Forssman pentasaccharide and polyvalent Galβ1→4GlcNAc as major ligands with affinity for *Caragana arborescens* agglutinin

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Abstract The binding properties of *Caragana arborescens* agglutinin (CAA, pea tree agglutinin) were studied by enzyme linked lectinosorbent assay (ELLSA) and by inhibition of CAA-glycan interaction. Among glycoproteins (gps) tested, CAA reacted strongly with asialo bird nest gp, asialo rat sublingual gp, human Tamm-Horsfall Sd(a$^+$) urinary gp (THGP) and asialo THGP that are rich in GalNAcα1→, GalNAcβ1→ and/or Galβ1→4GlcNAc residues. CAA also bound tightly with multivalent Galβ1→4GlcNAc (mII) containing glycoproteins (human blood group precursor gps, asialo fetuin) and asialo ovine salivary glycoprotein (Tn, GalNAcα1→Ser/Thr), but CAA reacted poorly or not at all with sialylated glycoproteins tested. Of the sugars tested for inhibition of binding, Forssman pentasaccharide (F_p, GalNAcα1→3GalNAcβ1→3Galα1→4Galβ1→4Glc) was the best. It was about 2.3, 9.5 and 52.6 times more active than Galβ1→4GlcNAc, GalNAc and Gal, respectively, and about 1.9 times more active than tri-antennary Galβ1→4GlcNAc (Tri-II). These results suggest that this agglutinin is mainly specific for F_p, mII and Tn clusters. This property can be used to detect human abnormal glycotopes related to F_p and unmasked mII/Tn clusters and to study cell growth and differentiation given the lack of toxicity of this lectin toward mouse fibroblast cells.

Key words: Carbohydrate specificity; Forssman pentasaccharide; *Caragana arborescens* agglutinin

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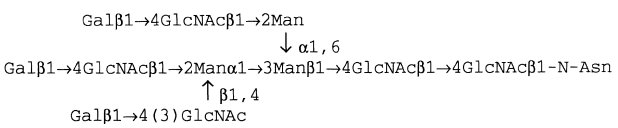
Abbreviations: gp, glycoprotein; THGP, Tamm-Horsfall glycoprotein; BN, bird nest glycoprotein; OSM, ovine submandibular glycoprotein (major); RSL, rat sublingual glycoprotein (major); ELLSA, enzyme linked lectinosorbent assay; Sugars: Gal, D-galactopyranose; Glc, D-glucopyranose; LFuc, L-fucopyranose; GalNAc, 2-acetamido-2-deoxy-D-galactopyranose; GlcNAc, 2-acetamido-2-deoxy-D-glucopyranose; Lectin: CAA, *Caragana arborescens* agglutinin; Applied lectin determinants [36–38]: F_p, GalNAcα1→3GalNAc β1→3Gal α1→4Galβ1→4Glc; II, Galβ1→4GlcNAc; mII, poly(multi)valent II, including bi-, tri- and tetra-antennary and cluster form of II; Tn, GalNAcα1→Ser/Thr.
Tri-II, tri-antennary Galβ1→4GlcNAc:



1. Introduction

The study of lectins is increasing due to their ever wider importance in biology, immunology and medicine [1–3]. In the early 1980s, lectins of the same apparent monosaccharide specificity were found to possess different reactivities toward different oligosaccharide chains [4–8], and to have differential affinities for animal cells and mammalian glycoproteins [5–9]. This implies that lectins have their own binding specificity which extends beyond the monosaccharide unit. Indeed, many lectins have broad spectra of binding properties and possess dual or multiple affinities for various disaccharides [5–7,10].

The lectin used for this study was purified from the pea tree, *Caragana arborescens* (CAA). It is a glycoprotein with high hemagglutinating activity [11]. It is composed of two polypeptides with apparent molecular weight near 30 000. In the middle of the 1970s, the biochemical property of CAA has been well characterized [11]. However, the combining site of this lectin has not yet been fully determined. In order to understand better the biological roles of CAA and for it to be useful as an investigative tool for biomedical and immunochemical studies, it is important to establish its carbohydrate binding properties. In the present report, the carbohydrate specificity of CAA was studied by enzyme linked lectinosorbent assay (ELLSA) and by inhibition of lectin-glycoprotein binding. The results show that this lectin strongly recognizes exposed GalNAcα1→3GalNAc (F), multi-valent Galβ1→4GlcNAc (mII) and cluster forms of GalNAcα1→Ser/Thr (Tn) glycotopes, especially Forssman pentasaccharide.

2. Materials and methods

2.1. Lectin

C. arborescens agglutinin (CAA) was purchased from Sigma Chemical Company (St. Louis, MO, USA).

2.2. Glycoproteins and polysaccharide

Cyst human blood group active glycoproteins (MSS, Mcdon, Tighe and Beach) were prepared from human ovarian cyst fluid as described previously [12–15]. Ovine and rat salivary glycoproteins were purified according to the method of Tettamanti and Pigman [16] and its modification [17–19]. The crude salivary gland mucus glycoprotein (native BN) of the Chinese swiftlet (genus *Collocalia*) was extracted at 66°C with distilled H_2O for 20 min from commercial bird nest (BN) [20,21]. Tamm-Horsfall glycoprotein, which was kindly provided by Dr. W.M. Watkins, University of London, Royal Postgraduate Medical School, Hammersmith Hospital, London, UK, was isolated from the urine of one single donor (W.T.J.M.) with Sd(a$^+$) blood group by the method of Tamm and Horsfall [22] with 0.58 M NaCl. The precipitated material was lyophilized, its lipid content removed with 9:1, 2:1 and 1:2 chloroform-methanol treatment, and further purified as de-

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scribed [22]. Fetuin and mannan were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

For desialylation, a glycoprotein sample in 0.01 N HCl was hydrolyzed at 80°C for 90 min and dialyzed against distilled H_2O [16,23]. A cyst P-1 glycoprotein (Cyst Mcdon P-1) was the non-dialyzable fraction of the cyst gp after hydrolysis in pH 1.5 at 100°C for 2 h [12–15].

2.3. Sugars used as inhibitors

Monosaccharides, their derivatives and most oligosaccharides were purchased from Sigma Chemical Company (St. Louis, MO, USA) and GalNAcα1→3GalNAcβ1→3Galα1→4Galβ1→4Glc (F_p) was from Accurate Chemical and Scientific Corporation (Westbury, NY, USA)

Tri-antennary Galβ1→4GlcNAc glycopeptides and Tn glycopeptides were prepared from asialo fetuin and asialo OSM respectively. The procedures were as described previously [24,25].

2.4. Lectinochemical assays

The assay was performed according to the procedures described by

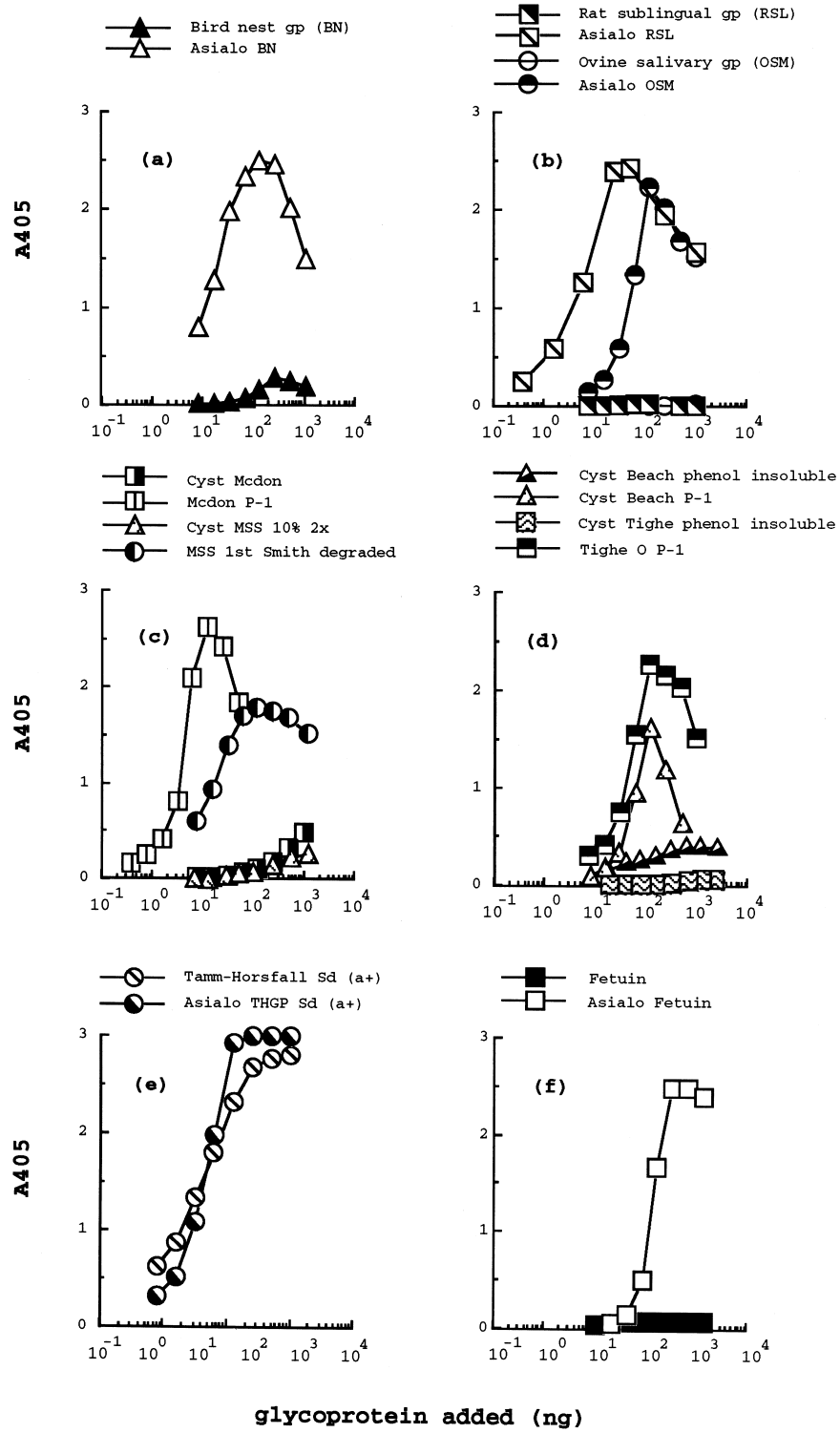

Fig. 1. Binding of CAA to microtiter plates coated with serially diluted glycoproteins. The lectin was used at a constant amount of 20 ng/well. Total volume 50 μl. A_{405} was read at 2 h.

Duk et al. [26]. The volume of each reagent applied to the plate was 50 μl/well, and all incubations, except for coating, were performed at room temperature. The reagents, if not indicated otherwise, were diluted with TBS containing 0.05% Tween 20 (TBS-T). The TBS buffer or 0.15 M NaCl containing 0.05% Tween 20 was used for washing the plate between incubations.

For inhibition studies, the serially diluted inhibitor samples were mixed with an equal volume of lectin solution containing a fixed amount of CAA. The control lectin sample was diluted two-fold with TBS-T. After 30 min at room temperature, the samples were tested by the binding assay, as described by Duk et al. [25,26]. The inhibitory activity was estimated from the inhibition curve and is expressed as the amount of inhibitor (nmol/well) giving 50% inhibition of the control lectin binding.

3. Results

3.1. CAA-glycoform interaction

The interaction patterns of CAA with glycoproteins, as studied by a microtiter plate lectin-enzyme binding assay (ELLSA), are illustrated in Fig. 1 and its binding profile

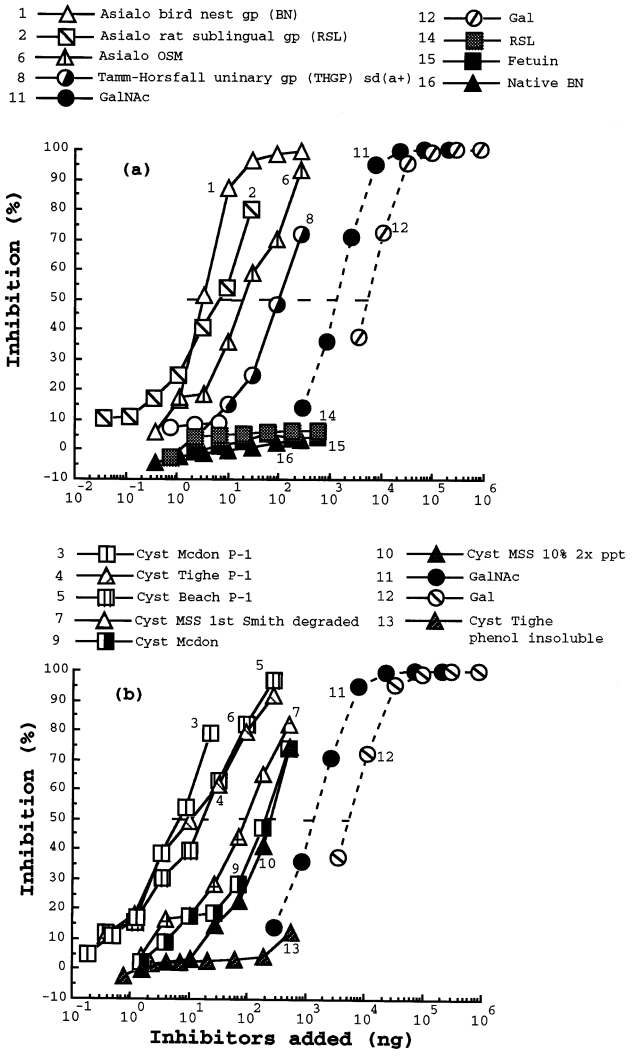

Fig. 2. Inhibition of CAA binding to cyst Mcdon P-1 gp coated plates with various glycoproteins. The quantity of cyst Mcdon P-1 glycoprotein used to coat was 12.5 ng per well. The amount of lectin used for the inhibition assay was 20 ng per well. Total volume: 50 μl. A_{405} was read at 2 h. (a) Salivary gps, urinary gp and fetuin; (b) human ovary cyst gps.

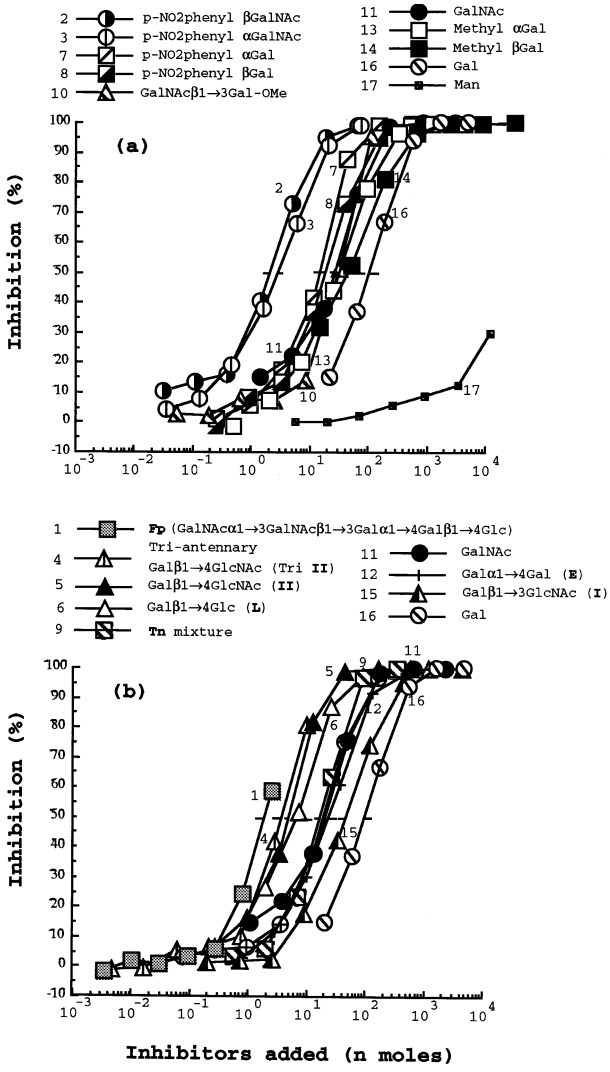

Fig. 3. Inhibition of CAA binding to cyst Mcdon P-1 gp coated ELLSA plates with monosaccharides, and oligosaccharides. Conditions as in Fig. 2. (a) Monosaccharides and their derivatives; (b) mammalian oligo- and disaccharides.

with glycoproteins is shown in Table 1. Among glycoproteins tested, CAA reacted strongly with most GalNAcα/β→ and Galβ1→4GlcNAc containing glycoproteins. These include asialo bird nest glycoprotein (BN) (Fig. 1a), asialo rat sublingual gp (Fig. 1b), human blood group precursor equivalent gps (cyst Mcdon P-1 and MSS 1st Smith degraded, Fig. 1c; cyst Tighe P-1 and Beach P-1, Fig. 1d), native Tamm-Horsfall Sd(a^+) glycoprotein and its asialo product (Fig. 1e) and asialo fetuin (Fig. 1f). They reached absorbance values at 405 nm over 1.7 within 2 h, but were inactive with most sialic acid containing and blood group A, B, H active glycoproteins. To overcome the possible problem of lack of quantitation of glycoproteins absorbed onto the microwells, the inhibitory powers of these glycoproteins were examined and described in the next section.

3.2. Inhibition of CAA-glycoform interaction by various glycoproteins

The abilities of various glycoproteins to inhibit the binding

Table 1
Binding of CAA to human blood group A, B, H, Ii and Le[b] active glycoproteins(gps), sialo and asialo glycoproteins by ELLSA[a]

Order of activities	Curve in Fig. 1	Glycoprotein (lectin determinants[b]; blood group specificity)	Maximum A_{405} absorbance	Binding intensity[c]
1	a	Asialo bird nest gp (II, E, T, F_α)	2.5	+++++
2	b	Asialo RSL (II)	2.5	+++++
3	c	Cyst Mcdon P-1 (T, Tn, I, II)	2.6	+++++
4	e	Asialo THGP Sd(a^+)	3.0	+++++
5	e	Tamm-Horsfall urinary glycoproteins Sd(a^+) (THGP)	2.8	+++++
6	f	Asialo fetuin (II, T)	2.5	+++++
7	b	Asialo OSM (Tn)	2.4	++++
8	d	Cyst Tighe P-1 (I, II)	2.2	++++
9	c	Cyst MSS 1st Smith degraded (T, Tn, I, II)	1.8	+++
10	d	Cyst Beach P-1 (T, Tn, I, II)	1.7	+++
11	c	Cyst Mcdon (A_h)	0.5	+
12	d	Cyst Beach phenol insoluble (B)	0.4	+
13	c	Cyst MSS 2×(A_h[A_1])	0.3	+
14	a	Bird nest gp (BN, Sialyl II, E, T, F)	0.3	+
15	f	Fetuin (Sialyl II, T)	0.07	−
16	d	Cyst Tighe phenol insoluble (H, Le[b])	0.06	−
17	b	Rat sublingual major gp (RSL, Sialyl II)	0.02	−
18	b	Ovine salivary gp (OSM, Sialyl Tn)	0.01	−

[a]Twenty ng of biotinylated lectin was added to various concentrations of glycoprotein ranging from 0.12 ng to 10 μg.
[b]The symbol in parentheses indicates the human blood group activity (Table 2) and/or lectin determinants [4–6]: F, GalNAcα1→3GalNAc; A, GalNAcα1→3Gal; A_h, GalNAcα1→3[LFucα1→2] Gal; B, Galα1→3Gal; E, Galα1→4Gal; T, Galβ1→3GalNAc; Tn, GalNAcα1→Ser/Thr; I/II, Galβ1→3/4GlcNAc; L, Galβ1→4Glc.
[c]The results were interpreted according to the spectrophotometric absorbance value at 405 nm (i.e. OD_{405}) after 2 h incubation as follows: +++++, OD ≥ 2.5; ++++, 2.5 > OD ≥ 2.0; +++, OD: 1.9–1.5; ++, OD: 1.5–0.75; +, OD: 0.75–0.2; and −, OD: < 0.2.

of CAA with Mcdon P-1 were analyzed by ELLSA and shown in Fig. 2. The amounts of glycoprotein (ng) required for 50% inhibition are listed in Table 2.

Among the glycoproteins tested for inhibition of interaction, asialo bird nest gp from Chinese swiftlet (Genus Collocalia) (curve 1), asialo rat sublingual gp (curve 2) and Mcdon P-1, a mild acid hydrolyzed glycoprotein prepared from human ovarian cyst fluid (curve 3) were the best (Fig. 2a), which required less than 7.0 ng to inhibit 50% of the interaction. All other exposed Galβ1→4GalNAc, GalNAcα or GalNAcβ glycotope containing glycoproteins also inhibited well the CAA-Mcdon P-1 interaction (curves 4 to 9), while Cyst Tighe phenol insoluble (blood group H and Le[b] active gp), RSL, fetuin and native BN (curves 13 to 16) were inactive.

3.3. Inhibition of CAA-glycoform interaction by mono- and oligosaccharides

The ability of various sugar ligands to inhibit the binding of CAA with Mcdon P-1 as measured by ELLSA is shown in Fig. 3 and the nmol of ligands required for 50% inhibition of the lectin-glycan interaction are listed in Table 3.

Among the monosaccharides and oligosaccharides studied, the Forssman pentasaccharide (F_p, GalNAcα1→3GalNAcβ1→3Galα1→4Galβ1→4Glc) and *p*-NO_2-phenyl βGalNAc, (curves 1 and 2) were the best inhibitors, and about 1.9 and 2.3 times more active than Tri-antennary Galβ1→4GlcNAc, (Tri-II, curve 4 in Fig. 3b) and monomeric Galβ1→4GlcNAc (curve 5), respectively. F_p (curve 1) was 9.5 and 52.6 times more active than GalNAc (curve 11) and Gal

Table 2
Amount of glycoproteins giving 50% inhibition of binding of CAA (20 ng/50 μl) by Cyst Mcdon P-1 gp (12.5 ng/50 μl)[a]

Order of activities	Curve in Fig. 2	Inhibitor	Quantity giving 50% inhibition (ng)	Reciprocal of relative potency[b]
1	a	Asialo bird nest gp (II, E, T, F_α)	3.1	1774.3
2	a	Asialo RSL (II, Tn)	6.5	846.2
3	b	Cyst Mcdon P-1 (T, Tn, I, II)	7.0	785.7
4	b	Cyst Tighe P-1 (I/II[Ii])	12.0	458.3
5	b	Cyst Beach P-1 (T, Tn, I, II)	18.0	305.6
6	a	Asialo OSM (Tn)	20.0	27.5
7	b	Cyst MSS 1st Smith degraded (T, Tn, I, II)	95.0	57.8
8	a	THGP Sd(a^+)	100.0	55.0
9	b	Cyst Mcdon (A_h)	210.0	26.2
10	b	Cyst MSS 10% 2×ppt (A_h[A_1])	250.0	22.0
11	a, b	GalNAc	1.5×10^3	3.7
12	a, b	Gal	5.5×10^3	1
13	b	Cyst Tighe phenol insoluble (H, Le[b])	12.3% (> 555.6)	
14	a	RSL (Sialyl II)	6.2% (> 555.6)	
15	a	Fetuin (Sialyl II, T)	4.2% (> 277.8)	
16	a	Native bird nest gp (Sialyl II, E, T, F)	3.5% (> 277.8)	

[a]The inhibitory activity was estimated from the inhibition curve in Fig. 2 and is expressed as the amount of inhibitor giving 50% inhibition of the control lectin binding. Total volume 50 μl.
[b]Reciprocal of relative potency of sugars when Gal was taken as 1.0 [10].

Table 3
Amount of various saccharides giving 50% inhibition of binding of CAA (20 ng/50 μl) by Cyst Mcdon P-1 gp (12.5 ng/50 μl)[a]

Curve NO	Curve in Fig. 3	Inhibitor	Quantity giving 50% inhibition (nmol)	Reciprocal of relative potency[b]
1	b	GalNAcα1→3GalNacβ1→3Galα1→4Galβ1→4Glc (F_p)	1.9	9.5
2	a	*p*-NO_2-phenyl βGalNAc	1.9	9.5
3	a	*p*-NO_2-phenyl αGalNAc	2.4	7.5
4	b	Tri-antennary Galβ1→4GlcNAc (Tri-II)	3.6	5.0
5	b	Galβ1→4GlcNAc (II)	4.5	4.0
6	b	Galβ1→4Glc (L)	6.0	3.0
7	a	*p*-NO_2-phenyl αGal	11.0	1.6
8	a	*p*-NO_2-phenyl βGal	14.0	1.3
9	b	Tn mixture	15.0	1.2
10	a	GalNAcβ1→3Gal-OMe	18.0	1.0
11	a, b	GalNAc	18.0	1.0
12	b	Galα1→4Gal (E)	19.0	0.9
13	a	Methyl αGal	19.0	0.9
14	a	Methyl βGal	25.0	0.7
15	b	Galβ1→3GlcNAc (I)	45.0	0.4
16	a, b	Gal	100.0	0.2
17	a	Man	>3055.6 (29.9% inhibition)	

[a]The inhibitory activity was estimated from the inhibition curve in Fig. 3 and is expressed as the amount of inhibitor giving 50% inhibition of the control lectin binding. Total volume 50 μl.
[b]Reciprocal of relative potency of sugars when GalNAc was taken as 1.0 [10].

(curve 16), respectively, while GalNAcβ1→3Gal-OMe and GalNAc (curves 10 and 11) were equally active. Galβ1→4GlcNAc (II) was slightly more active than Galβ1→4Glc (L) (curve 5 vs. curve 6). *p*-NO_2-phenyl derivatives of Gal were about 1.7 times more active than the corresponding methyl derivatives (curve 7 vs. 13, 8 vs. 14 in Fig. 3a) indicating that hydrophobicity contributes somewhat in the binding. Man was tested up to 100× greater concentration than Gal, but was still inactive (negative control).

4. Discussion

In this report, we studied the binding reactivity of CAA by using our recently established ELLSA method, in which the lectin was biotinylated and binding was detected with alkaline phosphatase conjugated avidin [26]. To avoid the possible problem created by differences in immobilization of the glycoproteins on the microplate wells, the reactivities of the glycoproteins used in Fig. 1 (Table 1), were also tested for inhibitory power of lectin-glycoprotein binding (Fig. 2 and Table 2). Another purpose of this study was to examine the inhibition of lectin binding with a variety of well defined oligosaccharides in solution. The conclusions of this study indicate that (1) GalNAcα1→3GalNAc and complex Galβ1→4GlcNAc containing glycoproteins bind strongly to CAA (Figs. 1 and 2 and Tables 1 and 2); (2) when the inhibitory reactivities of CAA-glycoprotein binding by mono- and oligosaccharides were compared, it was shown that Forssman pentasaccharide is the most potent inhibitor; (3) the *p*-NO_2-phenyl derivatives of Gal were more active than the corresponding methyl derivatives (curves 7 vs. 13 and 8 vs. 14 in Fig. 3a), indicating that hydrophobic forces are important for binding (Table 3); (4) the combining site of CAA should be a shallow type or groove (Table 3), as this lectin is able to accommodate the monosaccharide at the non-reducing end of an oligosaccharide, Tn determinants, and probably internal GalNAcβ1→; and (5) its binding affinity toward mammalian structural features can be ranked in decreasing order as follows: Fp ≥ Tri-II and cluster forms of II ≥ L > GalNAc > E > I > Gal.

Both Tn determinant and F_p glycotopes are considered as markers for cancerous tissues [27–29]. The unusual enhancement of activity of F and Tn has been strongly associated with carcinogenesis [28,29].

The CAA-glycan profile obtained by this study can be used to detect human abnormal glycotopes related to F_p and unmasked mII/Tn clusters and to study cell growth and differentiation given the lack of toxicity of this lectin toward mouse fibroblast cells.

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References

[1] Van Damme, E.J.M., Peumans, W.J., Pusztai, A. and Bardocz (1997) Handbook of Plant Lectins: Properties and Biomedical Applications, John Wiley and Sons, Chichester.
[2] Girbes, T., Ferreras, M., Iglesias, R., Citores, L., de Torre, C., Carbajales, M.L., Jimenez, P., deBenito, F.M. and Munoz, R. (1996) Cell. Mol. Biol. 42, 461–471.
[3] Kumar, M.A., Timm, D.E., Neet, K.E., Owen, W.G., Peumans, W.J. and Rao, A.G. (1993) J. Biol. Chem. 268, 25176–25183.
[4] Wu, A.M. and Sugii, S. (1991) Carbohydr. Res. 213, 127–143.
[5] Wu, A.M. and Sugii, S. (1988) Adv. Exp. Med. Biol. 228, 205–263.
[6] Wu, A.M., Song, S.C., Sugii, S. and Herp, A. (1997) Ind. J. Biochem. Biophys. 34, 61–71.
[7] Wu, A.M. (1984) Mol. Cell. Biochem. 61, 131–141.
[8] Gallagher, J.T. (1984) Biosci. Rep. 4, 621–632.
[9] Lis, H. and Sharon, N. (1986) Annu. Rev. Biochem. 55, 35–67.
[10] Wu, A.M., Lin, S.R., Chin, L.K., Chow, L.P. and Lin, J.Y. (1992) J. Biol. Chem. 267, 19130–19139.
[11] Bloch, R., Jenkins, J., Roth, J. and Burger, M.M. (1976) J. Biol. Chem. 251, 5929–5935.
[12] Kabat, E.A. (1956) in: Blood Group Substances. Their Chemistry and Immunochemistry, pp. 135–139, Academic Press, New York.

[13] Wu, A.M., Kabat, E.A., Pereira, M.E.A., Gruezo, F.G. and Liao, J. (1982) Arch. Biochem. Biophys. 215, 390–404.
[14] Wu, A.M., Kabat, E.A., Nilsson, B., Zopf, D.A., Gruezo, F.G. and Liao, J. (1984) J. Biol. Chem. 259, 7178–7186.
[15] Wu, A.M. (1988) Adv. Exp. Med. Biol. 228, 351–394.
[16] Tettamanti, G. and Pigman, W. (1968) Arch. Biochem. Biophys. 124, 41–50.
[17] Herp, A., Wu, A.M. and Moschera, J. (1979) Mol. Cell. Biochem. 23, 27–44.
[18] Herp, A., Borelli, C. and Wu, A.M. (1988) Adv. Exp. Med. Biol. 228, 395–435.
[19] Wu, A.M., Herp, A., Song, S.C., Wu, J.H. and Chang, K.S.S. (1995) Life Sci. 57, 1841–1852.
[20] Wu, A.M., Song, S.C., Wu, J.H. and Kabat, E.A. (1995) Biochem. Biophys. Res. Commun. 216, 814–820.
[21] Wieruszeski, J.M., Michalski, J.C., Montreuil, J., Strecker, G., Peter-Katalinic, J., Egge, H., van Halbeek, H., Mutsaers, J.H.G.M. and Vliegenthart, J.F.G. (1987) J. Biol. Chem. 262, 6650–6657.
[22] Donald, A.S.R., Soh, C.P.C., Watkins, W.M. and Morgan, W.T.J. (1982) Biochem. Biophys. Res. Commun. 93, 1132–1139.
[23] Wu, A.M. and Pigman, W. (1977) Biochem. J. 161, 37–47.
[24] Wu, J.H., Song, S.C., Chen, Y.Y., Tsai, M.C., Kabat, E.A. and Wu, A.M. (1998) FEBS Lett. 427, 134–138.
[25] Wu, A.M., Song, S.C., Wu, J.H., Chang, K.S.S. and Kabat, E.A. (1997) Glycobiology 7, 1061–1066.
[26] Duk, M., Lisowska, E., Wu, J.H. and Wu, A.M. (1994) Anal. Biochem. 221, 266–272.
[27] Itzkowitz, S.H., Bloom, E.J., Kokal, W.A., Modin, G., Hakomori, S.I. and Kim, Y.S. (1990) Cancer 66, 1960–1966.
[28] Hakomori, S. (1989) Adv. Cancer Res. 52, 257–331.
[29] Ono, K., Hattori, H., Uemura, K.I., Nakayama, J., Ota, H. and Katsuyama, T. (1994) J. Histochem. Cytochem. 42, 659–665.