Interaction of a human blood group Sd(a−) Tamm-Horsfall glycoprotein with applied lectins

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Received 12 March 1996

Abstract **Unlike the human blood group Sd(a+) Tamm-Horsfall glycoprotein (THGP), the Sd(a−) one lacks terminal GalNAcβ1→ residues at the nonreducing ends. The binding properties of this glycoprotein and its asialo product with lectins were characterized by quantitative precipitin (QPA) and precipitin inhibition assays. Among 20 lectins tested by QPA, both native and asialo Sd(a−) THGP reacted best with *Abrus precatorius* and *Ricinus communis* and completely precipitated the lectin added. They also precipitated well *Wistaria floribunda* (WFA), *Glycine max* (SBA), *Bauhinia purpurea alba*, abrin-a and ricin, all of which recognize the Galβ1→4GlcNAcβ1→ sequence, although at different strength. The lectin-glycan interactions were inhibited by Galβ1→4GlcNAc and Galβ1→4Glc. When the precipitability of Sd(a−) THGP was compared with that of the Sd(a+) phenotype, the native Sd(a−) THGP exhibited a 40% lesser affinity for WFA, SBA, WGA and mistletoe lectin-I (ML-I). Mapping the precipitation and inhibition profiles of the present study and the results of THGP Sd(a+), it is concluded that Sd(a−) THGP showed a strongly diminished affinity for GalNAcβ1→ active lectins (SBA and WFA) than the Sd(a+) phenotype.**

Key words: Binding properties of applied lectins; Native glycoprotein; Asialo Tamm-Horsfall glycoprotein

1. Introduction

The blood group substances with Sd^a activity occur in most human secretions, with the highest concentration in urine and meconium [1–4]. It was found that the GalNAcβ1→ residues make an important contribution to the Sd^a determinant structure in Tamm-Horsfall glycoprotein (THGP) [5]. In our previous studies, we reported that a urinary THGP with human blood group Sd(a+) activity [5] contains ligands for GalNAcβ1→ and *N*-acetyllactosamine (Galβ1→4GlcNAc) active lectins [6] and toxic lectins [7]. However, the binding property of the rare phenotype Sd(a−) THGP, which is lacking GalNAcβ1→ residues at the terminal non-reducing ends of the carbohydrate chains [8] has not yet been studied. In order to clarify the binding role of the GalNAcβ1→ residue in the carbohydrate moiety of Sd(a+) THGP, we characterized the binding properties of Sd(a−) THGP before and after mild acid hydrolysis with a panel of lectins exhibiting a wide range of carbohydrate specificities (Table 1) by quantitative precipitin and precipitin inhibition assays. The results suggest that native Sd(a−) THGP has much less affinity for *Wistaria floribunda* (WFA), *Glycine max* (SBA), *Triticum vulgaris* (WGA) and mistletoe lectin-I (ML-I) as compared with the precipitability of Sd(a+) phenotype, and that both native and asialo Sd(a−) THGP contain important receptors for *N*-acetyllactosamine (Galβ1→4GlcNAc, II), and lactose (Galβ1→4Glc, L) specific lectins.

2. Materials and methods

2.1. Tamm-Horsfall glycoprotein

Tamm-Horsfall glycoprotein was prepared from the urine collected from a person with the Sd(a−) red cell phenotype [8]. The glycoprotein was isolated by the procedure of Tamm and Horsfall [9]. The native glycoprotein was subjected to mild acid hydrolysis at pH 2.0, 80°C for 90 min [10,11] and dialyzed against dH_2O. The nondialysable fraction is defined as Sd(a−) asialo THGP.

2.2. Sugar inhibitors

Galβ1→4GlcNAc, Galβ1→4Glc and GlcNAc were purchased from Sigma Chemical Co., St. Louis, MO, USA.

2.3. Lectins

Maclura pomifera (MPA), *Helix pomatia* (HPA) and *Wistaria floribunda* (WFA) lectins were purified by adsorption to insoluble polyleucyl hog gastric (A+H) mucin [12–14] and eluted by melibiose [15], GalNAc [16] and lactose [17], respectively. *Abrus precatorius* (APA) and abrin-a, prepared by Drs. L.P. Chow and J.Y. Lin, Institute of Biochemistry, College of Medicine, National Taiwan University, Taipei, Taiwan, were purified from the seeds of *Abrus precatorius* (jequirity bean) by Sepharose 4B and DEAE-cellulose column chromatographies [18]. The mistletoe lectin-I (ML-I) was isolated from ground plant material mistletoe grown on the locust tree (*Robinia pseudoacacia*) by acid treated agarose affinity chromatography with 0.15 M NaCl as eluant [19]. All other lectins used in this study were purchased from Sigma.

2.4. Lectinochemical assays

Quantitative precipitin and precipitin inhibition assays were performed by a microprecipitation technique [20] using 5.1–6.3 μg of lectin nitrogen mixed with varying amounts of glycoprotein. The mixture was incubated at 37°C for 1 h and kept at 4°C for 1 week. The total N in the washed precipitates was estimated by the ninhydrin method [21].

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Abbreviations: 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; NeuAc, *N*-acetyl-neuraminic acid; THGP, Tamm-Horsfall glycoprotein; asialo THGP, asialo Tamm-Horsfall glycoprotein; II, Galβ1→4GlcNAc; L, Galβ1→4Glc. Abbreviations of lectins and lectin determinants are given in Table 1.

SSDI S0014-5793(96)00320-1

Table 1
Gal, GalNAc, GlcNAc, Man and LFuc specific lectins, and their determinants tested for binding properties of Tamm-Horsfall Sd(a−) glycoprotein and its mild acid hydrolyzed product

No.	Lectin (agglutinin)	Monosaccharide specificity	Determinants[a] (active carbohydrate sequence)
1	*Helix pomatia* (HPA)	GalNAc only	F > A(≥ A_h) ≥ Tn, T
2	*Wistaria floribunda* (WFA)	GalNAc > Gal	A(≥ A_h), F > Tn, I(II)
3	*Glycine max* (soybean, SBA)	GalNAc > Gal	A(≥ A_h), Tn & I(II)
4	*Arachis hypogaea* (peanut, PNA)	Gal only	T > > I(II)
5	*Abrus precatorius* (APA)	Gal > GalNAc	T > I/II > E > B > Tn
6	*Bauhinia purpurea alba* (BPA)	GalNAc > Gal	T > I(II) & Tn
7	*Maclura pomifera* (MPA)	GalNAc > Gal	T > Tn
8	*Artocarpus integrifolia* (jacalin, AIL)	Gal > GalNAc	T > Tn ⋙ I(II)
9	Abrin-a	Gal	E
10	*Ricinus communis* (ricin, RCA_2)	Gal, GalNAc	T, I/II, L > E & B
11	Mistletoe lectin-I (ML-I)	Gal	E, L, T, I/II
12	*Ricinus communis* (RCA_1)	Gal only	II > I > B > T > > Tn
13	*Triticum vulgaris* (wheat germ, WGA)	GlcNAc	C_3, C_4 > C_2
14	*Phaseolus lunatus* (lima bean, LBL)	GalNAc > Gal	A_h(> A) ≫ Tn
15	*Psophocarpus tetragonolobus* (PTA)	GalNAc > Gal	A_h ≥ A > B,F
16	*Concanavalia ensiformis* (Con A)	Man	Mβ1→4C
17	*Lens culinaris* (lentil, LCL)	Man	Mβ1→4C
18	*Lotus tetragonolobus* (LTL)	Fuc	II_y > L_y
19	*Ulex europaeus*-I (UEL-I)	Fuc	II_h > II_y
20	*Ulex europaeus*-II (UEL-II)	Fuc	II_h > L_y

[a]Carbohydrate specificity of lectins as expressed by lectin determinants. F, GalNAcα1→3GalNAc; A, GalNAcα1→3Gal; A_h, GalNAcα1→3(LFucα1→2)Gal; Tn, GalNAcα1→Ser/Thr; B, Galα1→3Gal; E, Galα1→4Gal; I/II, Galβ1→3/4GlcNAc; L, Galβ1→4Glc; T, Galβ1→3GalNAc; C, GlcNAcβ1→4GlcNAc (chitin disaccharide); II_y, LFucα1→2Galβ1→4(LFucα1→3)GlcNAc; II_h, LFucα1→2-Galβ1→4GlcNAc; L_y, LFucα1→2Galβ1→4(LFucα1→3)Glc; M, the trimannosidic core structure in *N*-linked glycoproteins.

3. Results and discussion

Human THGP is the most abundant urinary protein in normal individuals [22–24]. Most THGPs carry the Sd(a+) blood group active determinant, GalNAcβ1→4(-NeuAcα2→3)Galβ1→4GlcNAcβ1→3Gal, indicating the presence of GalNAcβ1→4 at the nonreducing end and a repeating *N*-acetyllactosamine unit [5,25,26]. The Sd(a−) phenotype, which is lacking GalNAcβ1→ residues at the terminal nonreducing ends of the carbohydrate chains, is rare and comprises about 4% in the European population.

In order to prove that a terminal GalNAcβ1→ is the key sugar responsible for certain of the lectin binding properties of THGP Sd(a+) individuals, we investigated the lectin-binding characteristics of Sd(a−) THGP which lacks this sugar residue. In the present communication, the binding properties of the Sd(a−) THGP and its asialo product with a panel of lectins exhibiting a broad range of carbohydrate specificities were characterized by quantitative precipitin (QPA) and precipitin inhibition (QPIA) assays. During the past two decades, this system has been successfully used as a valuable tool to characterize the saccharide affinity of lectins [6,7,27–35], as such studies can provide insight into the specificities and size parameters of the lectin-glycan interactions. Among 20

Table 2
Comparative precipitation activities of Tamm-Horsfall glycoprotein, blood group type Sd(a−) with various applied lectins

No.	Lectin[a]	μgN lectin added	THGP Sd(a−)				Amount of GP required for 50% precipitation (μg)	
			Native		Asialo		THGP Sd(a−)	
			Max. lectin N precipitated μg(%)[b]	Difference % as compared with Sd(a+)	Max. lectin N precipitated μg (%)	Difference % as compared with Sd(a+)	Native	Asialo
1	WFA	5.1	1.8 (35.3)	−56.7	3.6 (71)	−7.0	–	13
2	SBA	5.6	2.5 (44.6)	−41.4	3.8 (66.6)	−49.4	–	19
3	APA	5.5	6.1 (110)	−8.0	6.7 (123)	+10.0	4.5	7.5
4	BPA	6.3	3.2 (50.5)	+29.5	4.8 (75.4)	−18.6	30	15
5	Abrin-a	5.8	3.1 (54.1)	−26.9	5.3 (91.9)	−3.1	27.5	13
6	Ricin	6.0	4.1 (68.3)	+58.3	5.6 (94.0)	−18.0	18	12
7	ML-I	5.1	1.3 (25)	−61.0	2.3 (44.2)	−13.8	–	–
8	RCA_1	5.5	6.6 (120)	+28.0	6.2 (112)	+24.0	9.5	4
9	WGA	5.6	3.0 (53.2)	−48.8	0.2 (4.0)	−4.9	27.5	–
10	Con A	5.0	0	−20.0	0.7 (14.6)	−1.4	–	–

[a]Only results of active lectins are shown.
[b]The values in parentheses indicate the % of μg N precipitated at maximum or at 30 μg glycoprotein when the amount of lectin N added is expressed as 100%.

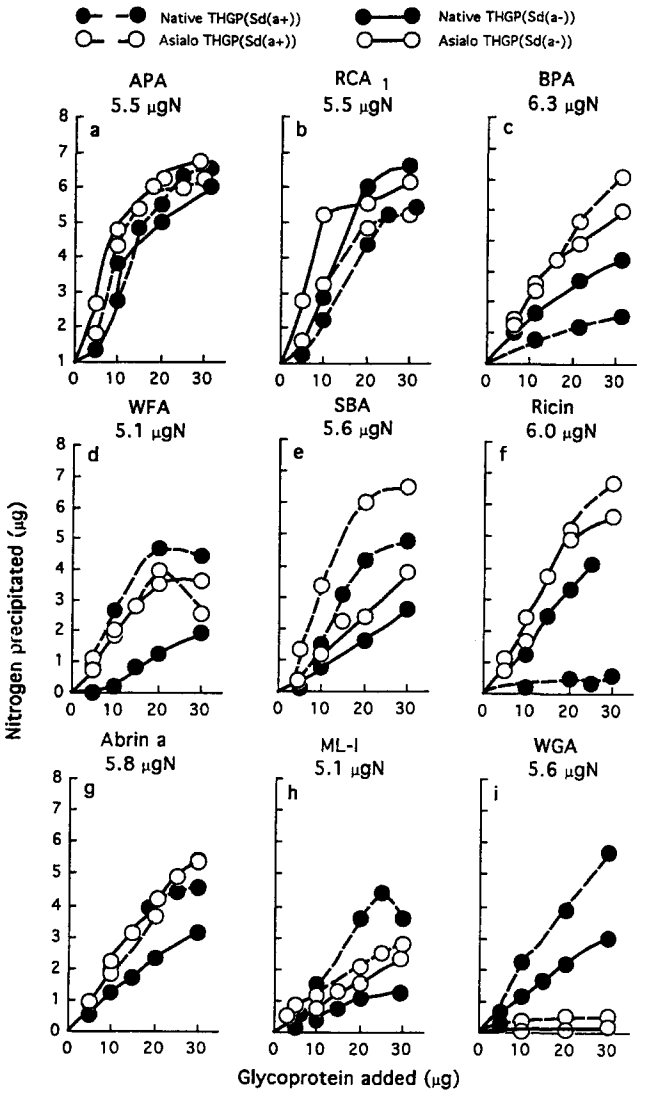

Fig. 1. Quantitative precipitin curves of native and asialo Sd(a+) and Sd(a−) Tamm-Horsfall glycoproteins with various lectins. The amount of lectin nitrogen added ranged from 5.1 to 6.3 μg. Total volume: 300 μl. *Helix pomatia*, *Arachis hypogaea* (PNA), *Maclura pomifera*, *Artocarpus integrifolia* (jacalin), *Phaseolus lunatus* (LBL), *Psophocarpus tetragonolobus*, *Lens culinaris*, *Lotus tetragonolobus*, *Ulex europaeus*-I, *Ulex europaeus*-II were inactive or showed insignificant reactivity.
←

lectins (Table 1) tested by QPA, both native and asialo Sd(a−) THGP reacted best with APA and RCA_1 and completely precipitated the lectin added (Fig. 1a,b). Less than 7.5 and 9.5 μg of glycoprotein was required to precipitate 50% of 5.5 μg APA nitrogen and 5.5 μg RCA_1 nitrogen, respectively (Table 2). They also precipitated well with *B. purpurea alba* (BPA, Fig. 1c), *W. floribunda* (WFA, Fig. 1d), *G. max* (SBA, Fig. 1e), ricin (Fig. 1f), abrin-a (Fig. 1g) and *T. vulgaris* (WGA, Fig. 1i), all of which recognize the Galβ1→4GlcNAcβ1→ sequence with variable strength (Table 1). Except for WGA, the asialo product also reacted significantly with the above lectins (Fig. 1).

The unexpected behavior of Sd(a−) THGP and its asialo products with APA and RCA_1 at the higher glycoprotein concentration is most likely due to the fact that a higher concentration of glycoprotein results in its broader dispersion within the medium, thereby providing greater accessibility to the lectin. This in turn requires a higher amount of glycoprotein to form a lectin complex and a precipitate (rather than having a small amount of glycoprotein being reacted with a larger proportion of lectin molecules). Thus, the higher percent precipitation values reflect the fact that the amount of nitrogen contributed by the glycoprotein in the complex was not corrected for.

In order to verify that the Sd(a−) THGP-lectin interactions occur through specific ligands rather than being nonspecific, three determinant structures, Galβ1→4GlcNAc (II), Galβ1→4Glc (L) and GlcNAc were used to inhibit the THGP-lectin association. As shown in Table 3, these interactions were inhibited by Galβ1→4GlcNAc and Galβ1→4Glc.

Comparison of the precipitation profiles of the Sd(a+) THGP-lectin and Sd(a−) THGP-lectin interactions, as shown in Fig. 1 and Table 2, revealed essential differences in binding properties between these two glycoproteins: native Sd(a+) THGP reacted strongly with WFA (Fig. 1d), SBA (Fig. 1e),

Table 3
Inhibition of Sd(a−) Tamm-Horsfall glycoprotein with various lectins by sugar inhibitors[a]

Lectin tested	Amount of lectin	Inhibition (%)[b]		
		2.9 μmol Galβ1→4Glc (L) added	2.0 μmol Galβ1→4GlcNAc (II) added	2.3 μmol GlcNAc added
(Native THGP) RCA_1	5.5 μg N	102	103	8.6
APA	5.5 μg N	85.2[c]	82.0[c]	0.6
(Desialized THGP) ricin	6.0 μg N	102	98.8	0
WFA	5.1 μg N	106	102	14.2
SBA	5.6 μg N	97.7	96.2	4.4
Abrin-a	6.0 μg N	96.3	94.7	19.7

[a]From 5.1 to 6.0 μg N lectin in 3.0 ml glass centrifuge tube was mixed with or without (control) 2.9 μmol Galβ1→4Glc, 2.0 μmol Galβ1→4GlcNAc and 2.3 μmol GlcNAc, respectively, as inhibitors. After incubation at 37°C for 30 min, 15 μg of Sd(a−) Tamm-Horsfall glycoprotein was added, and subsequently incubated at the same temperature for 1 h and at 4°C for 6 days.
[b]Percent 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 ×100.
[c]Percent inhibition, when 3.5 μmol L and 2.6 μmol II were added, respectively.

mistletoe lectin-I (ML-I) (Fig. 1h) and WGA (Fig. 1i) and precipitated over 86% of the lectin nitrogen added [6,7]. However, in comparison with the Sd(a+) THGP, the affinity of the native Sd(a−) THGP for these lectins decreased by more than 40% (Fig. 1i). On the contrary, the reactivity of Sd(a+) THGP with ricin [7] was increased by about 58% with Sd(a−) THGP (Fig. 1f). The reactivity of Sd(a−) THGP toward RCA_1 (Fig. 1b) and BPA (Fig. 1c) was also more than 28% higher than that of Sd(a+). The increment of these precipitation reactions can be explained by deshielding effects resulting from the absence of GalNAcβ1→ residues in the carbohydrate moiety of Sd(a−) THGP.

Mapping the precipitation and inhibition profiles between this study and the results of Sd(a+) THGP of the previous reports [6,7], it is concluded that native Sd(a−) THGP shows much less affinity for GalNAcβ1→ active lectins (SBA and WFA) than the Sd(a+) phenotype and that both native and asialo Sd(a−) THGP provide important ligands for II (Galβ1→4GlcNAcβ1→) and L (Galβ1→4Glc) active lectins.

Acknowledgements: This work was supported by Grants from the Chang-Gung Medical Research Project (CMRP No. 293), Kwei-san, Tao-yuan, Taiwan, the National Science Council (NSC 84-2331-B-182-016 and 85-2331-B-182-079), the National Health Institutes (DOH 85-HR-316 and DOH 84-HR-209), Department of Health, Taipei, Taiwan.

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