 1 from human lactotransferrin



Glycopeptide from human serotransferrin



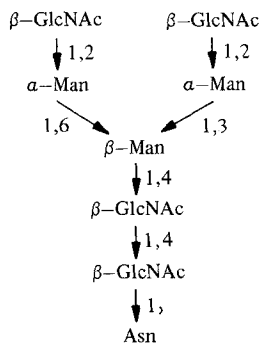
Asialo form of glycopeptide from human serotransferrin



Inhibitory dose with

Concanavalin A	0.026 mM	0.026 mM	0.026 mM
<i>Lens</i> agglutinin	0.0004 mM	0.003 mM	0.003 mM
<i>Vicia</i> agglutinin	0.0008 mM	0.013 mM	0.013 mM
<i>Pisum</i> agglutinin	0.0008 mM	0.013 mM	0.013 mM

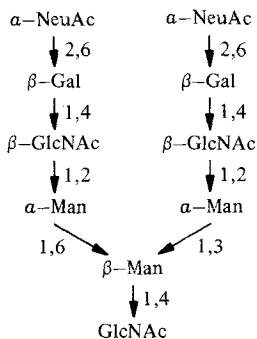
Asialoagalacto form of glycopeptide from human serotransferrin



Inhibitory dose with

Concanavalin A	0.015 mM	0.052 mM	0.0015 mM
<i>Lens</i> agglutinin	> 0.83 mM	0.415 mM	0.052 mM
<i>Vicia</i> agglutinin	> 0.83 mM	0.052 mM	0.207 mM
<i>Pisum</i> agglutinin	> 0.83 mM	n.d.	0.104 mM

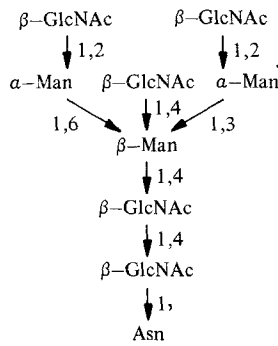
Sialoside 9



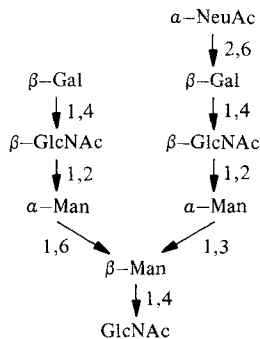
Inhibitory dose with

Concanavalin A	0.026 mM	0.026 mM	0.013 mM
<i>Lens</i> agglutinin	0.415 mM	0.087 mM	0.104 mM
<i>Vicia</i> agglutinin	0.104 mM	0.044 mM	0.052 mM
<i>Pisum</i> agglutinin	0.415 mM	n.d.	0.415 mM

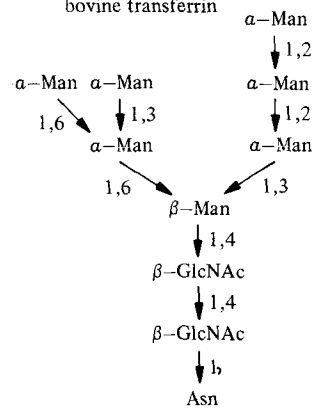
Glycopeptide from ovotransferrin



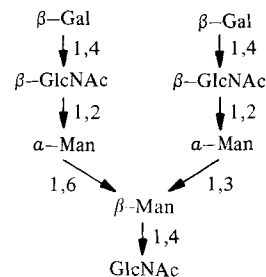
Sialoside 5



Glycopeptide from bovine transferrin



Asialo form of sialoside 9



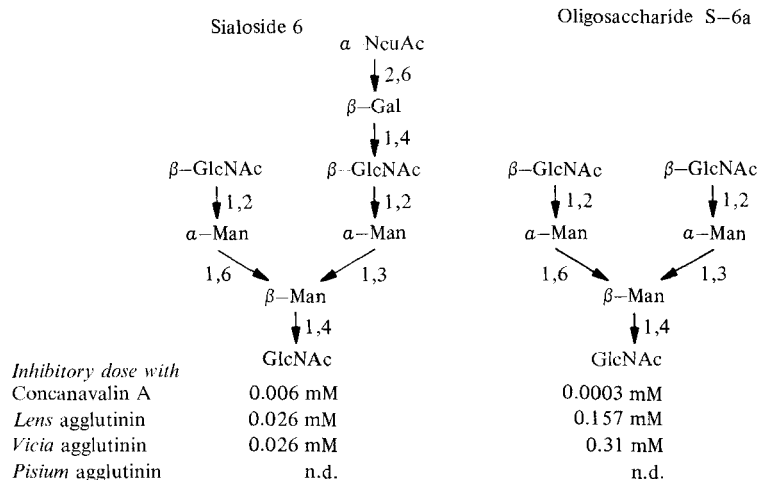


Fig. 3. Saccharidic determinants recognized by concanavalin A, and *Lens*, *Vicia* and *Pisum* agglutinins on glycans and oligosaccharides from glycoproteins with the N-glycosylamine linkage. Results are expressed as the minimum concentration of sugar (mM) necessary to completely inhibit one hemagglutinating dose

of the lectin seem confirmed. The oligosaccharidic sequence recognized by *Vicia* agglutinin must possess N-acetylglucosamine residues and the trimannosidic core with the α -(1 $\rightarrow$ 6)-linked fucose to fit perfectly with the combining site of the lectin, also suggesting a rather extended site. As shown in Table 3, *Vicia* agglutinin presents, as *Lens* agglutinin, a greater affinity for saccharidic sequences substituted by sialic acid residues bound in the C-6 position rather than in the C-3 position of galactose residues.

However, fine differences between the two lectins exist: the presence of the N-acetylglucosamine-asparagine sequence in the binding reaction of *Vicia* agglutinin does not seem to be as important as for *Lens* agglutinin since sialoside 9, derived from the human transferrin glycopeptide, is only eightfold less inhibitory. In the case of *Lens* agglutinin, it was 130-fold less inhibitory.

Pisum sativum Agglutinin

As for *Lens* and *Vicia* agglutinins, the best inhibitor found for *Pisum* agglutinin is also glycopeptide 1 from human lactotransferrin and as for the two other lectins, removal of the α -linked fucose residue gives a glycopeptide (like that from human serotransferrin) 17-fold less inhibitory (Fig. 3). These results are also in accord with those of Allen et al. [24] and Van Wauwe et al. [29] and are in favor of the existence of a hydrophobic area in or near the carbohydrate-binding site of the lectin. Moreover, as for *Vicia* agglutinin, the oligosaccharidic sequence recognized by *Pisum* agglutinin must possess N-acetylglucosamine sequences and the trimannosidic core, with this α -(1 $\rightarrow$ 6)-linked fucose residue to fit perfectly with the combining site of the lectin.

As in the case of *Vicia* agglutinin, sialoside 9 lacking the N-acetylglucosamine-asparagine sequence is also 32-fold less inhibitory than the glycopeptide itself (from human serotransferrin).

These results show that, if concanavalin A is quite different from the other three lectins, there are also fine differences between the *Lens*, *Vicia* and *Pisum* agglutinins as was previously pointed out [24].

Our results confirm for the last three lectins the existence of a hydrophobic area in or near the carbohydrate-binding site and this can be the cause of non-specific interactions

between the lectin and aromatic residues of glycopeptides or glycoproteins as described [30,31] for concanavalin A.

LECTINS WITH A SPECIFICITY FOR GALACTOSE AND N-ACETYLGALACTOSAMINE

Ricinus communis Agglutinin I

Results of the inhibition by various oligosaccharidic structures of hemagglutination by *Ricinus* agglutinin I are presented in Table 4.

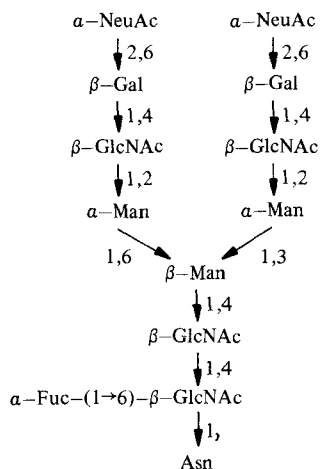
As shown in Fig. 4, the best inhibitor found for *Ricinus* agglutinin I is the asialo form of sialoside 9 which presents two β -(1 $\rightarrow$ 4)-linked galactose residues very accessible to the lectin. Sialoside 5, which possesses only one such residue, sialoside 6 in which the two galactose residues are lacking and sialoside 9, in which the galactose residues are substituted by α -(2 $\rightarrow$ 6)-linked sialic acid residues, are less inhibitory. These results show that *Ricinus* agglutinin I recognizes the N-acetylglucosamine sequence β -Gal-(1 $\rightarrow$ 4)-GlcNAc. Oligosaccharides S-6a and S-5b, which possess the same core structure as the above sialosides but without any β -linked galactose residue, are not inhibitory at all.

As shown in Table 3, *Ricinus* agglutinin I shows a greater affinity for saccharidic sequences possessing galactose residue(s) substituted by sialic acid residue(s) in the C-6 position rather than in the C-3 position. These results we preliminarily reported [4,5] have been confirmed in the meantime by Baenziger et al. [32]. Thus, as for the *Lens* and *Vicia* agglutinins, this observation can also be related to the higher rotational freedom of α -(2 $\rightarrow$ 6)-linkage, leaving in the case of *Ricinus* agglutinin I an N-acetylglucosamine sequence more accessible to the lectin.

Sialylated glycopeptides (from human serotransferrin and lactotransferrin) possess the same inhibitory power, but they are twofold more inhibitory than sialoside 9, suggesting a more rigid spatial configuration for the glycopeptides, maintaining an appropriate orientation of the saccharide sequence recognized by the lectin, in contrast to a random orientation for the sialoside itself.

One can also note that the two glycopeptides isolated from human lactotransferrin possess the same inhibitory

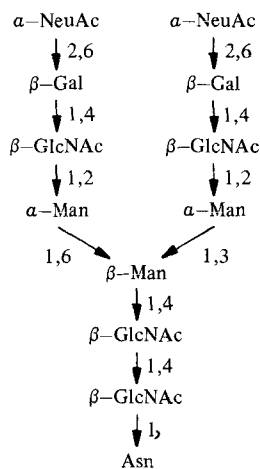
Glycopeptide 1 from human lactotransferrin



Inhibitory dose with

Ricinus agglutinin I 0.207 mM*Ricinus* agglutinin II > 0.415 mM

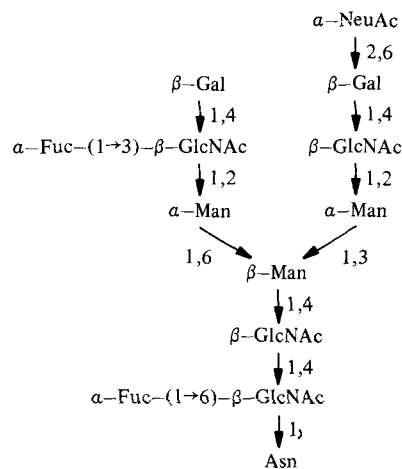
Glycopeptide from human serotransferrin



0.207 mM

0.207 mM

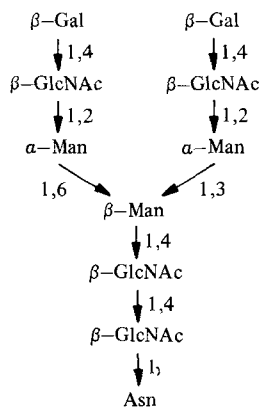
Glycopeptide 2 from human lactotransferrin



0.207 mM

0.104 mM

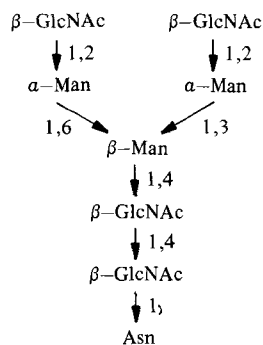
Asialo form of glycopeptide from human serotransferrin



Inhibitory dose with

Ricinus agglutinin I 0.104 mM*Ricinus* agglutinin II 0.104 mM

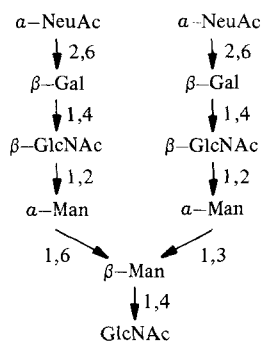
Asialoagalacto form of glycopeptide from human serotransferrin



> 0.83 mM

> 0.83 mM

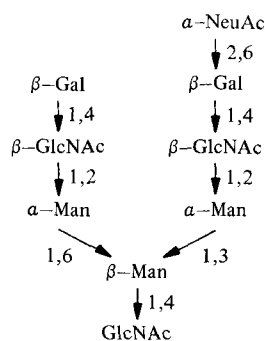
Sialoside 9



Inhibitory dose with

Ricinus agglutinin I 0.415 mM*Ricinus* agglutinin II 0.104 mM

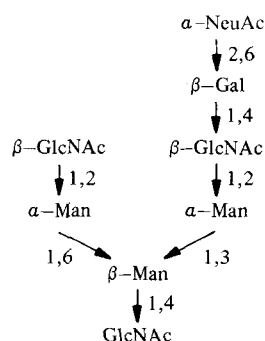
Sialoside 5



0.087 mM

0.051 mM

Sialoside 6



0.415 mM

0.104 mM

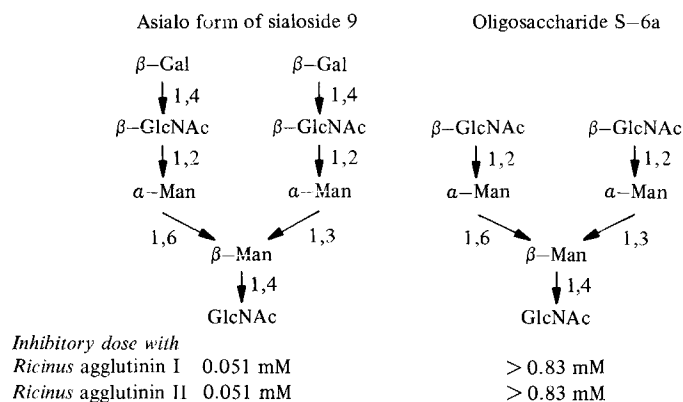


Fig. 4. Saccharidic determinants recognized by *Ricinus agglutinins I and II* on glycans or oligosaccharidic structures from glycoproteins with the *N*-glycosylamine linkage. Results are expressed as the minimum concentration of sugar (mM) necessary to completely inhibit one hemagglutinating dose

power in spite of an unsubstituted β -galactose residue in the case of glycopeptide 2. The presence of the α -(1 $\rightarrow$ 3)-linked fucose on the *N*-acetylglucosamine residue seems in this case to limit the accessibility of the *N*-acetylglucosamine sequence to the lectin.

Removal of the α -(2 $\rightarrow$ 6)-linked sialic acid residues from the human serotransferrin glycopeptide (asialo form) increases the inhibitory power twofold when compared to the sialylated form. Moreover, treatment of the desialylated product with β -D-galactosidase completely abolishes its inhibitory capacity.

It can also be noted that sialylated glycopeptides isolated from human transferrin and lactotransferrin possess the same inhibitory power. Thus, the fucose residue α -(1 $\rightarrow$ 6)-linked to the *N*-acetylglucosamine residue conjugated to asparagine seems to have no particular effect on the binding with the lectin. This result is in contrast to those of Adair and Kornfeld [33] who have found that a glycopeptide isolated from human IgG whose glycan structure is identical to that of the glycopeptide isolated from human lactotransferrin, was about threefold more inhibitory than the glycopeptide isolated from human transferrin, which lacks the α -(1 $\rightarrow$ 6)-linked fucose near the *N*-glycosidic linkage.

Ricinus communis Agglutinin II

Results of the inhibition by various oligosaccharidic structures of hemagglutination by *Ricinus agglutinin II* are presented in Table 4.

An important point is that the toxin *Ricinus agglutinin II*, is inhibited by *N*-acetylglucosamine whereas *Ricinus agglutinin I* is not. Otherwise, the carbohydrate-binding specificity of lectin II towards *N*-glycosidic-derived structures is very similar to that of lectin I: as shown in Fig. 4, the best inhibitor for *Ricinus agglutinin II* is also the desialylated sialoside 9 which presents two β -(1 $\rightarrow$ 4)-linked galactose residues very accessible to the lectin.

As for the lectin I, *Ricinus agglutinin II* also presents a greater affinity for saccharidic sequences with galactose residues substituted by α -(2 $\rightarrow$ 6)-linked rather than by α -(2 $\rightarrow$ 3)-linked sialic acid residues. These results differ from those of Baenziger et al. [32] who found indirectly, that in contrast to lectin I, lectin II could recognize both C-3 and C-6 substituted galactose residues in an *N*-glycosyl-type structure. However, in the same study, these authors found that neither lectin bound to *O*-glycosyl-type structures with sialic acid α -(2 $\rightarrow$ 3)-linked to the β -Gal-(1 $\rightarrow$ 3)-GalNAc units.

Table 4. Minimum concentration of various oligosaccharidic structures necessary to completely inhibit red cell agglutination by lectins specific for galactose and *N*-acetylglucosamine
n.d., not determined. For structures see Fig. 1 and 2

Compounds	Minimum concentration for inhibition with		
	<i>Ricinus</i> lectin		soybean lectin
	I	II	
	mM		
Galactose	2.5	0.625	1.25
<i>N</i> -Acetylglucosamine	> 5	0.312	0.019
Lactose	1.25	0.155	1.25
Melibiose	2.5	0.625	0.625
Human transferrin	> 0.008	> 0.008	> 0.008
Human lactotransferrin	> 0.008	> 0.008	> 0.008
Glycopeptide from human serotransferrin			
complete form	0.207	0.207	> 0.415
asialo form	0.104	0.104	0.415
asialoagalacto form	> 0.83	> 0.83	> 0.83
Glycopeptide from human lactotransferrin			
form 1	0.207	> 0.415	> 0.415
form 2	0.207	0.104	> 0.415
Neuraminyl-(α 2 $\rightarrow$ 3)-lactose	0.625	n.d.	n.d.
Neuraminyl-(α 2 $\rightarrow$ 6)-lactose	0.156	n.d.	n.d.
Oligosaccharide S-6a	> 0.83	> 0.83	n.d.
Oligosaccharide S-5b	> 0.83	> 0.83	n.d.
Sialoside 1	> 0.83	> 0.83	> 0.83
Sialoside 2	0.83	0.415	> 0.83
Asialo form of sialoside 2	0.415	0.207	0.415
Sialoside 3	0.83	0.312	> 0.83
Sialoside 4	0.415	0.104	> 0.83
Sialoside 5	0.087	0.051	0.415
Sialoside 6	0.415	0.104	> 0.83
Sialoside 9	0.415	0.104	0.415
Asialo form of sialoside 9	0.051	0.051	0.415

If the affinity of lectin II for *N*-acetylglucosaminic structures seems very similar to those of isolectin I, our results show that fine differences between the two lectins exist.

In contrast to the lectin I, *Ricinus agglutinin II* does not seem to require a proper orientation of the saccharidic

sequence recognized by the lectin, for sialoside 9 which possesses a random configuration as compared to the sialylated glycopeptide isolated from human transferrin is in fact two-fold more inhibitory.

It is also interesting to compare the inhibitory power of the glycopeptide from human serotransferrin to that of glycopeptides 1 and 2 from human lactotransferrin. The substitution of the *N*-acetylglucosamine residue taking part in the glycosylamine linkage by an α -(1 $\rightarrow$ 6)-linked fucose residue decreases the inhibitory power of this glycopeptide at least twofold. This result is also in contrast to that of Adair and Kornfeld [33] who have found that a glycopeptide isolated from human IgG was about four-fold more inhibitory than the glycopeptide isolated from human transferrin.

On the other hand, glycopeptide 2 from human lactotransferrin is at least fourfold more inhibitory than glycopeptide 1 from the same source. Here, in contrast to *Ricinus* agglutinin I, the α -(1 $\rightarrow$ 3)-linked fucose on the *N*-acetylglucosamine residue does not seem to limit the accessibility of the unsubstituted β -galactose residue to the lectin.

Differences between the two lectins towards glycopeptides with an *O*-glycosidic linkage, bearing β -Gal-(1 $\rightarrow$ 3)-GalNAc sequences have also been characterized by Baenziger et al. [32]. According to their results, *Ricinus* agglutinin II binds glycopeptides containing this sequence with greater association constant than agglutinin I, and can bind glycopeptides with terminal *N*-acetylgalactosamine residues, while agglutinin I cannot.

Soybean Agglutinin

Results of the inhibition by various oligosaccharidic structures of hemagglutination by soybean agglutinin are presented in Table 4.

The best inhibitor is *N*-acetylgalactosamine itself. Galactose and lactose are 70-fold less inhibitory than this sugar, and melibiose with a terminal non-reducing α -linked galactose is twofold more inhibitory than lactose.

These results are in good agreement with those of Pereira et al. [34] and Hammarström et al. [35] and confirm that soybean agglutinin presents the greatest affinity for *N*-acetylgalactosamine residues.

Desialylated glycopeptides or oligosaccharides (asialo form of the glycopeptide from human serotransferrin, asialo form of sialoside 9) of the *N*-acetylglucosaminic type, possessing exposed non-reducing β -(1 $\rightarrow$ 4)-linked galactose residues are slightly inhibitory as shown previously [36]. However enzymatic removal of these galactose residues (the asialoagalacto form of the glycopeptide from human serotransferrin) results in a complete loss of their inhibitory power. This point is contrary to the results of Irimura et al. [36] who find that porcine thyroglobulin β -glycopeptide, from which both sialic acid and galactose residues have been enzymically removed, is as good an inhibitor for soybean agglutinin as the desialylated glycopeptide.

LECTINS WITH A SPECIFICITY FOR *N*-ACETYLGLUCOSAMINE

Wheat-Germ Agglutinin

Results concerning the inhibition by various oligosaccharidic structures of hemagglutination by wheat-germ agglutinin, are presented in Table 5 and in Fig. 5.

Table 5. Minimum concentration of various oligosaccharidic structures necessary to completely inhibit red blood cell agglutination by lectins specific for *N*-acetylglucosamine

Compounds	Minimum concentration for inhibition with lectin from		
	wheat germ	<i>Solanum</i>	<i>Datura</i>
	mM		
<i>N</i> -Acetylglucosamine	> 15	> 15	> 15
Chitobiose	0.4	1.85	> 1.85
Chitotriose	0.0375	0.015	0.19
Human transferrin	> 0.008	> 0.008	> 0.008
Human lactotransferrin	> 0.008	> 0.008	> 0.008
Glycopeptide from human serotransferrin			
complete form	> 0.415	> 0.415	0.415
asialoagalacto form	> 0.415	> 0.415	> 0.415
Glycopeptide from human lactotransferrin			
form 1	> 0.415	0.207	0.207
form 2	> 0.415	0.207	0.207
Glycopeptide from hen ovomucoid	0.65	0.104	0.026
Glycopeptide from ovotransferrin	> 0.415	0.415	0.052
Sialoside 2	> 0.415	0.415	> 0.415
Asialo form of sialoside 2	> 0.415	0.415	> 0.415
Sialoside 9	> 0.415	0.207	> 0.415
Asialo form of sialoside 9	> 0.415	> 0.415	> 0.415

The best inhibitor is chitotriose, chitobiose being 10-fold and *N*-acetylglucosamine 100-fold less inhibitory. These results are in agreement with those of Allen et al. [37], Goldstein et al. [38] and Monsigny et al. [39] showing that the combining site of wheat-germ agglutinin appears to be complementary to a sequence of three β -(1 $\rightarrow$ 4)-linked *N*-acetylglucosamine residues.

Of the various oligosaccharidic structures examined for their capacity to inhibit wheat-germ agglutinin, only the β -glycopeptide isolated from ovomucoid (see Fig. 2) shows a weak inhibitory activity. This glycopeptide contains the chitobiose sequence attached to asparagine by a glycosylamine linkage and possesses five exposed non-reducing *N*-acetylglucosamine residues. On the other hand, neither a glycopeptide isolated from ovotransferrin which possesses the same core sequence and only four exposed non-reducing *N*-acetylglucosamine residues, nor the asialoagalacto form of glycopeptide isolated from human serotransferrin, and also possessing the same core sequence but only two exposed non-reducing *N*-acetylglucosamine residues, are inhibitory.

However, Goldstein et al. [38] have found that wheat-germ agglutinin could interact with the glyco-asparagines β -Man-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$)-Asn and β -GlcNAc-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$)-Asn with equivalent inhibitory power to chitobiose, suggesting that in the case of the two last glycopeptides, this common chitobiose-asparagine sequence is no more accessible to the lectin. Recently, it has been shown [40,42] that wheat-germ agglutinin is able to interact specifically with non-reducing terminal *N*-acetylneuraminic acid residues of glycoproteins and glycolipids, because of the structural similarity due to the acetamido group of *N*-acetylneuraminic acid, and to a lesser degree with *N*-acetylgalactosamine. These interactions also involved

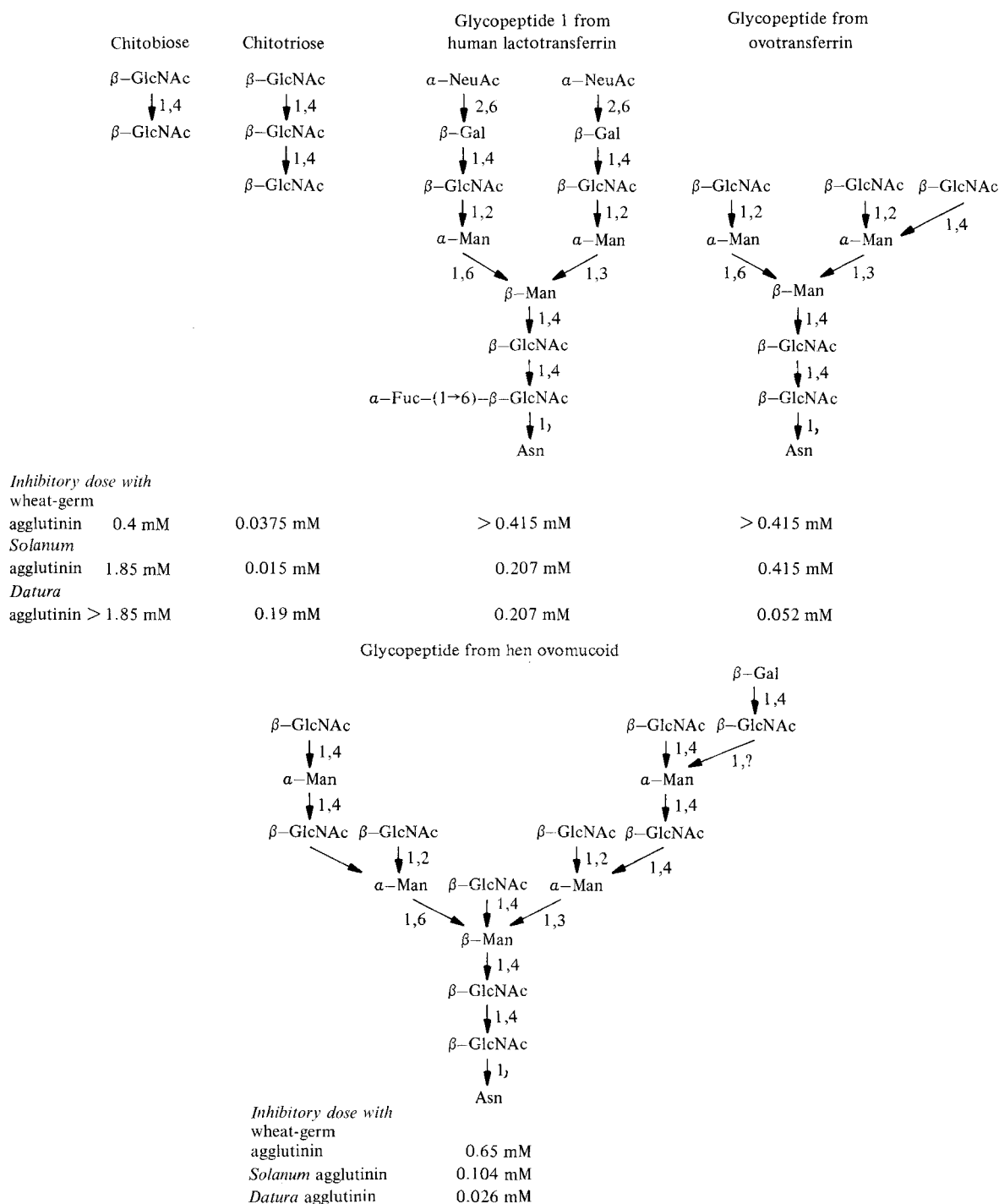


Fig. 5. Saccharidic determinants recognized by agglutinins from wheat germ, *Solanum* and *Datura* agglutinins on glycans and oligosaccharides from various glycoproteins. Results are expressed as the minimum concentration of sugar (mM) necessary to completely inhibit one hemagglutinating dose

a charge effect and what Monsigny et al. [41] call an 'avidity effect': glycoconjugates (glycoproteins, glycolipids or glycopeptides) with a high density of terminal non-reducing *N*-acetylglucosamine or *N*-acetylneuraminic acid residues will interact with wheat-germ agglutinin. This may be the case in the interaction with the β -glycopeptide isolated from ovomucoid which possesses five external non-reducing *N*-acetylglucosamine residues.

On the other hand, glycopeptides or oligosaccharides such as the glycopeptide from human serotransferrin or sialoside 9, which possess only two external *N*-acetylneuraminic acid residues, or the asialoagallo form of the glycopeptide from

human serotransferrin which possesses only two exposed non-reducing *N*-acetylglucosamine residues, are not inhibitory.

All these results show that the interactions between wheat-germ agglutinin and cell-surface glycoconjugates are very complex and have to be interpreted very carefully.

Solanum tuberosum Agglutinin

Results of the inhibition by various oligosaccharidic structures of hemagglutination by *Solanum* agglutinin, are shown in Table 5 and in Fig. 5.

The best inhibitor is, as for wheat-germ agglutinin, the chitotriose, chitobiose being 120-fold and *N*-acetyl-D-glucosamine at least 1000-fold less inhibitory. These results are slightly different from those of Allen et al. [43] who find that chitotriose is only twofold more inhibitory than chitobiose and that the best inhibitory is chitotetraose, suggesting that the *Solanum* agglutinin possesses an extended binding site as proposed for wheat-germ agglutinin [37].

Glycopeptide 1 from human lactotransferrin is ninefold more inhibitory than chitobiose, whereas the glycopeptide from human serotransferrin is not inhibitory at a maximal concentration of 0.415 mM. This result suggests that there is a hydrophobic area in or near the lectin-binding site which can interact with the methyl group of L-fucose in association with the recognition of the chitobiose sequence.

Like wheat-germ agglutinin, the *Solanum* agglutinin can bind external non-reducing *N*-acetylglucosamine residues, but only if there is a high density of such residues near the binding site: the β -glycopeptide isolated from ovomucoid, which possesses five external non-reducing *N*-acetylglucosamine residues is 18-fold more inhibitory than chitobiose. The glycopeptide from ovotransferrin, which possesses four such residues is fourfold less inhibitory and the asialoagalacto form of glycopeptide from human serotransferrin which possesses only two external *N*-acetylglucosamine residues is not inhibitory at a concentration of 0.415 mM.

As in the case of wheat-germ agglutinin, the binding site of *Solanum* agglutinin seems to be able to interact with non-reducing terminal *N*-acetylneuraminic acid residues, since sialoside 9, which possesses two such residues, is ninefold more inhibitory than chitobiose. However, it remains difficult to explain why the glycopeptide from human serotransferrin, which also possesses two external non-reducing *N*-acetylneuraminic acid residues, is not inhibitory at a maximal concentration of 0.415 mM.

Datura stramonium Agglutinin

As shown in Table 5, the best inhibitor is the β -glycopeptide isolated from ovomucoid which possesses five external non-reducing *N*-acetylglucosamine residues. The glycopeptide from ovotransferrin possessing only four of such *N*-acetylglucosamine residues is twofold less inhibitory and the asialoagalacto form of glycopeptide from human serotransferrin which possesses two external non-reducing *N*-acetylglucosamine residues is not inhibitory at a maximal concentration of 0.415 mM.

These results suggest that *Datura* agglutinin interacts with terminal non-reducing *N*-acetylglucosamine residues, but as for the wheat-germ and *Solanum* agglutinins, a high density of such residues seems to be necessary. *N*-acetylglucosamine itself is not inhibitory at a maximal concentration of 15 mM. Chitobiose and chitotriose are poor inhibitors for *Datura* agglutinin. Moreover, it has been found recently [44] that chitotetraose is fivefold more inhibitory than chitotriose, suggesting, as for the wheat-germ and *Solanum* agglutinins, an extended carbohydrate-binding site. The fact that glycopeptides 1 and 2 from human lactotransferrin are twofold more inhibitory than the glycopeptide from human serotransferrin suggests, as for the other lectins, a hydrophobic area in or near the lectin-binding site which could interact with the methyl group of L-fucose in association with the recognition of the chitobiose core sequence. In conclusion, the comparative study of the specificity of the three *N*-acetylglucosamine-binding lectins from wheat-germ, *Solanum* and *Datura*, shows

Table 6. Minimum concentration of various oligosaccharidic structures necessary to completely inhibit red blood cell agglutination by lectins specific for L-fucose

Compounds	Minimum concentration for inhibition with	
	<i>Lotus</i> lectin	<i>Ulex</i> lectin I
	mM	
L-Fucose	0.17	1.25
Human transferrin	> 0.008	> 0.008
Human lactotransferrin	> 0.008	> 0.008
Glycopeptide from human serotransferrin	> 0.415	> 0.415
Glycopeptide from human lactotransferrin		
form 1	0.415	> 0.415
form 2	0.207	> 0.415
Glycopeptide I	0.052	0.312
Glycopeptide II	0.207	> 0.415
Glycopeptide III	0.026	0.026
Fucoside 1	0.052	0.83
Fucoside 2	> 0.415	> 0.415
Sialoside 9	> 0.83	> 0.83

that very fine differences in their affinity towards defined saccharidic sequences exist.

LECTINS WITH A SPECIFICITY FOR L-FUCOSE

Lotus tetragonolobus Agglutinin

As shown in Table 6, the best inhibitor is the penta-fucosylated glycopeptide III (Fig. 1) which possesses four external non-reducing fucose residues α -(1 $\rightarrow$ 3)-linked to the *N*-acetylglucosamine of the four *N*-acetylglucosamine branches and one fucose residue α -(1 $\rightarrow$ 6)-linked to the *N*-acetylglucosamine of the glycosylamine linkage.

Since L-fucose itself is less inhibitory than glycopeptides III, I and fucoside 1, the specificity of *Lotus* agglutinin seems to be directed towards the α -(1 $\rightarrow$ 6)-linked fucose residue.

However, substitution of the structure of glycopeptide I decreases the inhibitory power of the glycopeptide: in fact, glycopeptide II is fourfold less inhibitory than fucoside 1 or glycopeptide I and glycopeptides 1 and 2 from human lactotransferrin which possess an α -(1 $\rightarrow$ 6)-linked fucose residue are eightfold less inhibitory. This finding seems, to indicate that substitution of the α -Fuc-(1 $\rightarrow$ 6)- β -GlcNAc-(1 $\rightarrow$)-Asn sequence limits the accessibility of this structure to the lectin. A similar conclusion is described by Pereira et al. [17] who have shown, using blood-group-active substances, related oligosaccharides isolated from urine and milk as well as various derivatives of L-fucose, that *Lotus* agglutinin is strongly specific for unsubstituted 'type-2 chains' containing an α -(1 $\rightarrow$ 2)-linked fucose residue on the terminal galactose, with or without another α -(1 $\rightarrow$ 3)-linked fucose residue substituting the penultimate *N*-acetylglucosamine residue. Substitution of the terminal non-reducing galactose with α -(1 $\rightarrow$ 3)-linked galactose destroys the inhibitory activity of the oligosaccharide, due to an inaccessibility of the α -(1 $\rightarrow$ 2)-linked fucose to the lectin.

The same authors have also shown that unsubstituted 'type-1 chains', possessing the same fucose substitutes but a β -Gal-(1 $\rightarrow$ 3)-GlcNAc or β -Gal-(1 $\rightarrow$ 3)-Glc core instead of

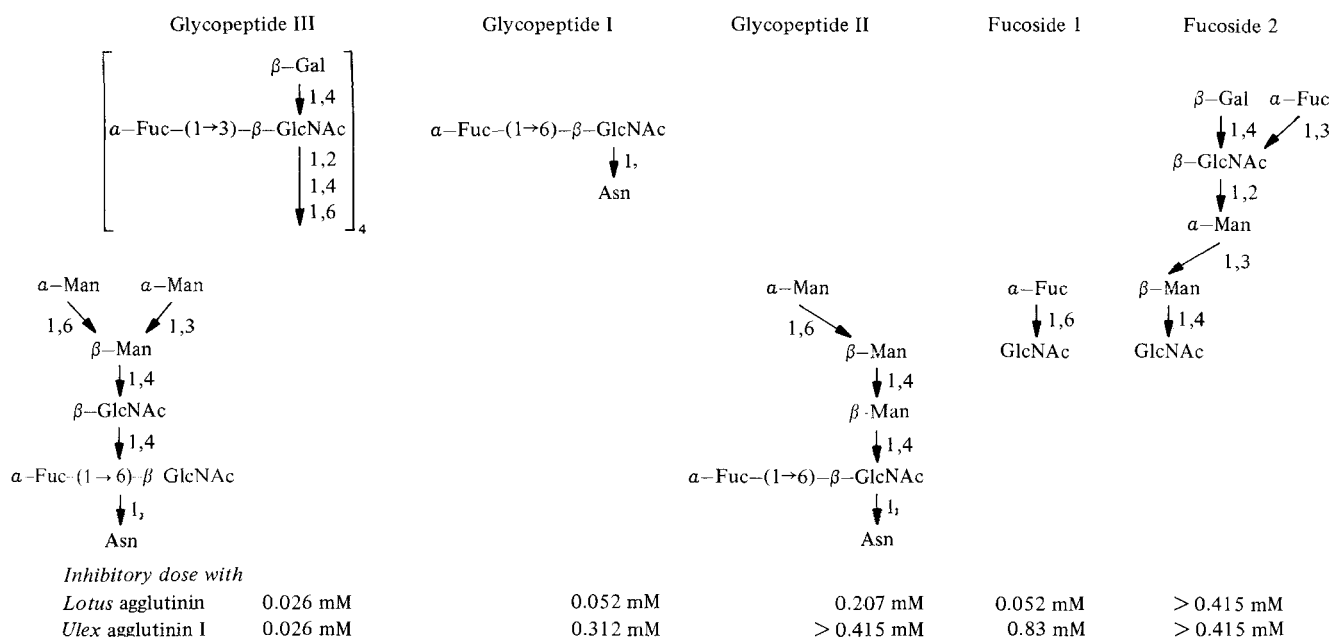


Fig. 6. Saccharidic determinants recognized by *Lotus* agglutinin and *Ulex* agglutinin I on glycans and oligosaccharides from glycoproteins with the N-glycosylamine linkage. Results are expressed as the minimum concentration of sugar (mM) necessary to completely inhibit one hemagglutinating dose

the β -Gal-(1 $\rightarrow$ 4)-GlcNAc or β -Gal-(1 $\rightarrow$ 4)-Glc core found in 'type-2 chains', are not inhibitory and this can also be explained by a spatial configuration of this core which makes the L-fucose residue inaccessible to the lectin.

In our results, it is noteworthy that fucoside 2 which lacks the α -Fuc-(1 $\rightarrow$ 6)- β -GlcNAc-(1 $\rightarrow$)-Asn sequence but possesses an external fucose α -(1 $\rightarrow$ 3)-linked to an N-acetylglucosamine residue, is not inhibitory at a maximal concentration of 0.415 mM. However, glycopeptide 2 from human lactotransferrin is twofold more inhibitory than glycopeptide 1 from the same source. This result seems to indicate, in the case of glycopeptide 2 from human lactotransferrin and glycopeptide III, the existence of an 'avidity effect' [41] in the interaction between *Lotus* agglutinin and these glycopeptides. The same result is also described by Pereira et al. [17] who have found that a difucosyl substituted 'type-2 chain' is two-fold more inhibitory than the monosubstituted 'type-2 chain' oligosaccharide.

Ulex europeus Agglutinin

Results of the inhibition by various oligosaccharidic structures of hemagglutination by *Ulex* agglutinin I are shown in Table 6 and Fig. 6.

As found previously [45], the major differences between the *Lotus* agglutinin and *Ulex* agglutinin I are that the latter presents higher affinity for type-O human red blood cells and that higher concentrations of inhibitors are necessary to obtain a complete hemagglutination inhibition in the presence of *Ulex* agglutinin I.

As for *Lotus* agglutinin, the best inhibitor found for *Ulex* agglutinin is glycopeptide III (Fig. 1). L-Fucose is a poor inhibitor and is 48-fold less inhibitory than glycopeptide III. As for *Lotus* agglutinin, substitution of the α -Fuc-(1 $\rightarrow$ 6)- β -GlcNAc-(1 $\rightarrow$)-Asn sequence decreases its inhibitory power, indicating limited accessibility to the lectin. Fucoside 2 which

lacks this sequence, but possesses an external fucose α -(1 $\rightarrow$ 3)-linked to an N-acetylglucosamine residue is not inhibitory at a maximal concentration of 0.415 mM. The relatively high inhibitory power of glycopeptide III can be then explained by the existence of an 'avidity effect'.

Recently, Pereira et al. [46], using various blood-group and milk oligosaccharides, have shown that the best inhibitor for *Ulex* agglutinin I is a blood-group-H monofucosyl oligosaccharide of the 'type-2 chain': α -Fuc-(1 $\rightarrow$ 2)- β -Gal-(1 $\rightarrow$ 4)- β -GlcNAc-(1 $\rightarrow$ 6)-R. This oligosaccharide is 900-fold more inhibitory than α -L-fucose itself. In contrast to the *Lotus* lectin, the addition of a second L-fucose α -(1 $\rightarrow$ 3)-linked to the N-acetylglucosamine or glucose residue reduces the inhibitory power of the oligosaccharide. This is why fucosyl oligosaccharides with 'type-1 chains' are as good inhibitors for *Ulex* agglutinin I as are fucosyl oligosaccharides with 'type-2 chains'.

All these results show that these two L-fucose-binding lectins, *Lotus* agglutinin and *Ulex* agglutinin I can be also very useful to characterize very fine differences in the glycan structure of cell-surface glycoconjugates.

CONCLUSIONS

This work represents a very indirect approach to the problem of lectin specificity since the best saccharide inhibitor found for a given lectin does not necessarily represent the true glycan site recognized by this lectin on a cell-surface glycoconjugate. In this connection, Adair et al. [33] have shown that surface glycopeptides isolated from red blood cells are 1200-fold more inhibitory for *Ricinus communis* agglutinin I than galactose itself. However in spite of this restriction, our results provide information on the specificities of some lectins commonly used as probes in the study of membrane glycoconjugates or for the fractionation of membrane glycoproteins. These results show that lectins con-

sidered 'identical' in terms of monosaccharide specificity, possess the ability to recognize fine differences in more complex structures.

Moreover, our results show that different lectins are able to recognize different saccharidic sequences, but belonging to the same glycan structure. As these sequences are likely to be common to numerous glycoproteins, including cell-membrane glycoproteins, the use of some of the lectins as a tool in the study of cell-surface carbohydrates seems to be limited. The same conclusion was drawn by Nilsson et al. [47] who found that different lectins bind to the same cell-surface glycoproteins. Kimura et al. [48] also arrive at this conclusion and show that commonly used lectins such as concanavalin A, *Lens* and wheat-germ agglutinins and *Ricinus* agglutinin I react with a broad spectrum of lymphocyte cell-surface glycoproteins.

The relative affinity of a lectin for a given glycan structure, as expressed in the different tables, can be compared with the true association constant of the lectin for this glycan: the lower is the minimum amount of the glycan necessary to inhibit hemagglutination by this lectin, the higher is the association constant of the lectin for this saccharide. Moreover, from these results, the ability of a given lectin insolubilized on Sepharose to fractionate oligosaccharides or glycopeptides containing the saccharide sequence recognized by the lectin can be predicted.

In addition, our results confirm previous data concerning the spatial configuration of the glycan moiety of glycoproteins. In fact, many lectins (*Lens* and *Vicia* agglutinins, and *Ricinus* agglutinins I and II) present a greater affinity for saccharidic sequences substituted by sialic acid residues bound in the α -(2 $\rightarrow$ 6) position of galactose rather than in the α -(2 $\rightarrow$ 3) position and this can be related to the high rotational freedom of α -(2 $\rightarrow$ 6)-linkage. Some lectins with an extended binding site, such as *Lens*, *Vicia* and *Pisum* agglutinins, react to a greater extent with their specific oligosaccharidic sequence, when this sequence belongs to a glycopeptide possessing the chitobiose-asparagine core than with the derived oligosaccharides lacking the β -GlcNAc-(1 $\rightarrow$)-Asn sequence. These results can be related to the fact that the chitobiose core gives to the whole glycan a more rigid conformation as opposed to the random configuration of the derived oligosaccharide; however, the presence of charges on asparagine could also be involved in the binding.

Finally, our results show that many lectins (from *Lens*, *Vicia*, *Pisum*, *Solanum* and *Datura*) seem to possess in or near their carbohydrate-binding site, a hydrophobic area such as that described for concanavalin A [25] and this could be the cause of non-specific hydrophobic interactions between the lectin and residues of glycopeptides or glycoproteins, as described for concanavalin A [30,31].

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