Defining the Carbohydrate Specificities of *Erythrina corallodendron* Lectin (ECorL) as Polyvalent Galβ1-4GlcNAc (II) > Monomeric II > Monomeric Gal and GalNAc

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Background: *Erythrina corallodendron* lectin (ECorL) is one of the potent applied lectins. In previous studies, the carbohydrate specificities of this lectin were limited to monosaccharides, simple oligosaccharides and several clusters. However, the polyvalent factor has not been investigated.

Methods: The binding properties at the combining sites of ECorL were characterized by sensitive enzyme-linked lectinosorbent (ELLSA) and inhibition assays, using our collection of ligands and polyvalent natural glycans with known glycotopes.

Results: Results of both binding and inhibition assays revealed a very high affinity between ECorL and Galβ1-4GlcNAc (II)-containing glycoproteins. Among soluble natural glycans tested for inhibition, the high-density polyvalent II glycotopes, such as *Streptococcus pneumoniae* type 14 capsular polysaccharide which is composed of repeating poly-II residues, resulted in 2.4×10^4, 1.4×10^3 and 8.6×10^2-fold higher affinities to ECorL than the monomeric Gal, linear II and tri-antennary II, respectively, at the non-reducing end in *N*-linked glycopeptides (Tri-II). The ECorL-glycan interaction was also strongly inhibited by most of the other high-density II-containing glycoproteins. Although GalNAc was as potent an inhibitor as Gal, its polyvalent structural units were poor inhibitors.

Conclusions: [1] Galβ1-4GlcNAc (II) and other Galβ1-related oligosaccharides are essential for binding. [2] Their polyvalent form in glycoproteins is the most important binding factor for ECorL, while II monomer and oligo-antennary II forms play only a limited role in binding. [3] Although GalNAc is more active than Gal for ECorL, its reactivity is not changed by polyvalent effects. This lectin may be used as a tool to study glycobiology in basic and medical sciences.

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Key words: ECorL, lectin, carbohydrate specificity, polyvalency

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Lectins are carbohydrate-specific reagents and biological recognition molecules. Those lectins that can be used as tools to study glycobiological systems are defined as 'applied' lectins. Many applied lectins have been widely used to study glycobiology and biomedicines.[1-5] These include the study of the structural and functional roles of cell surface carbohydrates, isolation of mutants resistant to the cytotoxic action of some lectins, and investigation of the distribution and mobility of cell surface glycoproteins on normal and malignant cells. They have also been used for blood grouping, for determination of secretor status, and as reagents for isolation and characterization of glycoproteins. In order to broaden the essential knowledge required for application and interpretation, the carbohydrate recognition factors of lectins need to be thoroughly characterized.

Erythrina corallodendron lectin (ECorL) is isolated and purified from the seeds of *Erythrina corallodendron* by an affinity chromatographic column consisting of lactose coupled to Sepharose.[6] This lectin is a dimeric glycoprotein (4.5% carbohydrate) of 60.2 kDa consisting of two identical subunits of 28 kDa.[6-8] Each subunit is glycosylated by two *N*-linked oligosaccharides. ECorL forms a non-canonical dimer, in which two monomers are arranged back-to-back, forming a handshake motif.[9] The lectin is Gal/GalNAc specific and exhibits a pronounced preference for GalNAc.[10] It agglutinates human erthrocytes of groups A, B, O and AB, with a slight preference for O-type cells and is mitogenic for human peripheral blood lymphocytes but does not stimulate mouse thymocytes or splenocytes.[6] The best natural ligand for ECorL is the H-2 (LFucα1-2Galβ1-4GlcNAc) blood type determinant fucosyl-N-acetyllactosamine.[11,12] ECorL has been studied in terms of specific recognition of carbohydrates and was limited to oligo-antennary Galβ1-4GlcNAc (II) as the most active ligand.[6,10,12,13] It reacts more strongly with fucosyllactose and fucosyllactosamine than with *N*-acetyllactose, lactose, *N*-acetylgalactosamine and galactose.[6,10,12-14] However, the affinity of ECorL for many mammalian sugar structural units and the effects of polyvalent glycotopes on their binding to the lectin have not been thoroughly investigated. Therefore, the recognition factors of ECorL were analyzed in this study using our collection of ligands and polyvalent natural glycans with known glycotopes and a sensitive enzyme-linked lectinosorbent assay (ELLSA).

MATERIALS AND METHODS

Lectin

Biotinylated *Erythrina corallodendron* lectin (ECorL) was purchased from Vector Laboratories (Burlingame CA, USA).

Glycoproteins and polysaccharides

The glycoproteins tested were prepared from human ovarian cyst fluid, saliva, and hog gastric mucosa.[15-21] Active substances of the blood groups A, B, H, Le^a, Le^b and Ii were purified from human ovarian cyst fluid (HOC) by digestion with pepsin, precipitation with increasing concentrations of ethanol and extraction of the dried ethanol precipitates with 90% phenol. The insoluble fraction was named after the blood group substance (e.g. Cyst Tighe phenol insoluble, where "Tighe" denotes the HOC sample code). A similar principle was applied to other HOC collections (e.g. Beach, MSS, and N-1, etc). The supernatant was fractionally precipitated by addition of 50% ethanol in 90% phenol to the indicated concentrations.[15] The designation "10 (or 20) % (ppt)" denotes a fraction precipitated from phenol at an ethanol concentration of 10 (or 20)%; "2x" signifies that a second round of phenol extraction and ethanol precipitation was carried out (e.g. Cyst MSS 10% 2x and Cyst N-1 Le^a 20% 2x). The carbohydrate chains of HOC consisted of multiple saccharide branches attached by *O*-glycosidic linkages at their internal reducing ends to serine or threonine residues of the polypeptide backbone.[17-19,22] In general, the "P-1" fractions (e.g. Cyst Beach P-1 and Cyst Tighe P-1) represent the nondialyzable portion of the blood group substances after mild hydrolysis at pH 1.5-2.0 for 2 h which removes most of the L-fucopyranosyl end groups, as well as some blood group A and B active oligosaccharide side-chains.[16,23,24] P-1 fractions from HOC gps which exposed the internal structures equivalent to those on the blood group precursors are defined as "precursor equivalent gps" (Fig. 1).

Hog gastric mucin #4, a blood group A + H substance, was derived from crude hog stomach mucin as described previously (Fig. 2).[25] Treatment of mucin #4 with HCl (pH 2, 100°C, 90 min) yielded hog gastric mucin #9, while acid hydrolysis (pH 1.5, 100°C, 2 and 5 hrs) yielded hog gastric mucins #14

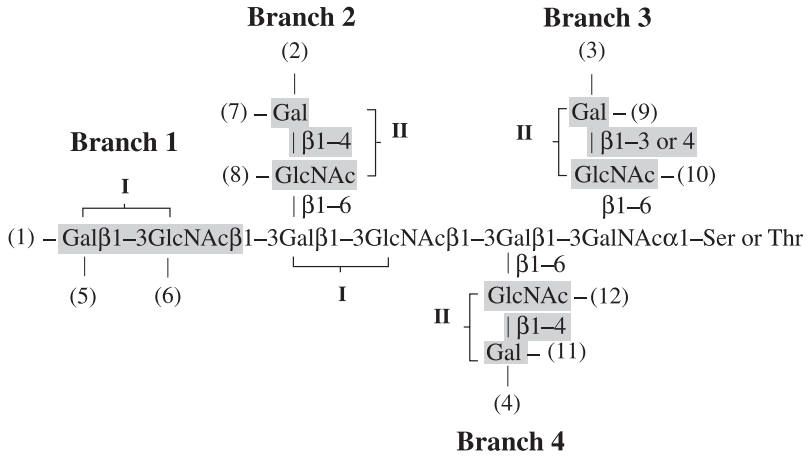

Fig. 1 Proposed representative carbohydrate side chains of blood group active glycoproteins, prepared from the human ovarian cyst fluid. This structure represents the internal portion of carbohydrate chains to which various human blood group determinants are attached. The four-branched structure (1 to 4) shown is the representative internal portion of the carbohydrate moiety of blood group substances to which the residues responsible for A, B, H, Le^a, and Le^b activities are attached. This structure also represents precursor blood group of active glycoproteins[20] and can be prepared by Smith degradation of A, B, H active glycoproteins, purified from human ovarian cyst fluids.[18-20,24] Numbers in parentheses indicate the site of attachment for the human blood group A, B, H, Le^a, and Le^b determinants. These determinants as well as the structural units at the nonreducing end are the sources of lectin reactive A/A_h, B, I/II, T, and Tn determinants.[14] This megalo-saccharide of twenty-four sugars has not been isolated. However, most of the carbohydrate chains isolated are parts of this structure. Shaded areas (II) are proposed to be the reactive glycotopes for ECorL.

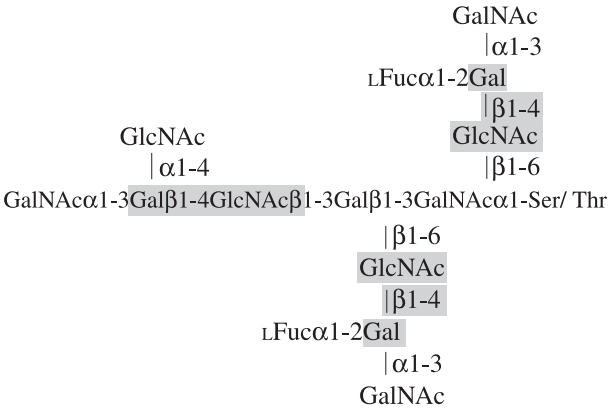

Fig. 2 Proposed structure of carbohydrate side chains of Hog A + H gastric glycoproteins.[32] Shaded areas (II) are proposed to be the reactive glycotopes for ECorL.

and 21, respectively. Extensive hydrolysis led to destruction of blood group activities.[26]

Human α_1-acid glycoprotein was purchased from Sigma (St. Louis, MO, U.S.A.). Human α_1-acid glycoprotein contains tetra-, tri- and di-antennary complex type glycans in a ratio of 2:2:1 (Fig. 3).[27,28] Fetuin (Gibco Laboratories, Grand Island, NY, USA), which is the major glycoprotein in fetal calf serum, has six oligosaccharide side chains per molecule, three *O*-glycosidically-linked to Ser/Thr and three *N*-glycosidically-linked to Asn[29] which contains tri- and di-antennary complex type glycans in a ratio of 1:2.

Tamm-Horsfall gp (THGP) which was kindly provided by the late Dr. W.M. Watkins, was isolated with 0.58 M NaCl from the urine of a single donor (W.T.J.M.) with the Sd (a^+) blood group by the method of Tamm and Horsfall.[30,31]

Mucus gp (or native bird-nest gp), the so-called nest-cementing substance from the salivary gland of Chinese swiftlets (genus *Collocalia*),[32] was extracted with distilled water at 60°C for 20 min from commercial bird-nest substance (Kim Hing Co., Singapore).

Ovine, bovine, porcine and human submandibular/salivary mucins or glycoproteins (OSM, BSM, PSM and HSM) were purified according to the method of Tettamanti and Pigman[33] with modifications.[34,35] About 75% of the carbohydrate side chains of asialo OSM were GalNAcα1-Ser/Thr (Tn). Asialo

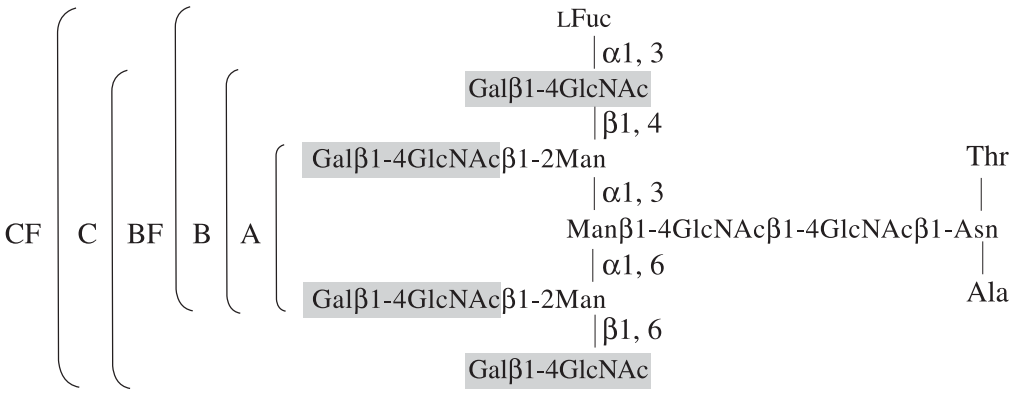

Fig. 3 Structure of human plasma α_1-acid glycoprotein. The primary structure of class A, B, BF, C and CF carbohydrate units of the glycosylation site in human plasma α_1-acid glycoprotein[27] is indicated in the above structure for asialo-orosomucoid. The carbohydrate units of this asialoglycoprotein can be grouped into compounds with a biantennary (class A), triantennary (class B), and triantennary II structure with a fucose residue (class BF). Shaded areas (II) are proposed to be the reactive glycotopes for ECorL.

PSM contains Galβ1-3GalNAcα1- (T_α) together with Tn and GalNAcα1-3Gal (A) sequences, as most of the outer fucosyl residues and sialic acids are cleaved by mild acid hydrolysis. Native ASG-Tn,[36] a salivary glycoprotein of the nine-banded armadillo (*Dasypus novemcinctus mexicanus*) containing only Tn (GalNAcα1-Ser/Thr) as carbohydrate side chains, was isolated from 0.01 M phosphate buffered saline (PBS) pH 6.8 gland extract after removal of ASG-A, which is one of the sialoglycoproteins in armadillo salivary glands.[37]

The anti-freeze gp was from an Antarctic fish (*Trematomus borchgrevinki*) which contains only T_α as carbohydrate chains[38] was provided by Dr. R. E. Feeney (Department of Food Science and Technology, University of California, Davis, CA, USA) through the late Dr. E. A. Kabat (Columbia Medical Center, New York, NY, USA).

The *Pneumococcus* type XIV polysaccharide was isolated from *Streptococcus pneumoniae* capsule,[39] was a generous gift from the late Dr. E.A. Kabat (Fig. 4).

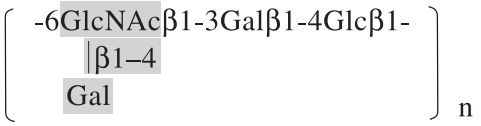

Fig. 4 Repeating units of the *Pneumococcus* type 14 capsular polysaccharide. The shaded area (II, Galβ1-4GlcNAc) is proposed to be the reactive glycotope for ECorL.

Sugars used for inhibition studies

Mono-, di- and oligo-saccharides were purchased from Sigma or prepared by Dextra (Berkshire, UK). Tri-antennary II glycopeptides (Tri-II) were prepared from asialo fetuin by pronase digestion and repeatedly fractionated by BioGel P-4 400 mesh column chromatography.[40] The Tn clusters used for this study were mixtures of Tn containing glycopeptides from OSM in the filterable fraction (Molecular mass cut-off < 3000).[41]

Microtiter plate lectin-enzyme binding assay

ELLSA was performed according to the procedures described.[42,43] The volume of each reagent applied to the plate was 50 μl/well, and all incubations, except for coating, were performed at room temperature (20°C). The reagents, if not indicated otherwise, were diluted with Tris-HCl buffered saline containing 0.05% Tween 20 (TBS-T). TBS buffer or 0.15 M NaCl containing 0.05% Tween 20 was used for washing the plate between incubations.

Ninety six-well microtiter plates (Nunc, MaxiSorp, Vienna, Austria) were coated with gps in 0.05 M carbonate buffer, pH 9.6, and incubated overnight at 4°C. After washing the plate, biotinylated lectins (50 ng) were added to each well and incubated for 30 min. The plates were washed to remove

unabsorbed lectin and ExtrAvidin/alkaline phosphatase solution (Sigma, diluted 1:10,000) was added. After 1 h, the plates were washed at least four times and incubated with *p*-nitrophenyl phosphate (Sigma 104 phosphatase substrate 5 mg tablets) in 0.05 M carbonate buffer, pH 9.6, containing 1 mM $MgCl_2$ (1 tablet/5 ml). The absorbance was read at 405 nm in a microtiter plate reader, after 4 h incubation with the substrate.

For inhibition studies, serially diluted inhibitor samples were mixed with an equal volume of lectin solution containing a fixed amount of lectin. The control lectin sample was diluted two-fold with TBS-T. After 30 min incubation at 20°C, samples were tested in the binding assay, as described above. The inhibitory activity was estimated from the inhibition curve and is expressed as the amount of inhibitor (ng or nmol/well) giving 50% inhibition of the control lectin binding.

All experiments were done in duplicate or triplicate, and data are presented as the mean value of the results. The standard deviation did not exceed 12% and in most experiments was less than 5% of the mean value. For the binding experiments, the control wells, where coating or addition of biotinylated lectin was omitted, gave low absorbance values (below 0.1), indicating that blocking the wells before lectin addition was not necessary when Tween 20 was present in the TBS.

RESULTS

ECorL-glycan interaction

The interaction patterns of ECorL with blood group active substances and gps and glycans by ELLSA are shown in Fig. 5 and the binding profiles for glycoproteins are illustrated in Table 1. Among the glycoproteins tested, several high-density Galβ1-4/3GlcNAc (II/I) containing gps reacted best with ECorL. These include asialo bird nest gp (Fig. 5B), and blood group ABH(O) precursor equivalent gps prepared from human ovarian cyst fluid (cyst OG 10% 2x ppt in Fig. 5D, cyst Tighe P-1 in Fig. 5C; Fig. 1). They required less than 95 ng to reach 1.5 absorbance units at A_{405}. ECorL also bound strongly with glycans, including many structure II or II/I-containing *N*-linked and *O*-linked gps (asialo human α_1-acid gp, Fig. 5A and asialo Tamm-Horsfall Sd. (+) urinary glycoprotein, Fig. 5B; asialo fetuin, Fig. 5A) and cyst N-1 Le^a 20% 2x (Fig. 5D). This lectin reacted moderately to slightly with other II/I-containing *N*-linked gps (asialo bovine α_1-acid gp, Fig. 5A), some human blood group ABH precursor equivalent gps (cyst Mcdon P-1, Fig. 5C, cyst Beach P-1, Fig. 5C, cyst MSS 1st Smith degraded, Fig. 5C, cyst Tij 20% of 2nd 10% 2x, Fig. 5D), human blood group ABH active gp (cyst Beach, Fig. 5D) and mild-acid treated hog mucin #9, #14, #21 and #4 (Fig. 5E). ECorL was found to be weakly reactive or inactive with Tn-containing gps (asialo BSM, asialo PSM, asialo OSM, asialo HSM, and asialo ASG, Fig. 5F) and sialic acid containing glycoproteins, such as human α_1-acid, fetuin (Fig. 5A), bird nest gp (Fig. 5B) and cryptic Tn-containing gps (PSM, BSM, OSM, HSM and ASG, Fig. 5F).

Inhibition of ECorL-glycoform interaction by various polyvalent glycotopes in glycoproteins

To exclude the possibility that the affinity differences of these gps were due to plate adsorption discrepancies, the binding affinity was also examined by inhibition assay as described in Materials and Methods. The ability of various glycoproteins to inhibit the binding of ECorL with an oligo-antennary Galβ1-4GlcNAc (II)-containing gp (asialo human α_1-acid gp) was also analyzed by ELLSA. The inhibition profile is shown in Fig. 6 and the amounts of glycoprotein (nanogram) required for 50% inhibition are listed in Table 2.

Among the glycans tested for inhibition, *Pneumococcus* type 14 capsular polysaccharide, composed of repeating poly II residues (Fig. 4), was the most powerful inhibitor requiring only 0.7 ng to inhibit the interaction. It was 2.4×10^4, 1.4×10^3 and 8.6×10^2 times more active than Gal, monomeric II and tri-antennary II, respectively (curve 1 vs. curves 32, 29 and 28, Fig. 6A and Table 2). Most of the other high-density II/I or II-containing gps were also potent inhibitors. These included asialo bird nest gp (curve 2, Fig. 6A), blood group precursor equivalent II/I-containing gps (curves 3-6 and 10-12, Fig. 6B), mild-acid hydrolyzed hog gastric mucin A+H substances (hog gastric mucin #14 and #21, curves 7 and 8, Fig. 6A), and *N*-linked multi-antennary II-containing gps (asialo human α_1-acid gp, curve 14 and asialo THGP, curve 16, Fig. 6A). Their activities were between 9.4×10^2 and 1.3×10^4 times higher than that of monomeric Gal (curves 2-14, 16 vs. curve 32,

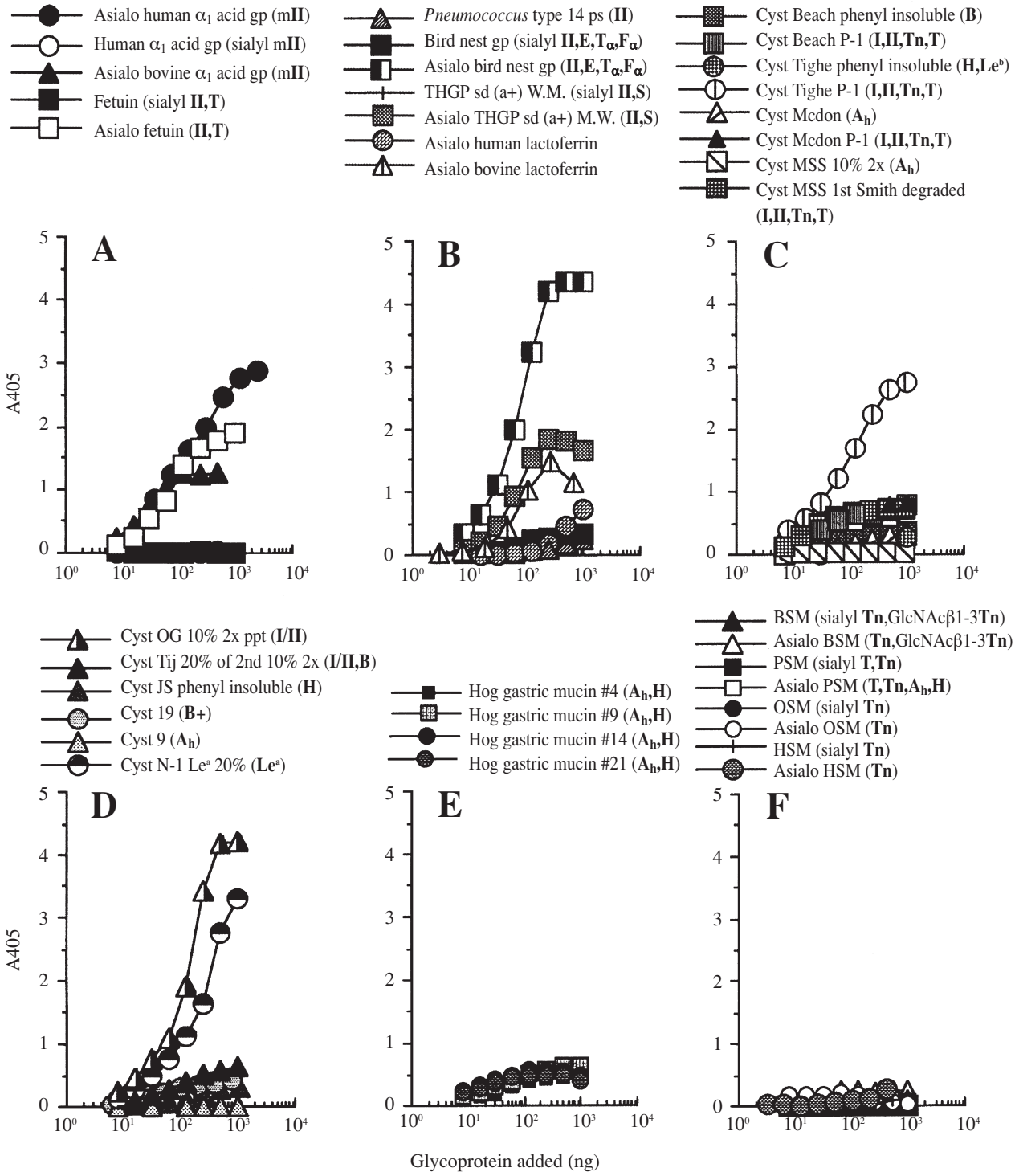

Fig. 5 Binding of ECorL to microtiter plates coated with serially diluted human blood group A, B, H, Le^a and Le^b active glycoproteins, sialo- and asialoglycoproteins. The amount of ECorL used was 50 ng per well. Total volume of the assay was 50 μl. A_{405} was recorded after 4 h incubation.

Table 1. Binding of ECorL to Human Blood Group A, B, H, Le^a and Le^b Active Glycoproteins (gps), Sialo- and Asialo Glycoproteins as Determined by ELLSA*

Graph in Fig. 5	Glycoprotein (lectin determinants†; blood group specificity)	1.5 (A_{405}) unit (ng)	Maximum A_{405}	
			Absorbance reading‡	Binding intensity‡
Blood group precursor equivalent gps				
D	Cyst OG 10% 2x ppt (I/II)	95.0	4.2	+++++
C	Cyst Tighe P-1 (I, II, T, Tn)	95.0	2.8	+++++
C	Cyst Mcdon P-1 (I, II, T, Tn)	–	0.8	+
C	Cyst Beach P-1 (I, II, T, Tn)	–	0.8	+
C	Cyst MSS 1st Smith degraded (I, II, T, Tn)	–	0.7	+
E	Hog gastric mucin # 14 (I/II)	–	0.6	+
D	Cyst Tij 20% 2X (I/II, B)	–	0.6	+
E	Hog gastric mucin # 21 (I/II)	–	0.5	+
Oligo-antennary Galβ1-4GlcNAc (II) in *N*-linked gps				
A	Asialo human α_1-acid gp (mII)	150.0	2.9	+++++
A	Asialo fetuin (mII, T)	180.0	1.9	+++
A	Asialo bovine α_1-acid gp (mII)	–	1.3	++
Poly II containing polysaccharide				
B	*Pneumococcus* type 14 ps (II)	–	0.3	±
GalNAcβ1-4Galβ1-4GlcNAc-containing gps				
B	Asialo THGP Sd. (a^+) W. M. (II, S)	120.0	1.8	+++
Galα_1-4Galβ1-4Galβ1-4GlcNAc-containing gp				
B	Asialo bird nest gp (II, E, T_α, F_α)	45.0	4.4	+++++
GalNAcα_1-Ser/Thr (Tn)/ Galβ1-3GalNAcα_1-Ser/Thr (T)-containing gps				
F	Asialo PSM (T, Tn, A_h, H)	–	0.6	+
	Asialo ASG (Tn)	–	0.4	±
F	Asialo BSM (Tn, GlcNAcβ1-3Tn)	–	0.3	±
F	Asialo HSM (Tn)	–	0.3	±
F	Asialo OSM (Tn)	–	0.2	±
	Active antifreeze gp (T_α; M.W. 1.0-2.1 $\times 10^4$)	–	0.0	–
	Inactive antifreeze gp (T_α; M.W. 2.6-3.8 $\times 10^3$)	–	0.0	–
Sialylated (crypto) II, T/Tn-containing gps				
B	THGP Sd. (a^+) W. M. (II, S)	–	0.4	±
B	Bird nest gp (sialyl II, E, T_α, F_α)	–	0.3	±
F	BSM (sialyl Tn, sialyl GlcNAcβ1-3Tn)	–	0.1	–
F	HSM (sialyl Tn)	–	0.05	–
A	Human α_1-acid gp (sialyl mII)	–	0.03	–
A	Fetuin (sialyl II, T)	–	0.03	–
	ASG-A (sialyl Tn)	–	0.01	–
A	Bovine α_1-acid gp (mII)	–	0.0	–
F	PSM (sialyl T, Tn)	–	0.0	–
F	OSM (sialyl Tn)	–	0.0	–
Blood group ABH and Lewis-containing gps				
D	Cyst N-1 Le^a 20% 2x (Le^a)	200.0	3.3	+++++
E	Hog gastric mucin # 9 (A_h, H)	–	0.6	+
E	Hog gastric mucin # 4 (A_h, H)	–	0.5	+
D	Cyst 19 (A, B)	–	0.4	±
C	Cyst Beach phenol insoluble (B)	–	0.4	±
C	Cyst Mcdon (A_h)	–	0.3	±
C	Cyst Tighe phenol insoluble (H, Le^b)	–	0.3	±
D	Cyst JS phenol insoluble (H)	–	0.3	±
C	Cyst MSS 10% 2x (A_h)	–	0.04	–
D	Cyst 9 (A_h)	–	0.02	–

Abbreviations: *: 50 ng of biotinylated ECorL was added to various glycoprotein concentrations ranging from 1ng to 2.5 μg/50μl; †: The symbol in parentheses indicates the human blood group activity and/or lectin determinants;[49] I/II: Galβ1-3/4GlcNAc; Tn: GalNAcα1-Ser/Thr; T: Galβ1-3GalNAc; A_h: GalNAcα1-3[$_L$Fucα1-2]Gal; B: Galα1-3Gal; H: $_L$Fucα1-2Gal; ‡: The results were interpreted according to the measured A_{405} after 4 h incubation; +++++: O.D. ≥ 2.5; ++++: 2.5 > O.D. ≥ 2.0; +++: 2.0 > O.D. ≥ 1.5; ++: 1.5 > O.D. ≥ 1.0; +: 1.0 > O.D. ≥ 0.5; ±: 0.5 > O.D. ≥ 0.2; and –: O.D. < 0.2. Other glycans that showed an A_{405} nm value of less than 0.01 were: mannan; colominic acid; pectin-A and C.

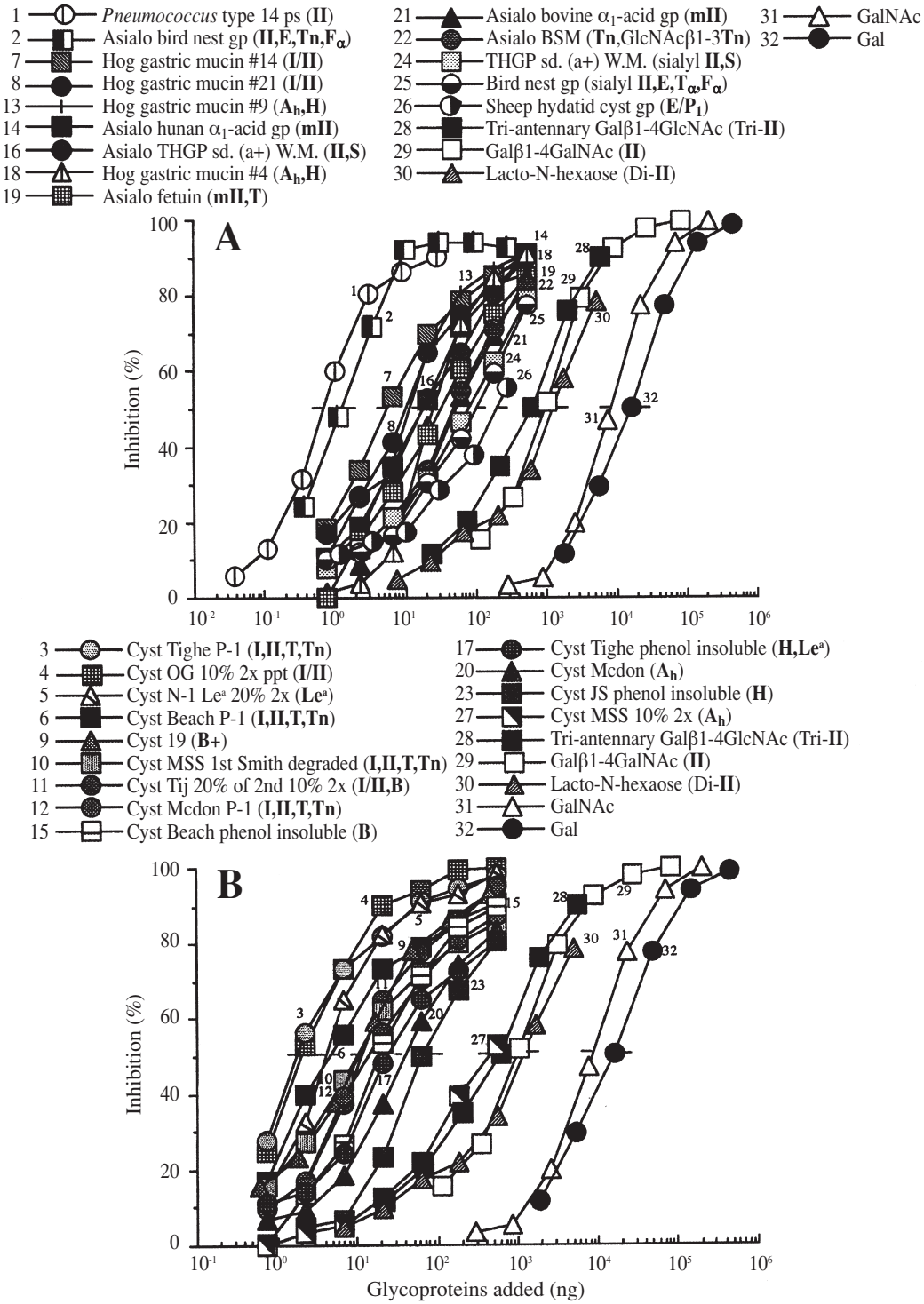

Fig. 6 Inhibition of ECorL binding to a Galβ1-4GlcNAc-containing glycoprotein (asialo human α_1-acid gp) coated on ELLSA plates by various glycoproteins. The amount of glycoprotein in the coating solution was 100 ng per well. The lectin (50 ng per well) was pre-incubated with an equal volume of serially diluted inhibitors. The final ECorL content was 25 ng per well. Total volume was 50 μl. A_{405} was recorded after 2 h incubation.

Table 2. Amount of Different Gps Giving 50% Inhibition of Binding of ECorL (25ng/50μl) to Asialo Human α_1-acid Gp (100ng/50μl)*

Curves or identification no.	Curves in Fig. 6	Glycoprotein/glycans	Quantity giving 50% inhibition (nanograms)	Relative potency†
1	A	*Pneumococcus* type 14 ps (II)	0.7	2.4×10^4
2	A	Asialo bird nest gp (II, E, T_α, F_α)	1.3	1.3×10^4
3	B	Cyst Tighe P-1 (I, II, T, Tn)	1.9	8.9×10^3
4	B	Cyst OG 10% 2x ppt (I/II)	2.0	8.5×10^3
5	B	Cyst N-1 Le^a 20% 2x (Le^a)	4.0	4.3×10^3
6	B	Cyst Beach P-1 (I, II, T, Tn)	5.0	3.4×10^3
7	A	Hog gastric mucin # 14 (I/II)	5.5	3.1×10^3
8	A	Hog gastric mucin # 21 (I/II)	10.0	1.7×10^3
9	B	Cyst 19 (A, B)	10.0	1.7×10^3
10	B	Cyst MSS 1st Smith degraded (I, II, T, Tn)	10.0	1.7×10^3
11	B	Cyst Tij 20% of 2nd 10% 2x (I/II, B)	12.0	1.4×10^3
12	B	Cyst Mcdon P-1 (I, II, T, Tn)	12.0	1.4×10^3
13	A	Hog gastric mucin # 9 (A_h, H)	12.0	1.4×10^3
14	A	Asialo human α_1-acid gp (mII)	18.0	9.4×10^2
15	B	Cyst Beach phenol insoluble (B)	18.0	9.4×10^2
16	A	Asialo THGP Sd. (a^+) W.M. (II, S)	18.0	9.4×10^2
17	B	Cyst Tighe phenol insoluble (H, Le^b)	25.0	6.8×10^2
18	A	Hog gastric mucin # 4 (A_h, H)	25.0	6.8×10^2
19	A	Asialo fetuin (mII, T)	30.0	5.7×10^2
20	B	Cyst Mcdon (A_h)	40.0	4.3×10^2
21	A	Asialo bovine α_1-acid gp (mII)	50.0	3.4×10^2
22	B	Asialo BSM (Tn, GlcNAcβ1-3Tn)	50.0	3.4×10^2
23	B	Cyst JS phenol insoluble (H)	60.0	2.8×10^2
24	A	THGP Sd. (a^+) W.M. (II, S)	80.0	2.1×10^2
25	A	Bird nest gp (sialyl II, E, T_α, F_α)	100.0	1.7×10^2
		BSM (sialyl Tn, sialyl GlcNAcβ1-3Tn)	180.0	94
		Asialo HSM (Tn)	180.0	94
26	A	Sheep hydatid cyst gp	200.0	85
		Asialo OSM (Tn)	250.0	68
		Asialo ASG (Tn)	300.0	57
27	B	Cyst MSS 10% 2x (A_h)	400.0	43
28	A,B	Galβ1→4GlcNAcβ1→2Man ↓α1-6 Galβ1→4GlcNAcβ1→2Manα1→3Manβ1→4GlcNAcβ1→4GlcNAcβ1-N-Asn ↑β1-4 Galβ1→4GlcNAc Tri-antennary Galβ1-4GlcNAc (Tri-II)	600.0‡	28
29	A,B	Galβ1-4GlcNAc (II)	1000.0	17
30	A,B	Galβ1→4GlcNAc ↓β1-6 Galβ1→4Glc Lacto-*N*-hexaose ↑β1-3 [LNH; Di-II or IIβ1-6(IIβ1-3)L] Galβ1→4GlcNAc	1300.0‡	13
31	A,B	GalNAc	15000.0	1.1
32	A,B	Gal	17000.0	1.0
		Human α_1-acid gp (sialyl mII)	> 555.6 (12.1%)	–
		Asialo PSM (T, Tn, A_h, H)	> 555.6 (9.0%)	–
		Fetuin (sialyl II, T)	> 555.6 (4.4%)	–

*: The inhibitory activity was estimated from the inhibition curve in Fig. 6 and is expressed as the amount of inhibitor giving 50% inhibition; Total volume was 50 μl; †: Relative potency = Quantity of Gal (curve 32) required for 50% inhibition is taken as 1.0 / Quantity of sample required for 50% inhibition; ‡: Based on carbohydrate content; §: The inhibitory potency of inactive glycoproteins is expressed as the maximum amount of gps tested that yield inhibition (in parenthesis) below 50%; Other gps in which 278-556 ng were used for inhibition of ECorL-gp binding but did not reach 50% inhibition: HSM, ASG, OSM, PSM, active antifreeze gp; mannan; pectin-A, pectin-C, colominic acid.

Fig. 6, Table 2) and up to 7.7×10^2 and 4.6×10^2 times more active than those of monomeric II and Tri-II (curves 28 and 29, Fig. 6, Table 2), respectively. The reaction was also strongly inhibited by hog gastric mucin A+H substances (curves 13 and 18, Fig. 6A), human blood ABH active gps (curves 9,15,17,20 and 23, Fig. 6B), poly oligo-antennary II-containing *N*-linked gps (curves 19 and 21, Fig. 6A) and asialo BSM (curve 22, Fig. 6A). They were up to 1.4×10^3 times more reactive than Gal. Although monomeric GalNAc was active, even slightly more active than Gal (curve 31 vs. curve 32), most of its polyvalent forms did not contribute to binding. These included Tn- or T-containing gps such as asialo HSM, asialo PSM and active antifreeze gp (Table 2). Except for THGP and bird nest gp (curves 24 and 25), most sialic acid-containing gps (such as human α_1 acid, fetuin and OSM, Table 2) were either weakly active or inactive, indicating that sialic acid has a masking effect.

Inhibition of ECorL-glycoform interaction by mono- and oligo-saccharides

The ability of various sugars to inhibit the binding of ECorL by an oligo-antennary Galβ1-4GlcNAc-containing gp (asialo human α_1-acid gp) is shown in Fig. 7 and the amounts of ligands (expressed as nanomoles) required for 50% inhibition of the lectin-glycan interaction are listed in Table 3. Among the II, II-oligosaccharides and mammalian multi-II antennary glycotopes tested, human blood group type II precursor (Galβ1-4GlcNAc, II) was 32 times more active than Gal, indicating that GlcNAcβ1- added to carbon-4 of Gal plays a very important role in binding (curve 6 vs. curve 34, Fig. 7A). Bi-antennary hexasaccharides IIβ1-6(Iβ1-3)L (lacto-*N*-neohexaose, LNnH), tri-antennary Galβ1-4GlcNAc at the non-reducing end in *N*-linked glycopeptides (Tri-II) and bi-antennary IIβ1-6(IIβ1-3)L (Di-II) were up to 4.2 and 133 times more efficient than II disaccharide and Gal, respectively (curves 1 to 3 vs. curves 6 and 34, Fig. 7A), suggesting that most bi- or oligo-antennary glycotopes can enhance the binding reactivities. When IIβ1-6 was added to Gal of L (Galβ1-4Glc) in Iβ1-3L (type 1) and IIβ1-3L (type 2), the reactivity of these hexasaccharides IIβ1-6(Iβ1-3)L and IIβ1-6(IIβ1-3)L increased 28.3 and 1.5-fold (curve 1 vs. curve 8 and curve 3 vs. curve 4, Table 3); IIβ1-3L (type 2) and Iβ1-3L (type 1) were 1.4 and 1.3 times better than Galβ1-4/3GlcNAc (II/I) (curve 4 vs. curve 6 and curve 8 vs. curve 10, Table 3), indicating that the affinity of ECorL for glycoforms can be enhanced by formation of bi-antennary II and the length of the carbohydrate sequence in Galβ1-containing ligands. IIβ1-3L (type 2), human blood group I Ma trisaccharide and II were nearly as active, and were 8.8-12.2 times more active than Galβ1-3GlcNAc (I) (curves 4 to 6 vs. curve 10, Fig. 7A and Table 3), suggesting that the major combining sites of ECorL are II. Galβ1-4Glc (L) and Galβ1-4Man (curves 7 and 23) were about 2.4 and 3.3 times more active than Galβ1-3GlcNAc (I) and Galβ1-6GlcNAc, respectively (curves 7 and 23 vs. curves 10 and 29); the preference of ECorL for the linkage of subterminal sugar, in decreasing order, is as follows: Galβ1-4 > Galβ1-3 > Galβ1-6.

The effect of various substitutions on Galβ1-4GlcNAc (II) and Galβ1-4Glc (L) containing compounds on the binding is also shown in Table 3. Galβ1-4[Fucα1–3]GlcNAc (Le^x) and Galβ1-4[Fucα1-3]Glc (3-Fucosyllactose) were poor inhibitors, indicating that adding Fucα1-3 to subterminal GlcNAc of Galβ1-4GlcNAc (II) and Glc of Galβ1-4Glc (L) blocks interaction; Fucα1-2Galβ1-4Glc (2'-Fucosyllactose) was 1.7 times more active than Galβ1-4Glc (L) (curve 22 vs. curve 7), implying that Fucα at the 2-position of the Gal of Galβ1-4Glc (L) slightly promotes the reactivity.

All mammalian GalNAcα/β- disaccharide structural units such as Tn mix (curve 11), P (GalNAcβ1-3Gal, curve 14), F (GalNAcα1-3GalNAc, curve 15), A (GalNAcα1-3Gal, curve 17) and S (GalNAcβ1-4Gal, curve 18) were poor inhibitors. Of the Gal and GalNAc derivatives examined, *p*-nitrophenyl βGal was the best inhibitor and was 1.4 and 11.4 times more active than *p*-nitrophenyl αGal and Gal, respectively (curve 20 vs. curve 24 and curve 34). For methyl Gal glycosides, the α-anomer was a 2.3 times better inhibitor than β-anomer (curve 27 vs. curve 31). *P*-nitrophenyl α- and β- Gal were 3.0 and 10 times more active than methyl α- and β- Gal, respectively (curve 24 vs. curve 27 and curve 20 vs. curve 31), indicating that hydrophobic forces may participate in ECorL-carbohydrate interaction.

DISCUSSION

During the past two decades, several reports on

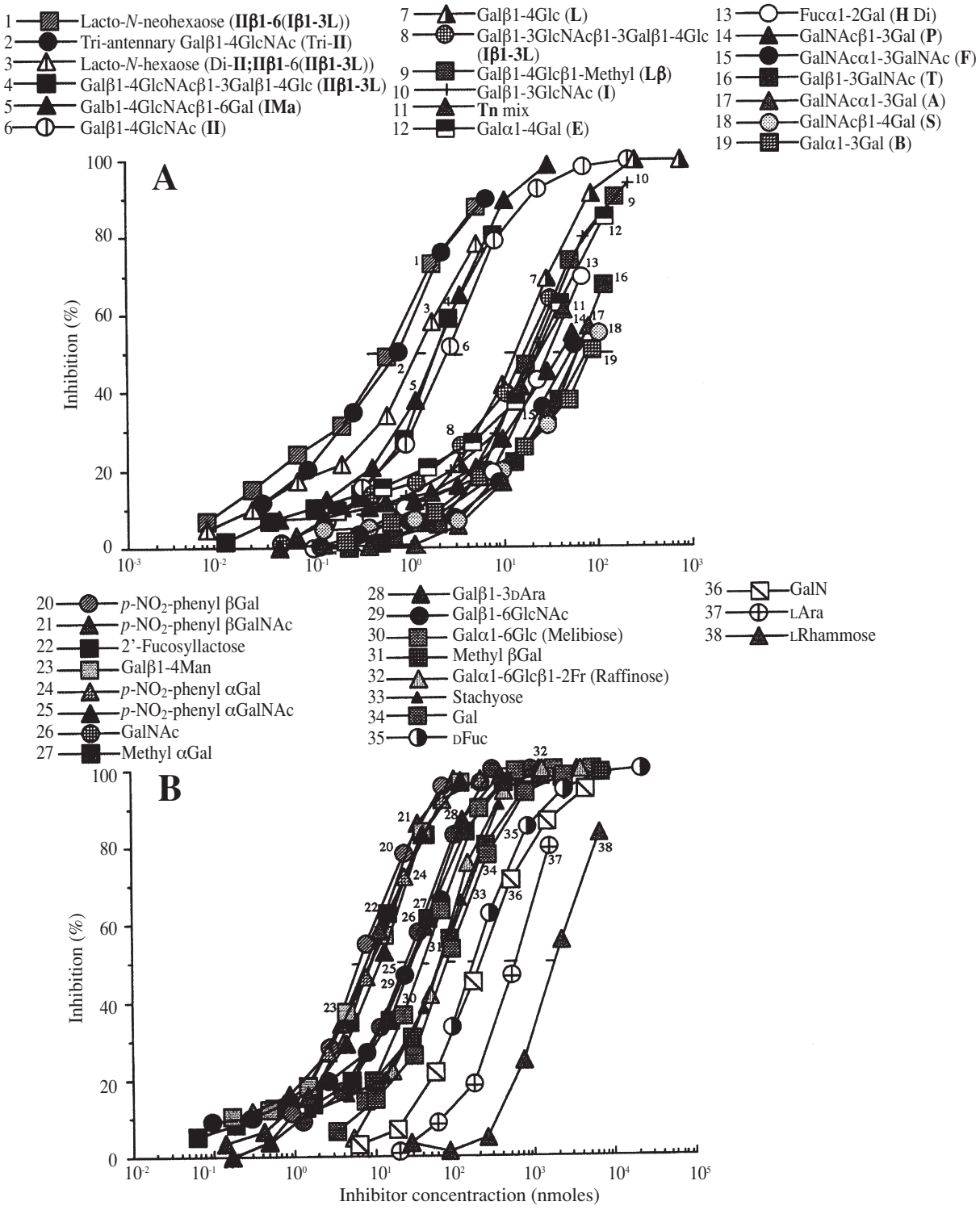

Fig. 7 Inhibition of ECorL binding to a Galβ1-4GlcNAc-containing glycoprotein (asialo human α_1-acid gp) coated on ELLSA plates by various saccharides. The quantity of glycoprotein in the coating solution was 100 ng per well. The quantity of ECorL used for the inhibition assay was 25 ng per well. Total volume was 50 μl. A_{405} was recorded after 2 h incubation.

Table 3. Amount of Various Saccharides Giving 50% Inhibition of Binding of ECorL (25ng/50μl) to Asialo Human α_1-acid Gp (100ng/50μl)*

Curves No.	Curves in Fig. 7	Saccharides		Quantity giving 50% inhibition (nanomoles)	Relative potency†
Galβ1-4Glcβ-/Galβ1-4/3GlcNAcβ-containing (L_β/II_β/I) ligands					
1	A	Galβ1→4GlcNAc ↓β1-6 Galβ1→4Glc ↑β1-3 Galβ1→3GlcNAc	Lacto-*N*-neohexaose [LNnH; IIβ1-6(Iβ1-3)L]	0.6	133.3
2	A	Galβ1→4GlcNAcβ1→2Manα1→3Manβ1→4GlcNAcβ1→4GlcNAcβ1-N-Asn ↓α1-6 ↑β1-4 Galβ1→4GlcNAc	Tri-antennary Galβ1-4GlcNAc (Tri-II)	0.7	114.3
3	A	Galβ1→4GlcNAc ↓β1-6 Ga1β1→4Glc ↑β1-3 Galβ1→4GlcNAc	Lacto-*N*-hexaose [LNH; Di-II or IIβ1-6(IIβ1-3)L]	1.2	66.7
4	A	Galβ1-4GlcNAcβ1-3Galβ-4Glc (Type 2, IIβ1-3L)		1.8	44.4
5	A	Galβ1-4GlcNAcβ1-6Gal (Human blood group I Ma trisaccharide)		2.0	40.0
6	A	Galβ1-4GlcNAc (II)		2.5	32.0
7	A	Galβ1-4Glc (L; Lactose)		15.0	5.3
8	A	Galβ1-3GlcNAcβ1-3Galβ1-4Glc (Type 1, Iβ1-3L; Lacto-*N*-tetraose)		17.0	4.7
9	A	Galβ1-4Glcβ1-Methyl (Lβ)		20.0	4.0
10	A	Galβ1-3GlcNAc (I)		22.0	3.6
Other mammalian carbohydrate structural units					
11	A	Tn-containing glycopeptides from ovine salivary gp (Tn mix, M.W. < 3.0×10^3)		25.0	3.2
12	A	Galα1-4Gal (E)		25.0	3.2
13	A	LFucα1-2Gal (H disaccharide)		30.0	2.7
14	A	GalNAcβ1-3Gal (P)		40.0	2.0
15	A	GalNAcα1-3GalNAc (F)		50.0	1.6
16	A	Galβ1-3GalNAc (T)		60.0	1.3
17	A	GalNAcα1-3Gal (A)		60.0	1.3
18	A	GalNAcβ1-4Gal (S)		70.0	1.1
19	A	Galα1-3Gal (B)		80.0	1.0
		Galα1-3Galβ1-4GlcNAc (B active II)		80.0	1.0
		Galβ1-3GalNAcα1-benzyl (T_α)		100.0	0.8
		Galβ1–4[Fucα1–3]GlcNAc (Le^x)		> 42.2 (22.4%)‡	–
Monosaccharides and their derivatives					
20	B	*p*-NO_2 phenyl βGal		7.0	11.4
21	B	p-NO_2 phenyl βGalNAc		8.0	10.0
22	B	Fucα1-2Galβ1-4Glc (2'-Fucosyllactose)		9.0	8.9
23	B	Galβ1-4Man		9.0	8.9
24	B	*p*-NO_2 phenyl αGal		10.0	8.0
25	B	*p*-NO_2 phenyl αGalNAc		12.0	6.7
26	B	GalNAc		25.0	3.2
27	B	Methyl αGal		30.0	2.7
28	B	Galβ1-3DAra		30.0	2.7
29	B	Galβ1-6GlcNAc		30.0	2.7
30	B	Galα1-6Glc (Melibiose)		40.0	2.0
31	B	Methyl βGal		70.0	1.1
32	B	Galα1-6Glcβ1-2Fruf (Raffinose)		70.0	1.1
33	B	Galα1-6Galα1-6Glcβ1-2Fruf (Stachyose)		70.0	1.1
34	A, B	Gal		80.0	1.0
35	B	DFuc		180.0	0.4
36	B	GalN		200.0	0.4

Table 3. (continued)

Curves No.	Curves in Fig. 7	Saccharides	Quantity giving 50% inhibition (nanomoles)	Relative potency†
37	B	LAra	600.0	0.1
38	B	LRhamnose	1800.0	0.04
		Man	18000.0	0.004
		Glc	250000.0	0.003
		Galβ1-4[Fucα1-3]Glc (3-Fucosyllactose)	> 45.6 (8.3%)	–
		GlcNAc	> 14350.0 (25.9%)	–
		LFuc	> 7742.5 (30.7%)	–
		DAra	> 1375.6 (2.6%)	–

*: The inhibitory activity was estimated from the inhibition curve in Fig. 7 and is expressed as the amount of inhibitor giving 50% inhibition. Total volume was 50μl; †: Relative potency = Quantity of Gal (curve 34) required for 50% inhibition is taken as 1.0 / Quantity of sample required for 50% inhibition; ‡: The inhibitory potency of inactive saccharides is expressed as the maximum amount of sugars tested that yield inhibition (in parenthesis) below 50%.

the protein crystal structure and binding properties of ECorL have been published.[7-9] However, the effects of polyvalency on the binding activities of many mammalian sugar structural units with ECorL have not been well characterized. In the present study, the recognition factors of ECorL were examined, using our collection of ligands and polyvalent glycotopes of natural glycans by enzyme-linked lectinosorbent assay, which is a sensitive and time/reagent-saving method.[42,43] To avoid potential problems caused by differences in absorption of glycoproteins to the microplate wells, the reactivities of the glycoproteins used in Fig. 5 (Table 1) were tested for their ability to inhibit lectin-glycoprotein binding (Fig. 6 and Table 2). Overall, the inhibitory profile, expressed as the amount of glycans (in nanograms) required to inhibit 50% of the ECorL-glycoprotein interaction, is in agreement with the interaction profile (Table 1 vs. Table 2). As shown in Fig. 5 and Table 1, the strength of ECorL-glycan interaction showed some variations among the II containing glycans. This may be due to an absorption problem of glycans to microplate wells, especially in the case of *Streptococcus pneumoniae* type 14 polysaccharide. Therefore, our interpretations of binding reactivities of glycans in this report are based on the data from the molecular mass inhibition assay (Table 2).

Among the soluble natural glycans tested, *Pneumococcus* type 14 polysaccharide, composed of repeating units with II (Galβ1-4GlcNAc) as side chains (Fig. 4), was the best inhibitor. It was 2.4×10^4, 1.4×10^3 and 8.6×10^2 times more active than Gal, monomeric II and tri-antennary II (Tri-II), respectively. Most polyvalent I/II-containing gps, especially human blood group precursor gps and hog gastric mucin, were also powerful inhibitors. These included some human ovarian cyst gps (cyst Tighe P-1,cyst OG 10% 2x ppt, Beach P-1, cyst MSS 1st Smith degraded, Cyst Tij 20% of 2nd 10% 2x and cyst Mcdon P-1, curves 3, 4, 6 and 9-12, Fig. 6B). They were 67 to 5.3×10^2 and 40 to 3.2×10^2 times more active inhibitors than monomeric II and Tri-II, respectively (curves 29 and 28, Fig. 6). The tremendous increase in inhibitory potency for ECorL from free monomeric sugar to glycoproteins suggests the importance of polyvalent II glycotopes in ECorL binding. Furthermore, the ability of ECorL to recognize human blood-group ABH and Lewis active cyst gps (cyst N-1 Le^a 20% 2x, cyst Beach phenol insoluble, cyst Tighe phenol insoluble, cyst Mcdon, cyst JS phenol insoluble; curves 5, 15, 17, 20 and 23, Fig. 6) implies the possibility that incomplete carbohydrate chains of exposed precursor I/II residues at the nonreducing end exist in these cyst gps[26] and ECorL can tolerate this terminal α2-linked fucose residue from Gal without a change in binding strength.[10,12,13] ECorL reacted weakly or not at all with gps containing sialylated multivalent II/I determinants (Tables 1 and 2). The poor or negative reactivities of ECorL with sialylated gps can be ascribed to the bulkiness and/or charge on sialic acid or could be because the sialic acid sterically prevents the access of the determinant sugars to the lectin combining site, or both. Certainly, the density of sialic acid may play an important role in the masking effect on lectin-carbohydrate interactions.

The inhibitory profile of simple oligosaccharides in decreasing order on a nanomole basis is as follows: IIβ1-6(Iβ1-3)L > Tri-II > Di-II > IIβ1-3L > Galβ1-4GlcNAcβ1-6Gal (human blood group I Ma trisaccharide) > Galβ1-4GlcNAc (II) > *p*-NO_2 phenyl βGal > *p*-NO_2 phenyl βGalNAc > Fucα1-2Galβ1-4Glc > Galβ1-4Man > *p*-NO_2 phenyl αGal > *p*-NO_2 phenyl αGalNAc > Galβ1-4Glc (L; Lactose) > Iβ1-3L > Galβ1-4Glcβ1-methyl (Lβ) > Galβ1-3GlcNAc (I) > Tn-containing glycopeptides, Galα1-4Gal (E), GalNAc > LFucα1-2Gal (H disaccharide), methyl αGal, Galβ1-3DAra, Galβ1-6GlcNAc > GalNAcβ1-3Gal (P), Galα1-6Glc (Melibiose) > GalNAcα1-3GalNAc (F) > Galβ1-3GalNAc (T), GalNAcα1-3Gal (A) > GalNAcβ1-4Gal (S), methyl βGal, Galα1-6Glcβ1-2Fruf (Raffinose), Galα1-6Galα1-6Glcβ1-2Fruf (Stachyose) > Galα1-3Gal (B), Galα1-3Galβ1-4GlcNAc (B active II) > Galβ1-3GalNAcα1-benzyl (T_α) > Gal; Galβ1–4[Fucα1–3] GlcNAc (Le^x), and Galβ1-4[Fucα1-3]Glc (3-Fucosyllactose) were inactive. Thus, the relationship among II, T and Tn has also been defined. Although monomeric GalNAc is more active than Gal, most of its polyvalent forms do not increase binding (Tables 1 and 2).

It is of interest to compare the binding properties of RCA_1 and ricin with that of ECorL. Table 4 shows that (i) although they all belong to Galβ1-4GlcNAc (II) specific lectins, the inhibition profiles of these lectins toward the monosaccharides were different. As shown in Table 4, the reactivity order was Gal < GalNAc for ECorL (relative potency [RP] of Gal/GalNAc was 0.3); Gal > GalNAc for ricin (RP: 2.0); and Gal ≫ GalNAc for RCA_1 (RP: 83.3).[44] (ii) ECorL, RCA1 and ricin all bound strongly with polyvalent II (Galβ1-4GlcNAc) structures in *N*-linked gps (asialo human α_1-acid gp, asialo fetuin) and in *O*-linked gps (glycoproteins from human ovarian cyst fluid). (iii) RCA_1 showed strong binding for *Pneumococcus* type 14 polysaccharide, but both ECorL and ricin showed weak binding which can be attributed to the poor absorbance of most of polysaccharides on microplate wells. (iv) The reactivity of lectins toward polyvalent Tn/T gps can be ranked as ricin ≫ RCA_1 > ECorL. (v) In ECorL and RCA_1, the hydrophobicity surrounding α/β- anomeric Gal enchances binding, whereas in ricin, the hydrophobic interaction is important only for binding of β-anomeric Gal (Table 4).

Summarizing the current and previous studies, the following conclusions can be drawn: [1] Galβ1-4GlcNAc and other Galβ1-related oligosaccharides are essential for binding. [2] Their polyvalent form in glycoproteins is the most important binding factor for ECorL. The polyvalency effect can be expressed in decreasing order as follows: polyvalent II and its related oligomers ≫ oligo-antennary II > monomeric II. Polyvalence in binding is also found in other plant and animal lectins, such as *Ricinus communis* agglutinin 1 (RCA_1),[44] *Ricinus communis* toxic protein (ricin),[45] *Pseudomonas aeruginosa* II lectin,[46] and galectin-4 from rat gastrointestinal tract.[47] [3] The shape of the ECorL combining site should be a shallow cleft, recognizing Galβ1-4GlcNAc as the major binding site with an additional one to four sugar subsites. [4] It is most complementary to Galβ1-4GlcNAcβ1-3Galβ1-4Glc (IIβ1-3L) for a linear sequence, and to Galβ1-4GlcNAcβ1-6(Galβ1-3GlcNAcβ1-3)Galβ-4Glc (lacto-*N*-neohexaose, LNnH, IIβ1-6[Iβ1-3]L) for a branch form. [5] ECorL has a preference for the β-anomer of Gal at the non-reducing end of oligosaccharides with a Galβ1-4

Table 4. Variations in Binding to Monosaccharides and Their Glycosides among ECorL, RCA_1 and Ricin[44,45]*

Lectin	Carbohydrate specificities	RP† of Gal/GalNAc	Sugar anomerism in: (RP of β- and α-anomers)			Hydrophobicity in: (RP of *p*-nitrophenyl and methyl glycosides)	
			p-NO_2 phenyl Gal	Methyl Gal	*p*-NO_2 phenyl GalNAc	Gal α-anomer	Gal β-anomer
ECorL	II > I > E, B	0.3	1.4	0.4	1.5	3.0	10.0
RCA_1	II ≥ I > E, B	83.3	2.0	2.4	Inactive	4.5	3.8
Ricin	T > I /II, Tn	2.0	2.0	0.9	1.0	1.0	2.2

*: Values are calculated from moles required for 50% inhibition; †: Expressed as reciprocal of relative potency.

linkage > Galβ1-3 > Galβ1-6. [6] Since the ratio of the Galβ-anomer of *p*-nitrophenyl glycosides per methyl glycosides is 10.0 (Table 4), the hydrophobic forces of Galβ- may also contribute to binding. [7] Although GalNAc is a better inhibitor than Gal for ECorL, its polyvalent forms are poor enhancing factors. [8] ECorL exhibits a higher affinity for Fucα1-2Galβ1-4Glc than for Galβ1-4Glc (L), showing that Fucα linked to the C2-position of non-reducing terminal Gal does not block, or even increase reactivity. [9] The carbohydrate specificity of ECorL for mammalian carbohydrate structural units can be mapped as follows: Tri-II > Di-II > I Ma > II > L > I > Tn, E > H > P > F > T, A > S > B > Le^x (inactive).

These results should be helpful for establishing the functional role of ECorL and for characterizing glycotopes of mammalian glycans. The ability of this lectin to bind polyvalent II -specific oligosaccharides may make it a useful biochemical and medical tool for study of the structural and functional roles of cell surface carbohydrates by lectino-histochemistry,[48] for detection of disease-related alterations of glycan synthesis and for use as a cell marker for diagnostic purposes.[1-5]

Acknowledgments

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定義來自珊瑚刺桐種子之凝集素的碳水化合物專一性爲多價性 Galβ1-4GlcNAc (II) > 單體 II >單體 Gal 和 GalNAc

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背 景： 珊瑚刺桐凝集素(ECorL) 是有效能的應用凝集素之一。過去的研究中，對它在碳水化合物專一性的了解僅限於單醣、簡單性的寡醣及少許的聚合醣，然而於多價因子(polyvalent factor) 上對結合性質的影響尚未被深入研究。

方 法： 利用靈敏的酵素免疫法和抑制分析法，以及收集到的結合分子和天然多價性醣體，來偵測ECorL 結合位置的特性。

結 果： 從酵素免疫的結合及抑制分析的結果顯示 ECorL 對於含有 Galβ1-4GlcNAc (II) 的醣蛋白有極高的親和力。在抑制分析後所有測試的醣蛋白中，以含有高密度多價性 II 醣表位的 *Streptococcus pneumoniae* type 14 的夾膜多醣體具有最強的抑制效果，其抑制能力分別是 Gal, II 及 tri-antennary II 的 2.4×10^4、1.4×10^4、8.6×10^2 倍。另外 ECorL 也與其它具有高密度 II 的醣蛋白有很強的親和力。雖然單醣 GalNAc 與 Gal 一樣都是有結合性之抑制劑，但GalNAc 的多價性結構的醣蛋白卻是極差的抑制劑。

結 論： [1] ECorL 對碳水化合物的專一性主要是在含有 Galβ1-4GlcNAc (II) 和其他 Galβ1- 相關的寡醣；[2] 醣蛋白中多價形式在 ECorL 與碳水化合物結合是扮演最重要的結合因子；[3] 雖然對 ECorL 而言，單醣 GalNAc 比 Gal 有較好的抑制能力，但多價效應卻無法提高它的反應性。此應用凝集素可作爲基礎科學和臨床醫學的研究工具。
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關鍵詞： 珊瑚刺桐凝集素，凝集素，碳水化合物專一性，多價性

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