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Binding and Precipitating Activities of *Erythrina* Lectins with Complex Type Carbohydrates and Synthetic Cluster Glycosides. A Comparative Study of the Lectins from *E. Corallodendron, E. Cristagalli, E. Flabelliformis,* and *E. Indica*

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***Erythrina* lectins possess similar structural and carbohydrate binding properties. Recently, tri- and tetra-antennary complex type carbohydrates with non-reducing terminal galactose residues have been shown to be precipitated as tri- and tetravalent ligands, respectively, with certain *Erythrina* lectins [Bhattacharyya L, Haraldsson M, Brewer CF (1988) Biochemistry 27:1034-41]. The present work describes a comparative study of the binding and precipitating activities of four *Erythrina* lectins, *viz., E. corallodendron, E. cristagalli, E. flabelliformis,* and *E. indica,* with multi-antennary complex type carbohydrates and synthetic cluster glycosides. The results show that though their binding affinities are very similar, the *Erythrina* lectins show large differences in their precipitating activities with the carbohydrates. The results also indicate significant dependence of the precipitating activities of the lectins on the core structure of the carbohydrates. These findings provide a new dimension to the structure-activity relationship of the lectins and their interactions with asparagine-linked carbohydrates.**

In recent years lectins have been isolated and characterized from more than a dozen species of the genus *Erythrina* [1, 2]. The lectins have strikingly similar physicochemical properties and show immunological cross-reactivity [1-7]. They are glycoproteins of molecular weight

Abbreviations: EAL, ECorL, ECL, EFL, and EIL represent the lectins from the seeds of *Erythrina arborescens, E. corallodendron, E. cristagalli, E. flabelliformis,* and *E. indica,* respectively; AFOS, the tri-antennary complex type oligosaccharide from asialofetuin; AFGP, the tri-antennary glycopeptide from asialofetuin; MeβGal, methyl β–D-galactopyranoside.
Unless stated otherwise all sugars are in the D-configuration.

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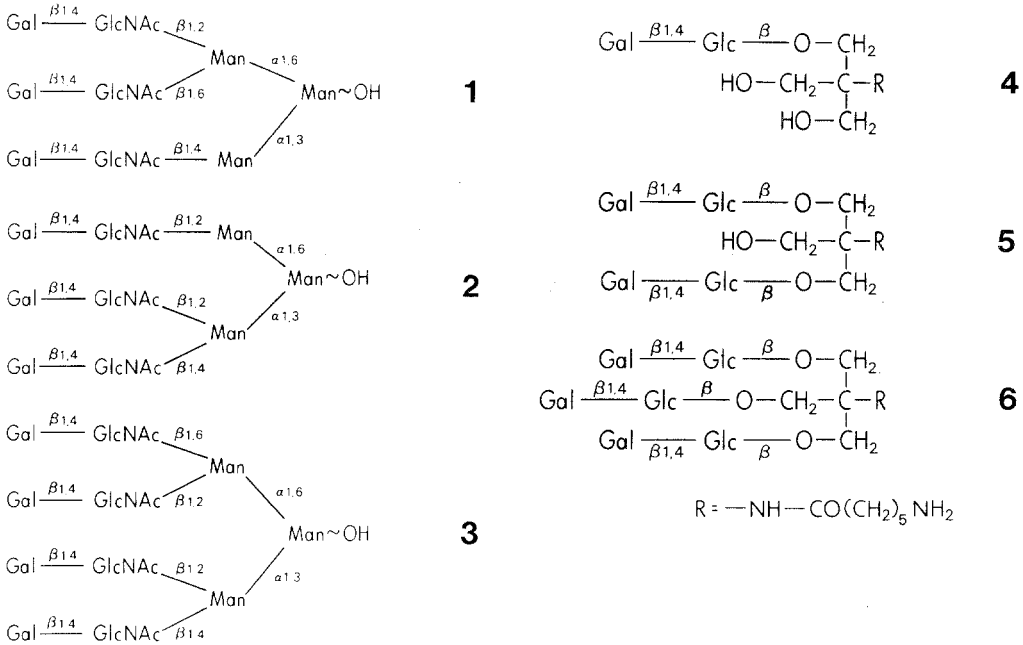

Figure 1. Structures of (a) the tri- and tetra-antennary complex type oligosaccharides, **1, 2,** and **3**, and (b) the cluster glycosides **4, 5,** and **6.**

57,000-68,000 and consist of two identical or nearly identical subunits. The lectins have a highly conserved *N*-terminal sequence, valine being the *N*-terminal amino residue in all cases [2, 6, 7]. The major oligosaccharide chains were isolated from *E. cristagalli* (ECL) and *E. corallodendron* (ECorL) lectins and shown to be structurally identical [8].

Studies of the binding of *Erythrina* lectins to simple sugars [5, 9-12] show that the disaccharide *N*-acetyllactosamine exhibits the highest affinity, where most of the binding energy is contributed by the non-reducing terminal galactose residue [9]. *Erythrina* lectins have also been shown to bind to certain asparagine-linked carbohydrates including bi-, tri-, and tetra-antennary complex type oligosaccharides and glycopeptides containing *N*-acetyllactosamine residues at the non-reducing terminii [5, 9-13]. Recently, the tri- and tetra-antennary complex type carbohydrates were demonstrated to be multivalent for certain galactose specific lectins, including the lectins from *E. indica* (EIL) and *E. arborescens* (EAL), and precipitate the proteins [9, 13]. Furthermore, recent studies with synthetic cluster glycosides, which have no structural resemblance to the complex type oligosaccharides other than having β-galactose residues at the non-reducing terminii [17], show that the tri-antennary cluster glycoside precipitates EIL as a trivalent ligand [14].

Table 1. Inhibition of hemagglutination by *Erythrina* lectins.

Sugar	Minimum concentration required for complete inhibition of 4 hemagglutinating doses[a] of			
	ECL	ECorL	EFL	EIL
MeβGal	14 mM	14 mM	7.0 mM	3.1 mM
Lactose	1.7 mM	3.4 mM	0.8 mM	3.1 mM
N-Acetyllactosamine	0.4 mM	0.4 mM	0.4 mM	0.4 mM
1	30 μM	40 μM	30 μM	32 μM[b]
2	40 μM	40 μM	40 μM	40 μM[b]
3	40 μM	—[c]	40 μM	40 μM[b]
6	0.26 mM	0.26 mM	0.26 mM	0.30 mM[d]
AFOS	65 μM	65 μM	65 μM	65 μM
AFGP	70 μM	70 μM	70 μM	70 μM

[a] Osawa and Matsumoto [20].
[b] Taken from Bhattacharyya *et al.* [9].
[c] Insufficient amount of oligosaccharide to test.
[d] Taken from Bhattacharyya and Brewer [14].

These observations have prompted interest in the relationship between the binding affinities and precipitating activities of closely related lectins with complex type carbohydrates and synthetic cluster glycosides. In the present paper, the binding and precipitating activities of three *Erythrina* lectins, ECL, ECorL, and EFL, with tri- and tetra-antennary complex type oligosaccharides and cluster glycosides are investigated, and the results compared with similar data for two other *Erythrina* lectins, EIL and EAL [9, 13, 14]. The major finding is that although all of the lectins have similar binding affinities, their precipitating activities with multi-antennary complex type carbohydrates and cluster glycosides differ considerably.

Materials and Methods

The *Erythrina* lectins were prepared as described [3-5]. The protein concentrations were determined spectrophotometrically using the extinction coefficients of 13.4 for EIL [3], and 15.9 [4, 5] for the other *Erythrina* lectins, and expressed in terms of subunit. The syntheses of complex type oligosaccharides **1, 2**, and **3**, and cluster glycosides **4, 5,** and **6** (Fig. 1) have been reported [15-17]. The tri-antennary asialoglycopeptide (AFGP) and the corresponding oligosaccharide analog containing one core *N*-acetylglucosamine residue (AFOS) (with the same non-reducing terminal structure as **2**) were isolated from fetuin by a modification of the previous procedure [18] as described earlier [9]. AFOS was obtained during the purification of AFGP [18] due to the presence of endo-β-*N*-acetylglucosaminidase activity in commercial pronase [19]. Mono- and disaccharides were obtained from Sigma Chemical Co., St. Louis, MO, USA. The concentrations of the carbohydrates were measured by the phenol-sulfuric acid method [20] using mannose as the standard. The purity of the glycopeptide and the oligosaccharides was checked by 500 MHz ^{1}H-NMR spectroscopy [21].

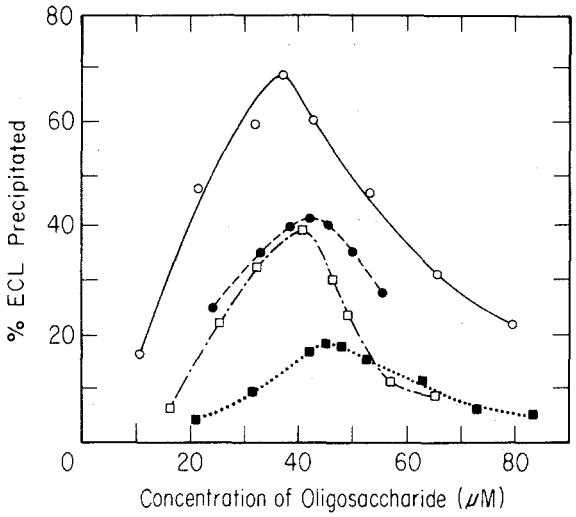

Figure 2. Precipitation of ECL with oligosaccharides **1** (●), **2** (□) **3** (○), and AFOS (■) at 22°C. See Table 2 for protein concentrations.

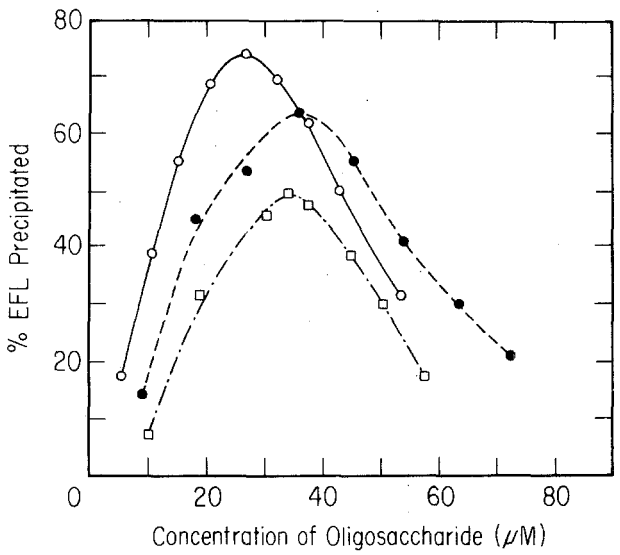

Figure 3. Precipitation of EFL with oligosaccharides **1** (●), 2 (□), and **3** (○) at 4°C. See Table 2 for protein concentrations.

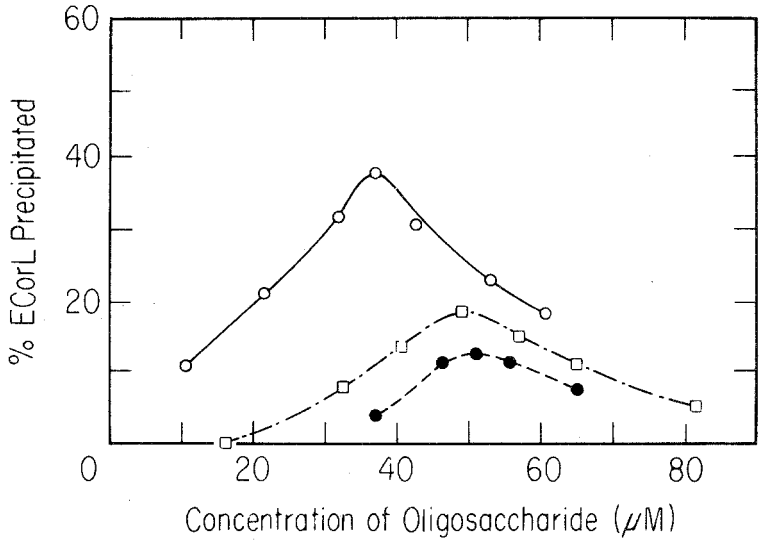

Figure 4. Precipitation of ECorL with oligosaccharides **1** (●), **2** (□), and **3** (○) at 4°C. See Table 2 for protein concentrations.

Hemagglutination Inhibition Assays

The assays were done at 22°C by the 2-fold serial dilution technique [22] in 10 mM sodium phosphate buffer, pH 7.2, containing 0.15 M NaCl, using a 3% (v/v) suspension of rabbit erythrocytes.

Quantitative Precipitation Assays

The assays were performed in 0.1 M Tris-HCl buffer, pH 7.2, containing 0.9 M KCl, 1 mM $MnCl_2$ and 1 mM $CaCl_2$ as described previously [23, 24].

Isoelectric Focusing

These were performed according to the method of Wrigley [25] using 7.5% gel and 1% ampholyte solution of the pH range 3-10.

Results and Discussion

Inhibition Assays

Table 1 shows the results of inhibition of hemagglutination by ECL, ECorL and EFL in the presence of simple sugars, complex type oligosaccharides **1, 2,** and **3**, and the glycoside **6**, as well as AFOS and AFGP. Inhibition data of **1, 2, 3,** and **6** for EIL were included for

Table 2. Stoichiometries of precipitation reactions of the *Erythrina* lectins with complex type oligosaccharides and glycopeptides.

Carbohydrates	Temperature (°C)	Lectin conc. (μM)[a]	Conc. of carbohydrates at equivalence point (μM)	Stoichiometry of precipitation reaction	Per cent lectin precipitated at equivalence point
		ECL			
1	22	131	42	1:3.1	41
2	22	131	41	1:3.2	39
	4	144	48	1:3.0	84
3	22	137	37	1:3.7	69
6	22	163		no precipitation	
	4	163	61	1:2.7	24
AFOS	22	135	45	1:3.0	18
	4	144	50	1:2.9	58
AFGP	22	144		no precipitation	
	4	141	51	1:2.8	37
		EFL[b]			
1	4	107	36	1:3.0	63
2	4	107	34	1:3.1	49
3	4	107	27	1:4.0	74
6	4	164		no precipitation	
AFOS	4	138	46	1:3.0	49
AFGP	4	134	41	1:3.3	36
		EIL			
2[c]	22	140	50	1:2.8	47
	4	140	48	1:2.9	80
AFOS	4	134	46	1:2.9	55
AFGP[c]	4	134	46	1:2.9	43
		ECorL[b]			
1	4	151	51	1:3.0	13
2	4	151	49	1:3.1	18
3	4	142	37	1:3.8	38
6	4	165		no precipitation	
AFOS	4	152		no precipitation	
AFGP	4	152		no precipitation	

[a] Lectin concentrations were calculated in terms of their subunit molecular weights of 34,000 for EIL [3], and 28,000 for the other lectins [4, 5].
[b] No precipitation at 22°C.
[c] Taken from Bhattacharyya *et al.* [9].

comparison [9, 13, 14]. The inhibitory concentrations of carbohydrates bear a linear relationship with their affinity constant values [26]. Thus, the results indicate that MeβGal and lactose have somewhat different affinities for most of the lectins. MeβGal binds best with EIL, while its affinities with ECL, ECorL, and EFL are 2-4 times weaker. Lactose binds 4-8 fold better than MeβGal with ECL, ECorL, and EFL, but equally well as MeβGal with EIL. *N*-Acetyllactosamine has the strongest affinity among the simple oligosaccharides tested and binds nearly equally well to all four *Erythrina* lectins. Oligosaccharides **1, 2,** and **3** bind with nearly 10-fold higher affinities than *N*-acetyllactosamine to the lectins. The data for **1,2,** and **3** binding to ECL and ECorL agree with results obtained by Debray *et al.* [12] for analogs of the oligosaccharides which contained an additional *N*-acetylglucosamine residue on the core β-mannose at the reducing terminus using *Erythrina* lectin-Sepharose columns. With each lectin, AFOS and AFGP are essentially equally potent, and have 5-6 fold higher affinity than *N*-acetyllactosamine. Glycoside **6** is also equally potent for binding with the three *Erythrina* lectins, but it has significantly lower affinity compared to the complex type oligosaccharides. The enhanced affinities of the complex type carbohydrates compared to *N*-acetyllactosamine, and **6** compared to lactose, are associated with an increase in the probability of binding due to the presence of multiple *N*-acetyllactosamine residues in the former molecules [9, 14].

*Quantitative Precipitation Studies with Oligosaccharides **1, 2, and 3***

Figs. 2, 3, and 4 show the results of quantitative precipitation analyses of ECL, EFL, and ECorL, respectively, with oligosaccharides **1, 2,** and **3**. The experimental uncertainty in the data is estimated to be ±3%. In each case, the precipitates were prevented from forming in the presence of 0.1 M MeβGal, and dissolved upon addition of the glycoside, indicating specificity of the precipitation reactions. The concentration of oligosaccharide at the equivalence point (point of maximum precipitation) [27] of each precipitation profile and the concentration of lectin subunit are shown in Table 2, together with the ratio of the concentrations. The ratio gives the stoichiometry of binding of the oligosaccharide to the lectin subunit [27].

Table 2 shows that oligosaccharides **1, 2**, and **3** precipitate with ECL at 22°C, and **2** precipitates more ECL at 4°C than at 22°C. In a preliminary experiment, **1** was found to give very weak turbidity with EFL at 22°C. However, at 4°C the oligosaccharide gave much stronger precipitation, and therefore, studies were carried out at the lower temperature (Fig. 3). The oligosaccharides do not give any visible precipitation with ECorL at 22°C. Even at 4°C, precipitation of ECorL is very weak compared to the other *Erythrina* lectins (Fig. 4).

The stoichiometry of the precipitation reaction between the oligosaccharides and the lectin subunits are approximately 1:3 for **1** and **2**, and 1:4 for **3** (Table 2).

Quantitative Precipitation Studies with Cluster Glycosides

Table 2 shows that ECL precipitates with tri-antennary cluster glycoside **6** at 4°C. The precipitation profile is broad indicating weak interaction (profile not shown). Similar results have been found for EIL [14]. Both EIL [14] and ECL do not precipitate with glycoside **6** at 22°C, nor do EFL or ECorL at either 22 or 4°C. The absence of precipitation between EIL or

ECL with glycoside **6** at 22°C is consistent with the lower affinity of the glycoside compared to the tri-antennary complex type oligosaccharides (Table 1). The lectins do not precipitate with the mono- and bi-antennary glycosides, **4** and **5**, at either 22 or 4°C.

Effect of the Size of Core Structure of Carbohydrates on Precipitating Activities of Lectins

Oligosaccharides **2**, AFOS, and AFGP have the same non-reducing terminii, but differ in the size of their core structures. Table 2 shows that the extent of precipitation of EIL and ECL at 4°C decreases with increasing size of the core region of the carbohydrates, even though the affinity of the lectin for all three carbohydrates is nearly the same. Similar results are found with EFL. ECL also precipitates more with oligosaccharide **2** than AFOS at 22°C, and does not precipitate with AFGP at this temperature. Furthermore, ECorL precipitates with the oligosaccharide **2** at 4°C, albeit weakly, but not with AFOS and AFGP. These findins demonstrate that the precipitating activities of the lectins are sensitive to the size of the core region of the carbohydrate(s), which may be due to steric factors involved in the formation of the precipitates.

Valency of the Carbohydrates

Table 2 shows that the stoichiometries of binding of the tri- and tetra-antennary carbohydrates to lectin subunits are 1:3 and 1:4, respectively, for all *Erythrina* lectins. Both EIL and ECL contain one carbohydrate binding site per subunit [3, 11]. Therefore, oligosaccharides **1, 2**, AFOS, and glycoside **6,** as well as glycopeptide AFGP are trivalent for ECL binding, while oligosaccharide **3** is tetravalent. Similar results are found for EIL [9, 14]. Data on the number of binding sites per subunit for ECorL and EFL are not available. However, the similarity in the structural and carbohydrate binding properties of the *Erythrina* lectins [1-6], and the stoichiometry data in the present study suggest that **1, 2**, AFOS, and AFGP are trivalent, and **3** is tetravalent for ECorL and EFL binding as well.

Precipitating Activities of the Lectins

Although their binding affinities (Table 1) are similar, the *Erythrina* lectins show significant differences in precipitation activities toward **1, 2, 3** or **6** (Table 2). EIL [9] and ECL precipitate with the oligosaccharides at both 4 and 22°C, EFL only at 4°C, and ECorL weakly at 4°C. A fifth *Erythrina* lectin, EAL, also weakly precipitates with the oligosaccharides at 4°C [9].

The tetra-antennary oligosaccharide **3** forms a larger amount of precipitate with each lectin than the tri-antennary oligosaccharides **1** and **2**. This is consistent with the higher valency of the tetra-antennary oligosaccharide compared to that of the two tri-antennary oligosaccharides [27]. However, the latter two carbohydrates display differences in their precipitation with the lectins. Although **1** and **2** possess similar affinities, **1** precipitates more EFL and EIL [9] than **2**. On the other hand, both oligosaccharides precipitate to nearly the same extent with ECL, while **2** precipitates more ECorL than does **1**. Thus, the precipitating activities of EFL, EIL, and ECorL are sensitive to the branching patterns of the two tri-antennary oligosaccharides, while ECL and EAL [9] are not.

ECL, EFL, and EIL, but not ECorL, precipitate with AFGP at 4°C. Thus, even though the affinities of the lectins for the glycopeptide are essentially the same, ECorL possesses no

precipitating activity with AFGP, while the other three lectins exhibit nearly equal precipitating activities with the glycopeptides. Similar results are found with the tri-antennary glycoside **6**. The glycoside has the same affinity with ECL, ECorL, EFL and EIL, however, it precipitates with EIL [14] and ECL at 4°C, but not with the other lectins.

The results raise the question as to the molecular mechanism(s) responsible for the different precipitating activities of the *Erythrina* lectins. In order to determine whether unfavourable charge interactions destabilize cross-linked complexes of certain lectins, isoelectric focusing experiments were carried out to determine the relative charge states of the proteins [25]. The results show that their isoelectric focusing patterns are very similar: each lectin shows three major bands with isoelectric points at approximately 5.0, 5.2 and 5.6. (The results for EIL were previously reported [3]). Thus, the overall charge of EIL, ECL, ECorL and EFL is the same at pH 7.2 and, therefore, the possibility of different charge states of the lectins influencing the stability of cross-linked complexes can be eliminated. Furthermore, results with EIL show that salt concentration does not influence the precipitation reaction [9], indicating that the overall charge of the protein does not have a significant effect.

Another factor which may influence the precipitating activities of the *Erythrina* lectins is the solubility of the individual proteins. However, in general, the solubility of globular proteins of similar molecular mass, amino-acid composition and overall charge are similar [28]. Lastly, structural differences between the lectins could effect the stability of their precipitation lattices. Although the lectins in the present study have similar affinities for carbohydrates, differences in the location of their binding sites or the geometry of the bound carbohydrate-protein complex could have a large influence on the structures of their respective cross-linked complexes. Thus, differences in the precipitating activities of the lectins may be related to the formation of different cross-linked lattices between the proteins and the carbohydrates. In this regard, evidence has been presented for the formation of different cross-linked lattices between the lectin concanavalin A and a series of closely related asparagine-linked glycopeptides [29, 30].

Conclusions

The present results together with our previous studies]9, 13, 14] demonstrate that five closely related lectins from different *Erythrina* species exhibit different precipitating activities with complex type carbohydrates. The cross-linking activities of these proteins may be of importance to the specificity of their interactions with multivalent carbohydrates since it was recently shown that concanavalin A forms homogeneous cross-linked complexes with certain bivalent asparagine-linked oligosaccharides [29, 30]. Preliminary studies indicate that certain *Erythrina* lectins possess similar properties (unpublished results). These results may relate to the biological properties of *Erythrina* lectins.

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