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NATIVE AND ASIALO-TAMM-HORSFALL GLYCOPROTEINS AS IMPORTANT LIGANDS FOR THE DETECTION OF GalNAcβ1→ AND Galβ1→4GlcNAc ACTIVE LECTINS

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SUMMARY: The binding properties of human Tamm-Horsfall Sd(a+) urinary glycoprotein(THGP) and asialo-THGP with various applied lectins was investigated by quantitative precipitin and precipitin inhibition assays. Both glycoproteins completely precipitated *Abrus precatorius* agglutinin(APA). They also reacted well with *Wistaria floribunda* (WFA), *Glycine max* (soybean, SBA), and *Ricinus communis* agglutinins and precipitated over 78% of the lectin nitrogen added, but reacted poorly or weakly with all α-anomeric GalNAc specific lectins, such as *Helix pomatia* (HPA), *Phaseolus lunatus* (lima bean, LBL), and *Maclura pomifera* (MPL) lectins. The glycoprotein-lectin interaction was inhibited by GalNAcβ1→,Galβ1→4GlcNAc, or by both. The findings suggest that Sd (a+) THGP and asialo-THGP are among the best water-soluble glycoprotein ligands for GalNAcβ1→ and Galβ1→4GlcNAc active lectins.

For the past two decades, we have been using water-soluble glycoproteins to study the binding properties of plant lectins, notably glycoconjugates with **A** (GalNAcα1→3Gal), **A_h** (GalNAcα1→3[Fucα1→2]Gal), **B** (Galα1→3Gal), **I/II** (Galβ1→3/4GlcNAc), **T** (Galβ1→3GalNAc) and **Tn** (GalNAcα1→Ser/Thr) determinants (1-8).

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Abbreviations used : Gal, D-galactopyranose; Glc, D-glucopyranose; LFuc or Fuc, L-fucopyranose; GalNAc, 2-acetamido-2-deoxy-D-galactopyranose; GlcNAc, 2-acetamido-2-deoxy-D-glucopyranose.

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However, glycoproteins with a combination of GalNAcβ1→ and Galβ1→4GlcNAc structures have sparingly been used because of a lack of availability of such glycoproteins.

The Tamm-Horsfall (TH) urinary glycoprotein (THGP) is an excellent source for the above glycoforms. In this report, the affinity of this compound and of its desialylated product (asialo-THGP) has been examined on a panel of lectins exhibiting a broad range of carbohydrate-binding specificities by quantitative precipitin and precipitin inhibition assays (9).

Human Tamm-Horsfall glycoprotein is glycosylphosphatidylinositol-anchor-linked membrane glycoprotein, but presumably the soluble secreted glycoprotein lacks the GPI-anchor (10). It is a polymeric macromolecule composed of about 70% protein and 30% carbohydrate. The carbohydrate moiety is linked to the protein core by an *N*-glycosidic linkage with heterogeneous oligosaccharides ranging from nonfucosylated, monosialylated diantennary chains to fucosylated, tetrasialylated, tetra-antennary chains (11). Most Tamm-Horsfall glycoproteins carry the Sd(a+) blood group active determinant GalNAcβ1→4(NeuAcα2→3) Galβ1→4GlcNAcβ1→3Gal, indicating the presence of a repeating *N*-acetyllactosamine unit (12,13), but the rare phenotype Sd(a-) lacks the terminal GalNAc residue (13,14).

The data reported here indicate that the Sd(a+) THGP and its desialylated product are excellent ligands for GalNacβ1→ and *N*-acetyllactosamine (Galβ1→4GlcNAc) specific lectins.

Materials and methods

Tamm-Horsfall glycoprotein 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 (15) with 0.58M NaCl. The precipitated material was lyophilized, its lipid content removed with 9:1, 2:1, and 1:2 chloroform-methanol, and further purified as described (13).

To determine the shielding effect of sialic acid on the terminal sugar residues of the carbohydrate side chains at the non-reducing ends, the native glycoprotein was subjected to mild acid hydrolysis at pH 2.0, 80°C for 90 min. The mild acid treated product is defined as asialo-THGP.

Sugar inhibitors

p-NitrophenylβGalNAc,Galβ1→4GlcNAc and GlcNAc were from Sigma Chemical Co., St.Louis, MO, USA.

Lectins

Ricinus communis agglutinin (RCA_1) was purchased from Boehringer Biochemical GmbH, Mannheim, Germany; *Arachis hypogaea* (peanut, PNA), *Glycine max* (SBA), *Phaseolus lunatus* (LBA), *P. tetragonolubus* (PTA), *B. purpurea alba* (BPA), *B. simplicifolia*-B_4 (BSI-B_4), *Lotus tetragonolobus* (LTL), *Ulex europaeus*-I (UEL-I), *Ulex europaeus*-II (UEL-II), *Lens culinaris* (LCL) and *C. ensiformis* agglutinins were from Sigma Chemical Co. *Maclura pomifera* (MPA), *Helix pomatia* (HPA) and *Wistaria floribunda* (WFA) lectins were purified by adsorption to insoluble polyleucyl hog gastric (A+H) mucin (9,16,17) and eluted by melibiose (18), GalNAc (19) and lactose (20), respectively. *Abrus precatorius* (APA), prepared by Drs. L.P.Chow and J.Y. Lin, Institute of Biochemistry, College of Medicine, National Taiwan University, Taipei, Taiwan, was purified from the seeds of *Abrus precatorius* (jequirity bean) by Sepharose 4B and DEAE-cellulose column chromatographies (21).

Immunochemical assays

Quantitative precipitin and precipitin inhibition assays were performed by a microprecipitation technique (22) using 5.1 to 6.3 μg of lectin nitrogen (N) for each determination; total N in the washed precipitates was estimated by the ninhydrin method (23).

Results and discussion

There are only fragmentary reports on the reactivity of the Tamm-Horsfall glycoprotein with lectins. It was shown that THGP from Sd(a+) donors is precipitated by *Dolichos biflorus* (24), but Sd(a-) THGP which lacks a terminal GalNAc residue, is inactive and that agglutination of human SD(a+)THGP by *Phaseolus vulgaris* is inhibited by GalNAc (25).

This paper expands the scope of the lectin-binding carbohydrate affinity of THGP with Sd(a+) blood group activity by quantitative precipitin and precipitin-inhibition studies of a wide variety of lectins which can provide insight into the specificities and size parameters of their combining site. During the past two decades, this system has been successfully used as a valuable tool to characterize the saccharide-binding affinity of lectins (1-8).

Based on the binding specificities studied with glycans by precipitin and precipitin-inhibition assays, Gal and/or GalNAc specific lectins have been divided into eight classes according to their affinity for disaccharides as all or part of the structures and as GalNAcα1→Ser/Thr of the peptide chain. These determinants can be coded as **A**, $\mathbf{A_h}$, **B**, **I/II**,

T, Tn, E and **L** determinants (1-8). However, more determinants are required to define the possible existence of the bioactive sites of glycoconjugates in a mammalian system. The human Tamm-Horsfall urinary glycoprotein (THGP) from an Sd(a+) donor was chosen for this study as it carries the GalNacβ1→ and Galβ1→4GlcNAc determinants as complete or incomplete structural features in the Sd(a+) blood group active sequence, GalNAcβ1→4(NeuAcα2→3)Galβ1→4GlcNAcβ1→3Gal (12,13) and because its detailed binding properties have not yet been thoroughly investigated. Thus, the goal of this study was to characterize the affinity of Sd(a+) THGP for a panel of lectins by the quantitative precipitin and precipitin-inhibition assays.

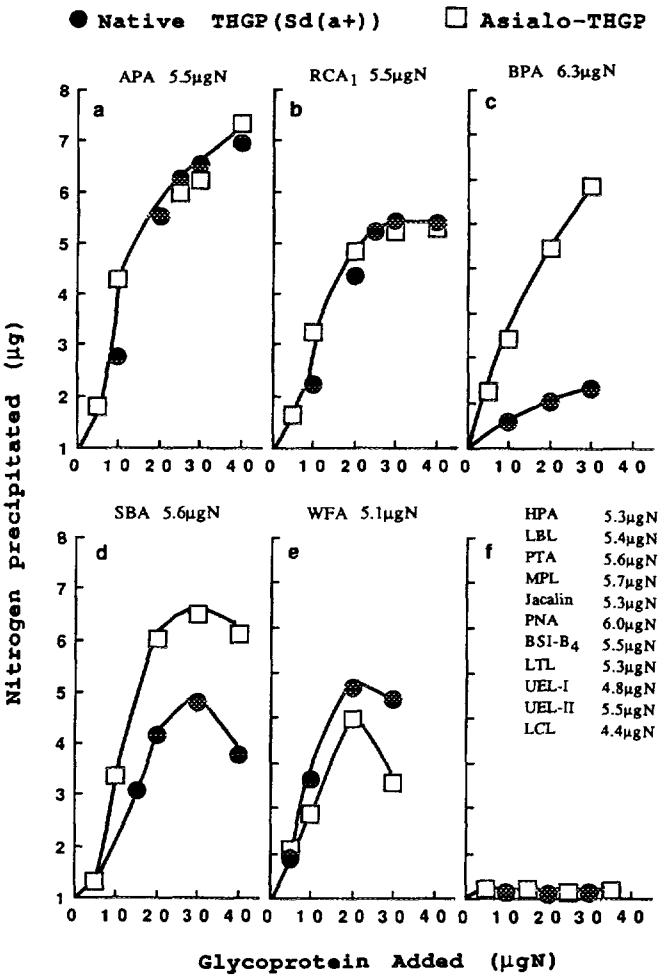

Fig. 1. Quantitative precipitin curves of native and asialo-Tamm-Horsfall glycoproteins with various applied lectins. The amount of lectin nitrogen added ranged from 4.4 to 6.3 μg, as indicated in Table 1. Total volume: 300 μl.

Table 1

Comparative precipitation activities of Tamm-Horsfall glycoprotein, blood type Sd(a+) with various applied lectins

No.	Lectins (agglutinins)	Proposed carbohydrate specificity[a]	Amount of lectin used for precipitation (μgN)	Maximum lectin N precipitated μgN (%) [b]		Amount of glycoprotein required for 50% precipitation (μg)	
				THGP	Asialo-THGP	THGP	Asialo-THGP
1	*A. precatorius* agglutinin (APA)	T>I/II>E>B>Tn	5.5	6.5(118)	6.2 (113)	10.0	7.0
2	*R. communis* agglutinin (RCA_1)	II>I>B>T>>Tn	5.5	5.4 (92)	5.2 (88)	13.0	9.0
3	*W. floribunda* (WFA)	$A(>A_h)$,F>Tn,I(II)	5.1	4.7 (92)	4.0 (78)	10.5	12.0
4	*G. max* (soybean, SBA)	$A(>A_h)$,Tn & I(II)	5.6	4.8 (86)	6.5(116)	15.0	9.0
5	*H. pomatia* (edible snail, HPA)	$F>A(\geq A_h)\geq Tn,T$	5.3	0.5 (9.4)	0.4 (7.5)	-	-
6	*P. lunatus* lectin (lima bean,LBL)	$A_h(>A)>>Tn$	5.4	0.1 (1.8)	0.2 (3.7)	-	-
7	*P. tetragonolobus* lectin (PTA)	$A_h \geq A>B,F$	5.6	0.1 (1.8)	0.1 (1.8)	-	-
8	*B. purpurea alba* (BPL)	T>I(II) & Tn	6.3	1.3 (21)	5.9 (94)	-	13.5
9	*M. pomifera* agglutinin (MPL)	T>Tn	5.7	0.1 (1.8)	0.1 (1.8)	-	-
10	*A. integrifolia* (jacalin)	T>Tn>>>I(II)	5.3	0.2 (3.8)	0.2 (3.8)	-	-
11	*A. hypogaea* (peanut, PNA)	T>>I(II)	6.0	0	0	-	-
12	*B. simplicifolia*-B_4 ($BSI\text{-}B_4$)	B	5.5	0.2 (3.6)	0.1 (1.8)	-	15.5
13	*L. tetragonolobus* lectin (LTL)	$II_y>L_y$	5.3	0.1 (1.9)	0.1 (1.9)	-	-
14	*U. europaeus*-I (UEL-I)	$II_h>II_y$	4.8	0	0.1 (2.0)	-	-
15	*U. europaeus*-II (UEL-II)	$II_h>L_y$	5.5	0.1 (1.8)	0	-	-
16	*Lens culinaris* (lentils, LCL)	$M\beta1\rightarrow4C$	4.4	0.1 (2.3)	0.2 (4.5)	-	-
17	*C. ensiformis* agglutinin (Con A)	$M\beta1\rightarrow4C$	5.0	1.0 (20)	0.8 (16)	-	-

[a] Carbohydrate specificity of lectins as expressed by lectin determinants **F**, $GalNAc\alpha1\rightarrow3GalNAc$; **A**, $GalNAc\alpha1\rightarrow3Gal$; $\mathbf{A_h}$, $GalNAc\alpha1\rightarrow3(LFuc\alpha1\rightarrow2)Gal$; **Tn**, $GalNAc\alpha1\rightarrow$Ser/Thr; **B**, $Gal\alpha1\rightarrow3Gal$; **E**, $Gal\alpha1\rightarrow4Gal$; **I/II**, $Gal\beta1\rightarrow3/4GlcNAc$; **L**, $Gal\beta1\rightarrow4Glc$; **T**, $Gal\beta1\rightarrow3GalNAc$; **C**, $GlcNAc\beta1\rightarrow4GlcNAc$ (chitin disaccharide); $\mathbf{II_y}$, $LFuc\alpha1\rightarrow2Gal\beta1\rightarrow4(LFuc\alpha1\rightarrow3)GlcNAc$; $\mathbf{II_h}$, $LFuc\alpha1\rightarrow2Gal\beta1\rightarrow4GlcNAc$; $\mathbf{L_y}$, $LFuc\alpha1\rightarrow2Gal\beta1\rightarrow4(LFuc\alpha1\rightarrow3)Glc$; **M**, the trimannosidic core structure in *N*-linked glycoproteins.

[b] The value in parentheses indicates the % of μgN precipitated at maximum or at 30 μg glycoprotein when the amount of lectin N added is expressed as 100%.

When the binding properties of native Sd(a+) THGP and asialo-THGP with various applied lectins by the precipitin assay were tested, it was revealed, as shown in Fig. 1a and Table 1, that both the native and the desialylated TH glycoproteins completely precipitated *Abrus precatorius* agglutinin (APA). Sd(a+)-THGP and asialo-THGP also reacted well with *Wistaria floribunda* (WFA), *Glycine max* (soybean, SBA) and *Ricinus communis* (RCA_1) agglutinins and precipitated more than 78% of the lectin nitrogen added (Figs. 1a,b,d,e and 2 and Table 1). When the binding properties of THGP before and after removal of sialic acid were compared, it was found that the reaction profile varies with the lectins tested. The reaction of BPA toward THGP increased dramatically after desialylation (Figs. 1c and 2) while the interaction with APA and RCA_1 remained unchanged (Fig. 1a,b) . The affinity of Sd(a+)THGP increased for SBA (Fig. 1d), and decreased slightly for WFA (Fig. 1e). However, these two glycoproteins reacted poorly or only weakly with all of the α-anomeric GalNAc specific lectins, such as *Helix pomatia* (HPA), *Phaseolus lunatus* (lima bean, LBL), *Artocarpus integrifolia* (jacalin), *Maclura pomifera* (MPL), *Arachis hypogaea* (PNA), and other lectins (Man- and LFuc-specific lectins (Fig. 1f).

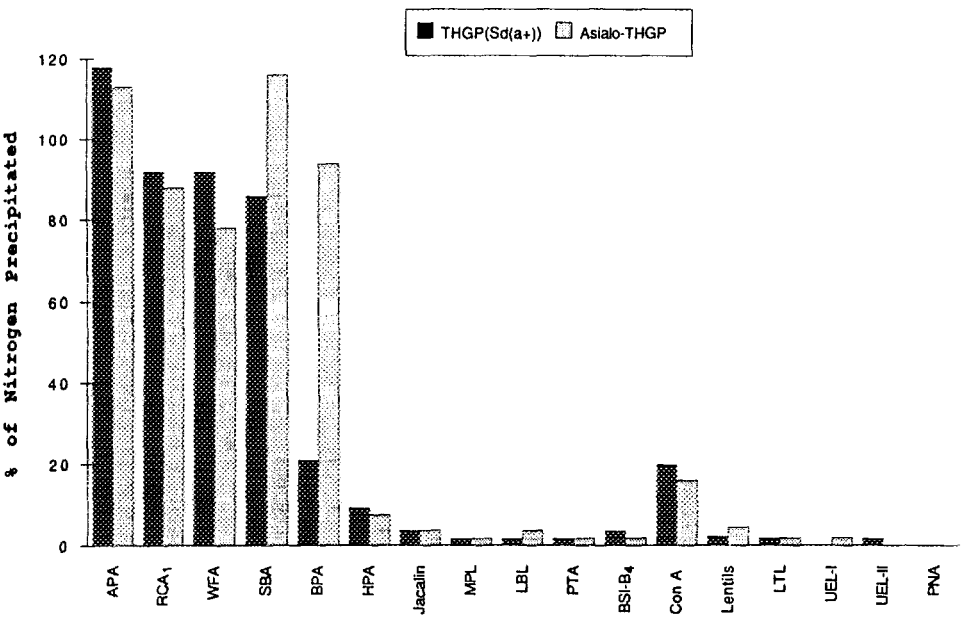

Fig. 2. Comparison of the binding of various lectins with native and asialo-Tamm-Horsfall glycoproteins. % of μg N precipitated at maximum with the amount of lectin added expressed as 100%.

Table 2

Inhibition of Tamm-Horsfall Glycoprotein Sd(a+)-Lectin Interaction by *p*-Nitrophenyl-β-D-GalNAc, Blood Group Type II (Galβ1→4GlcNAc) Sequence and GlcNAc[a]

Lectin tested	Amount of Lectin	Inhibition (%)[b]		
		0.95 μmole *p*-nitrophenyl-β-DGalNAc added	2.0 μmole Galβ1→4GlcNAc (II) added	2.5 μmole GlcNAc added
R. communis agglutinin (RCA_1)	5.5 μgN	8.0	102.2	16.7
A. precatorius agglutinin (APA)	5.5 μgN	0	63.0	4.2
W. floribunda agglutinin (WFA)	5.1 μgN	102.8	101.5	11.4
G. max agglutinin (SBA)	5.6 μgN	99.4	101.4	4.9

[a] A range from 5.1 to 5.6 μgN of lectins in a 3.0-ml glass centrifuge tube was mixed with or without (control) 0.95 μmole *p*-nitrophenyl-β-DGalNAc, 2.0 μmole Galβ1→4GlcNAc, and 2.5 μmole GlcNAc, separately, as inhibitors. After incubation at 37°C for 30 min, 15 μg of Tamm-Horsfall glycoprotein was added and subsequently incubated at the same temperature for 1 h and at 4°C for 6 days.

[b] % of inhibition = difference between A_{570} of nitrogen content in the precipitate without and with inhibitor added/A_{570} of nitrogen content in the precipitate without inhibitor added x 100.

In order to prove that the Sd(a+) THGP-lectin interaction occurs through lectin determinants rather than being nonspecific, two determinants, *p*-NO_2-phenylβGalNAc and Galβ1→4GlcNAc were used to inhibit its lectin association. When the inhibition assay was performed in the range of 5.1 to 5.6 μg N of lectin, (RCA_1, APA, WFA and SBA) and 15 μg of THGP, 63 to 100% of the precipitations were inhibited by 0.95 and 2.0 μmoles of *p*-NO_2-phenylβGalNAc and Galβ1→4GlcNAc, respectively, but not at all or only weakly by 2.5 μmoles of GlcNAc (Table 2).

From this reaction profile, it is suggested that the Sd(a+) Tamm-Horsfall glycoprotein and its desialylated form can be used as important ligands for the detection of GalNAcβ1→ and Galβ1→4GlcNAc active lectins.

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