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AFFINITY OF FOUR IMMOBILIZED *Erythrina* LECTINS TOWARD VARIOUS N-LINKED GLYCOPEPTIDES AND RELATED OLIGOSACCHARIDES*

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ABSTRACT

The behavior of *N*-acetyllactosamine-type oligosaccharides and glycopeptides on columns of four different *Erythrina* agglutinins immobilized on Sepharose was examined. The sugar-binding specificity of the four lectins is very similar and is directed toward unmasked *N*-acetyllactosamine sequences, the main difference between the four lectins being the relative strength of interaction of the lectins with a given glycan. Substitution of the *N*-acetyllactosamine sequences by sialic acid residues, either at O-3 or O-6 of galactose completely abolishes the affinity of the lectins for the saccharides. The presence of one or several α-Fuc-(1→3)-GlcNAc groups decreases or completely inhibits the interaction between the glycopeptides and the *Erythrina* lectins. Substitution of the β-mannose residue by an additional bisecting β-(1→4)-*N*-acetylglucosamine residue decreases the affinity of the lectins for these structures as compared to the unsubstituted ones. Surprisingly, the affinity of the lectins for the oligosaccharides tested is higher than for the corresponding glycopeptides. Our findings show that, after careful calibration with well-defined oligosaccharides and glycopeptides, the immobilized *Erythrina* agglutinin–Sepharose columns provide valuable tools for the fractionation of *N*-acetyllactosamine-containing oligosaccharides and glycopeptides.

INTRODUCTION

Lectins are carbohydrate-binding and cell-agglutinating proteins of non-immune origin, which have been isolated from a variety of plants, animals, and microorganisms (for reviews, see refs. 1–3). Certain classes of lectins are often similar in terms of monosaccharide specificity, but frequently these proteins differ in terms of oligosaccharide and/or glycopeptide specificity[4,5]. Therefore, before

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fractionation of oligosaccharides and glycopeptides of diverse origins by affinity chromatography using immobilized lectins, the precise structural requirements of the lectins should be carefully determined.

Recently, the specificity of *Erythrina cristagalli* agglutinin was examined by hapten inhibition of agglutination[6], by a precipitin test using precursor human ovarian-cyst blood-group substance with *I* and *i* activities[7], and by spectroscopic methods[8]. The lectin was demonstrated to be specific for the unsubstituted β-Gal-(1→4)-GlcNAc structure that is present in the unsialylated branches of *N*-acetyllactosamine (or complex)-type N-linked carbohydrate units of glycoproteins.

Lectins from nine other species of *Erythrina* of widely different origins have been shown recently, by hapten inhibition of hemagglutination, also to belong to the D-galactose/*N*-acetylgalactosamine specificity-group[9].

In this paper, we report the behavior of 27 *N*-acetyllactosamine-type oligosaccharides and glycopeptides on four different immobilized *Erythrina* agglutinins isolated from *E. cristagalli*, *E. corallodendron*, *E. lysistemon*, and *E. latissima*.

EXPERIMENTAL

Preparation of lectins. — Lectins from seeds of *E. cristagalli*, *E. corallodendron*, *E. latissima*, and *E. lysistemon* were purified by affinity chromatography on a column of lactose-derivatized Sepharose 4B as described[6,9]. The lectins were coupled to Sepharose 4B that had been CNBr activated according to the procedure of March *et al.*[10]. The amounts of lectins bound to Sepharose were estimated to be 3 mg per mL of gel, by subtracting the amount of unbound protein found in the supernatant and washing solutions after coupling. Protein was determined by the method of Lowry *et al.*[11].

Origin of glycopeptides and oligosaccharides. — Oligosaccharides **1–3**, shown in Table I, having ^{14}C-labeled, terminal non-reducing D-galactosyl groups, were gifts from Dr. F. Piller (Centre National de Transfusion Sanguine, Paris). Oligosaccharides **4–18** and glycopeptide **25** of Table I, were isolated by Dr. G. Strecker from the urines of patients with various lysosomal diseases[12]. Glycopeptides isolated from human serotransferrin (structure **19**), from human lactotransferrin (structures **20–22**), and from turtle-dove ovomucoid (structure **27**) were gifts from Professor G. Spik. Glycopeptides **23** and **24**, isolated from human α_1-acid glycoprotein, were obtained from Professor B. Fournet. Hybrid-type glycopeptide (structure **26**) isolated from hen ovalbumin was a gift of Dr. A. Kobata (Dept. of Biochemistry, University of Tokyo, Tokyo, Japan). The purity of all oligosaccharides and glycopeptides was established by ^1H-n.m.r. spectroscopy.

Labeling of glycopeptides and oligosaccharides. — Glycopeptides were labeled with [^{14}C]acetic anhydride (7 mCi/mmol, C.E.A., France) according to Koide *et al.*[13] and purified by gel filtration on a column of Biogel P2 equilibrated with water. Oligosaccharides **4–18** were labeled at the reducing-terminal *N*-

acetylglucosamine residues by reduction with tritiated potassium borohydride (50 Ci/mmol, C.E.A., France) according to ref. 14.

Affinity chromatography of oligosaccharides or glycopeptides on Erythrina *lectin–Sepharose columns.* — Radioactively labeled glycopeptides or related oligosaccharides ($2–30 \times 10^3$ d.p.m.; 0.1–10 nmol) were applied to the columns of lectin–Sepharose (1 × 10 cm) equilibrated at room temperature in a 10mM phosphate buffer pH 7.2 containing 0.15M sodium chloride (PBS). Elution was performed first with the foregoing buffer at a flow rate of 9 mL/h and then with PBS containing 0.15M D-galactose. Fractions of 1.5 mL were collected and aliquots counted by liquid scintillation in a Beckman LS-9000 scintillation counter. Recovery of radioactivity from the column was always >95%.

RESULTS AND DISCUSSION

The four elution profiles obtained when N-glycopeptides and related oligosaccharides are applied to immobilized *Erythrina* agglutinin–Sepharose columns are shown in Fig. 1. Oligosaccharides or glycopeptides are eluted at the void volume of the column (fraction FNR) because of a lack of interaction between the immobilized lectins and the saccharides. Other sugars, weakly interacting with the lectins, are eluted from the column by the starting buffer as retarded fractions (fractions FR) according to their affinity for the lectins: fractions FR1, FR2, and FR3 correspond to panels A, B, and C, respectively. Under the experimental conditions used, FR1 is eluted between fractions 1–15, FR2 between fractions 10–20, and FR3 as a broad peak between fractions 20–30.

There is a considerable overlap between fractions FR1 and FR2 when using a 1 × 10 cm column, which can be a limiting factor in the fractionation of oligosaccharides and glycopeptides of diverse origins. However, improvement in the separation of these fractions (FR1 and FR2) may be obtained by using a 0.5 × 20 cm column and by collecting 0.75-mL fractions (data not shown). The sharp elution profile obtained with 0.15M D-galactose in starting buffer (Peak FE; panel D) indicates a strong, specific interaction between the lectin and the bound saccharides.

Table I describes the behavior of 27 glycopeptides and oligosaccharides on the four immobilized *Erythrina* lectins. Generally, the four lectins interact with the same structural determinants, but the relative strengths of interaction, as demonstrated by the elution volumes, always follow the same order: *E. cristagalli* ≥ *E. corallodendron* > *E. lysistemon* ≥ *E. latissima*. The interactions of structures **13** or **23** are a good illustration of this fact. As previously shown by hapten-inhibition experiments of hemagglutination[6,9], the four *Erythrina* lectins belong to the D-galactose/*N*-acetyl-D-galactosamine specificity-group and they exhibit high preference for *N*-acetyllactosamine. The data presented in Table I confirm these results and further demonstrate that the four *Erythrina* lectins interact with all of the glycopeptides or oligosaccharides on condition that they possess at least one

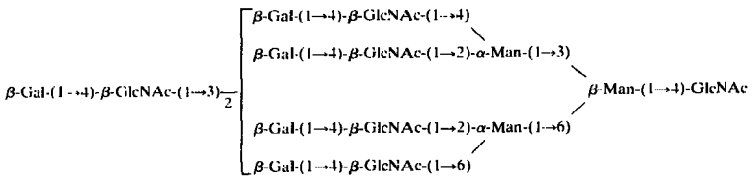
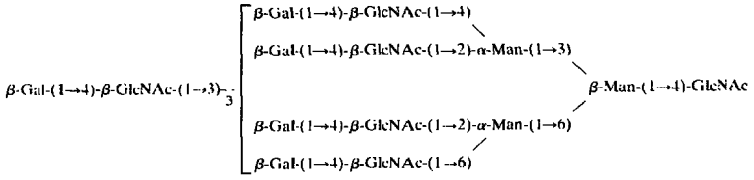
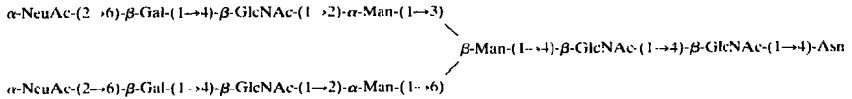
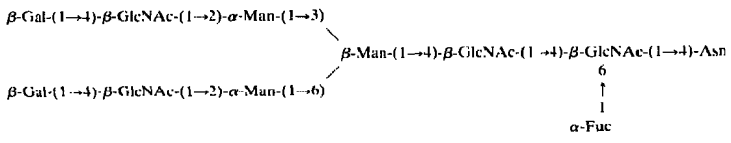
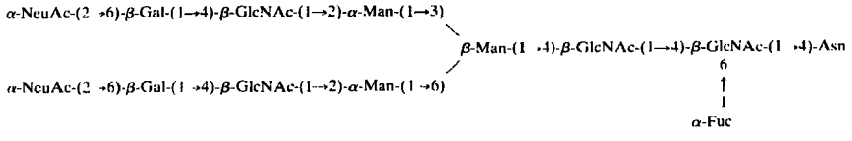
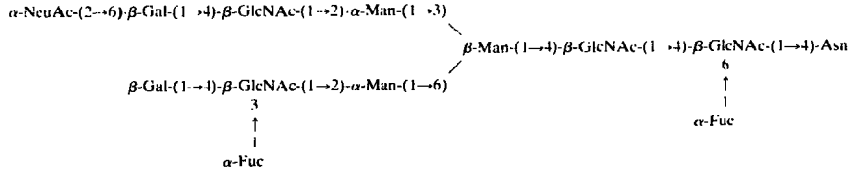
TABLE I

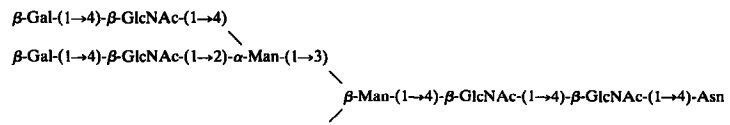
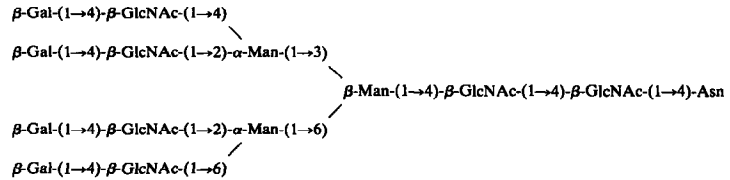
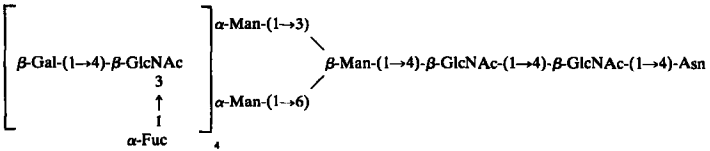
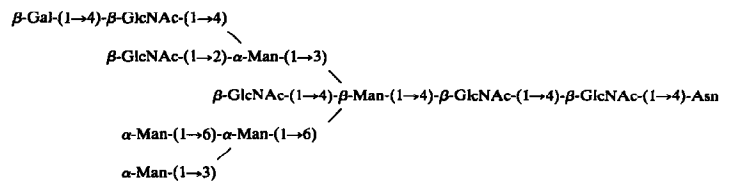
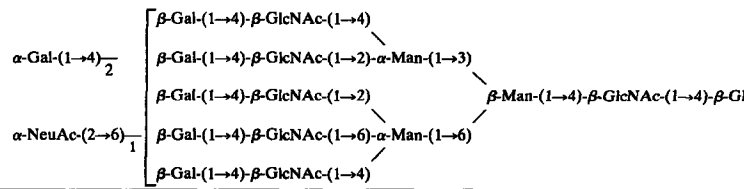
BEHAVIOR ON IMMOBILIZED *Erythrina* LECTIN-SEPHAROSE COLUMNS OF *N*-GLYCOSYLPEPTIDES AND RELATED OLIGOSACCHARIDES

Compound no.	*Glycan structure*	*E. cristagalli*	*E. corallodendron*	*E. lysistemon*	*E. latissima*
1	β-Gal-(1→4)-β-GlcNAc-(1→3)-β-Gal-(1→4)-GlcOMe	FR1	FR1	FR1	FNR
2	β-Gal-(1→4)-β-GlcNAc-(1→3)-β-Gal-(1→4)-β-GlcNAc-(1→3)-β-Gal-(1→4)-GlcOMe	FR1	FR1	FR1	FNR
3	β-Gal-(1→4)-β-GlcNAc-(1→3) β-Gal-(1→4)-GlcOMe β-Gal-(1→4)-β-GlcNAc-(1→6)	FR3	FR3	FR1	FR1
4	β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3)-β-Man-(1→4)-GlcNAc	FR2	ND	ND	ND
5	α-NeuAc-(2→3)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3)-β-Man-(1→4)-GlcNAc	FNR	FNR	FNR	FNR
6	α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) β-Man-(1→4)-GlcNAc α-Man-(1→6)	FNR	FNR	FNR	FNR
7	α-NeuAc-(2→3)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) β-Man-(1→4)-GlcNAc β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6)	FR1	FR1	FNR	FNR
8	α-NeuAc-(2→3)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) β-Man-(1→4)-GlcNAc α-NeuAc-(2→3)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6)	FNR	FNR	FNR	FNR
9	α-NeuAc-(2→3)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) β-Man-(1→4)-GlcNAc α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6)	FNR	FNR	FNR	FNR
10	α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) β-Man-(1→4)-GlcNAc α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6)	FNR	FNR	FNR	FNR

11
β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3)
β-GlcNAc-(1→4)-β-Man-(1→4)-GlcNAc
β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6)
FR2
FR1
FR1
FNR
12
β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3)
β-Man-(1→4)-GlcNAc
β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6)
FE
FR3
FR1
FR1
13
β-Gal-(1→4)-β-GlcNAc-(1→4)
β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3)
β-Man-(1→4)-GlcNAc
β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6)
FE
FE
FR2
FR1
14
β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3)
β-Man-(1→4)-GlcNAc
β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6)
β-Gal-(1→4)-β-GlcNAc-(1→6)
FE
FE
FR3
FR1
15
β-Gal-(1→4)-β-GlcNAc-(1→4)
β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3)
β-Man-(1→4)-GlcNAc
β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6)
β-Gal-(1→4)-β-GlcNAc-(1→6)
FE
FE
FE
FR3
16'
β-Gal-(1→4)-β-GlcNAc-(1→3)
β-Gal-(1→4)-β-GlcNAc-(1→4)
β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3)
β-Man-(1→4)-GlcNAc
β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6)
β-Gal-(1→4)-β-GlcNAc-(1→6)
FE
FE
FR1
FR1

TABLE 1 *(continued)*

Compound no.	*Glycan structure*	*E. cristagalli*	*E. corallodendron*	*E. lysistemon*	*E. latissima*
17[a]		FE	FE	FR1	FR1
18[a]		FE	FE	FE	FE
19		FNR	FNR	FNR	FNR
20		FR3	FR3	FR1	FR1
21		FNR	FNR	FNR	FNR
22		FR1	FR1	FNR	FNR

23		FE	FE	FR2	FR1
24		FE	FE	FR1	FR1
25		FNR	FNR	FNR	FNR
26		FR1	FR1	FR1	FNR
27[a]		FNR	FNR	FNR	FNR

[a]For oligosaccharides **16–18** and **27**, brackets around the antennae mean that the exact position of the mono- or oligosaccharide residues (1–3 residues) substituting the basic structures is not known.

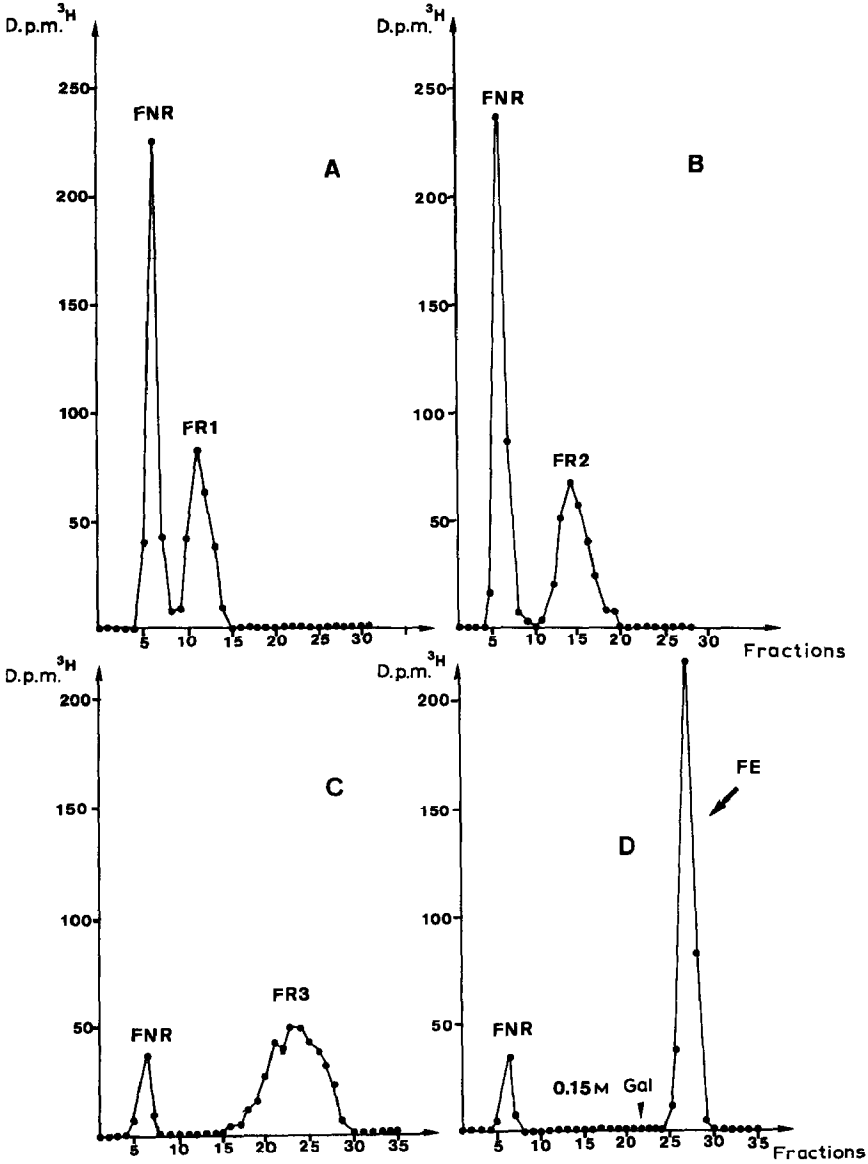


Fig. 1. Elution profiles of *N*-glycosylpeptides or related oligosaccharides on immobilized *Erythrina* lectin–Sepharose columns. Radioactively labeled glycopeptides or oligosaccharides (2–30 × 10^3 d.p.m.; 0.1 to 10 nmol) were applied to the lectin–Sepharose columns (1 × 10 cm) equilibrated at room temperature in a 10mM phosphate buffer pH 7.2 containing 0.15M sodium chloride (PBS). Elution was first performed with this buffer at a flow rate of 9 mL/h and then with PBS containing 0.15M D-galactose. Fractions of 1.5 mL were collected and aliquots counted by liquid scintillation in a Beckman LS-9000 scintillation counter. A: profile obtained when structure **11** (9000 d.p.m. ^{3}H) was applied to immobilized *Erythrina corallodendron* lectin–Sepharose column, giving the retarded fraction FR_1. B: profile obtained when structure **11** (9000 d.p.m. ^{3}H) was applied to immobilized *Erythrina cristagalli* lectin–Sepharose column, giving the retarded fraction FR_2. C: profile obtained when structure **12** (24000 d.p.m. ^{3}H) was applied to immobilized *Erythrina corallodendron* lectin–Sepharose column, giving the retarded fraction FR_3. D: profile obtained when structure **13** (67,000 d.p.m. ^{3}H) was applied to immobilized *Erythrina cristagalli* lectin–Sepharose column, giving the eluted fraction FE.

unmasked β-Gal-(1→4)-β-GlcNAc-(1→) sequence. The apparent lack of interaction of structures **1, 7**, or **26** with *E. lysistemon* and *E. latissima* could be explained by assuming that, similarly to immobilized concanavalin A and *Ricinus communis* agglutinin[15,16], immobilized *Erythrina* lectins only bind carbohydrates having association constants in solution greater than a minimal value; for concanavalin A and the *R. communis* lectin, this value was found[15,16] to be 4×10^6 M^{-1}.

Substitution of the *N*-acetyllactosamine sequences by sialic acid residues, either at O-3 or O-6 of galactose completely abolishes the affinity of the lectins for any saccharide.

Generally, the affinity between the *Erythrina* lectins and oligosaccharides increases with the number of *N*-acetyllactosamine sequences present in the carbohydrate structure. This is particularly clear in the interactions between immobilized *E. lysistemon* and structures **12–15**. Here again, however these interactions are modulated by the relative affinity of the immobilized lectins, and could be controlled by the amount of lectin coupled to Sepharose 4B.

It is noteworthy that immobilized *E. lysistemon* interacts more strongly with oligosaccharide **14** than with oligosaccharide **13**. Both are triantennary *N*-acetyllactosamine-type glycans, but in oligosaccharide **14**, one of the α-mannose residues is substituted by the third β-Gal-(1→4)-β-GlcNAc at O-6, whereas in oligosaccharide **13**, the substitution is at O-4. The freedom of rotation of carbohydrate structures around an α-(1→6) glycosidic linkage as compared to the relative rigidity of the α-(1→3)linkage[17] may explain the higher affinity of *E. lysistemon* observed for structure **14**. In contrast, using hapten inhibition of precipitin formation, Kaladas *et al.*[7] found that *E. cristagalli* interacted more strongly with oligosaccharide **13** than with oligosaccharide **14**.

The four lectins interact almost identically with the branched structure **3**, which possesses *I* activity and in which the two β-Gal-(1→4)-GlcNAc sequences are β-(1→3)- and β-(1→6)-linked to a galactose residue, and with the biantennary oligosaccharide **12** in which the sequences are β-(1→2)-linked to two mannose residues, *E. cristagalli* agglutinin presenting a slightly higher affinity for the biantennary oligosaccharide (structure **12**). This is in line with the finding that there is no difference in the susceptibility of human *I* and *i* erythrocytes to agglutination by the *E. cristagalli* lectin (Lis and Sharon, unpublished results).

The binding sites of the *Erythrina* lectins specifically recognize only one β-Gal-(1→4)-GlcNAc sequence, and the fact that oligosaccharides **3** or **12**, containing two such disaccharide structures, interact more strongly with the lectins confirms the ability of either sugar determinant to react with a single binding-site, as proposed previously[7]. However, oligosaccharide **2**, which possesses two such determinants but in a linear structure, interacts more weakly with the immobilized lectins than the branched biantennary oligosaccharides **3** or **12**, thus confirming that each binding-site accommodates only one *N*-acetyllactosamine residue. In this connection, it is noteworthy that the binding site of *Datura stramonium* agglutinin

appears to accommodate relatively large oligosaccharides and possesses the ability to interact with two repeating *N*-acetyllactosamine units[18].

Substitution of the β-mannose residue by an additional β-(1→4)-*N*-acetylglucosamine residue (structure **11**) decreases the affinity of the *Erythrina* lectins for this kind of structure as compared to the unsubstituted one (structure **12**). In this respect the *Erythrina* lectins are similar to RCA[19] and, to some extent, to Con A[15,19].

Surprisingly, the affinity of the four *Erythrina* lectins seems slightly higher for biantennary oligosaccharides (structure **12**) than for the corresponding glycopeptides (structure **20**). In the same way, *E. lysistemon* and *E. latissima* possess a lower affinity for the tetraantennary glycopeptide (structure **24**) than for the corresponding oligosaccharide (structure **15**). In contrast, many other lectins, e.g. *Lens culinaris* agglutinin or *Griffonia simplicifolia* agglutinin II, exhibit a higher affinity for glycopeptides than for related oligosaccharides[5,19]. This could be explained by the fact that the glycan–amino acid linkage leads to structure more rigid than those of the oligosaccharides[17]. However, the presence of charged groups on asparagine could also affect the binding.

The presence of one or several fucosyl residues linked α-(1→3) to the peripheral *N*-acetylglucosamine residues of multibranched saccharides of the *N*-acetyllactosamine type decreases (structure **22**) or completely abolishes (structure **25**) the interaction between the glycosylpeptide and the immobilized *Erythrina* lectins. Similar results have been previously obtained by hapten inhibition of hemagglutination induced by *E. cristagalli* agglutinin, showing that substitution of the β-Gal-(1→4)-GlcNAc sequence by α-L-fucose residues diminishes its inhibitory activity[7]. Such influence of α-Fuc-(1→3)-GlcNAc on binding to Con A and to erythro-agglutinating, as well as leuko-agglutinating lectins from *Phaseolus vulgaris*, has also been reported previously[20,21].

Immobilized *E. cristagalli*, *E, corallodendron*, and *E. lysistemon* possess a weak affinity toward a hybrid-type glycopeptide from hen ovalbumin (structure **26**), which presents a β-Gal-(1→4)-GlcNAc sequence accessible to the lectins. However, the *Erythrina* lectins do not show any affinity for a penta-antennary glycopeptide from turtle-dove ovomucoid (structure **27**), which possesses two unmasked *N*-acetyllactosamine sequences, and two *N*-acetyllactosamine sequences substituted each by an α-D-galactose residue and one such sequence substituted by an α-NeuAc residue, perhaps because of steric hindrance introduced by these substituting residues.

CONCLUSION

Our results confirm and extend previous results obtained by hapten inhibition of hemagglutination[6,9] and show that the four *Erythrina* lectins examined in this study are very similar with respect to specificity for *N*-acetyllactosamine-containing oligosaccharides, the main difference between the four lectins being their relative

strength of interaction with a given glycan. This similarity of lectins isolated from various species of *Erythrina*, as well as the amino acid-sequence homologies[9], indicates a high degree of conservation during evolution for these proteins. Various lectins from the *Viciae* tribe seem also to have been conserved at the level of their sugar-binding sites during evolution, as they exhibit a very similar fine specificity toward glycans of the *N*-acetyllactosamine type[22].

This study also shows that, after careful calibration with well-defined oligosaccharides and glycopeptides, the four immobilized *Erythrina* lectins could provide valuable tools for the fractionation and structural analysis of *N*-acetyllactosamine-containing oligosaccharides and glycopeptides of diverse origins.

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