Native and/or asialo–Tamm–Horsfall glycoproteins Sd(a+) are important receptors for *Triticum vulgaris* (wheat germ) agglutinin and for three toxic lectins (abrin-a, ricin and mistletoe toxic lectin-I)

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Abstract The binding properties of human Tamm–Horsfall Sd(a+) urinary glycoprotein (THGP) and asialo-THGP with *Triticum vulgaris* agglutinin(WGA) and three toxic lectins (abrin-a, ricin, and Mistletoe toxic lectin-I) were investigated by quantitative precipitin and precipitin inhibition assays. Both glycoproteins reacted strongly with abrin-a, precipitating over 80% of the lectin nitrogen tested. THGP also bound well to mistletoe toxic lectin-I and precipitated 86% of this lectin added, while the precipitability of its asialo product decreased by 28%. The native glycoprotein completely precipitated the WGA added, but its reactivity was reduced dramatically after desialylation. On the contrary, the poor reactivity of THGP with ricin increased substantially after removal of sialic acid and completely precipitated the lectin added. The glycoprotein–lectin interactions were inhibited by one or several of the following haptens, *p*-NO_2-phenylαGalNAc, *p*-NO_2-phenylβGalNAc, Galβ1→4GlcNAc, Galβ1→4Glc, GlcNAcβ1→4GlcNAc and/or GlcNAc. From the above results, it is concluded that native and/or asialo Tamm–Horsfall glycoproteins serve as important receptors for these three toxic lectins and for WGA.

Key words: Lectin reactivity of abrin-a, ricin, ML-I and wheat germ; Native and asialo–Tamm–Horsfall glycoproteins

1. Introduction

Human Tamm–Horsfall glycoprotein (THGP) is the most abundant protein in normal urine, yet its biological role remains obscure [1,2]. It is a glycosylphosphatidyl inositol-anchor-linked membrane glycoprotein, but presumably the secreted soluble form lacks the GPI-anchor [3]. This glycoprotein is a polymeric macromolecule with subunits of 8.0×10^4–1.0×10^5 M.W. and is composed of about 70% protein and 30% carbohydrate. The carbohydrate moiety is linked to the protein core by *N*-glycosidic linkages with heterogeneous oligosaccharides ranging from nonfucosylated, monosialylated diantennary chains to fucosylated, tetrasialylated, tetraantennary chains [4]. 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 [5,6], but the rare phenotype Sd(a−) lacks the terminal GalNAc residue [6,7].

THGP inhibits the mannose-dependent adhesion of *Escherichia coli* [1,2,8,9] and is a member of a structural glycoprotein family known to modulate cell adhesion [1]. However, little is known about the binding properties of THGP with toxic biomolecules, and/or the roles of sialic acid, *N*-acetyllactosamine, and chitin disaccharide (GlcNAcβ1→4GlcNAc, at the ends of the N-linked chains in the linkage region to the peptide) in the interaction of THGP with lectins. In this report, we characterized the binding properties of THGP, before and after mild acid hydrolysis, with three toxic lectins and *Triticum vulgaris* (WGA) agglutinin by both quantitative precipitin and precipitin inhibition assays.

The results suggest that the Sd(a+) THGP and/or its desialylated product contain important receptors for WGA and for three toxic lectins (abrin-a, ricin, and ML-I).

2. 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 [10] which involved repeated precipitation with 0.58 M NaCl. The precipitated material was dissolved in water, dialyzed and lyophilized. Lipid was removed by extraction with 9:1, 2:1, and 1:2 chloroform/methanol, and this material was further purified as described [6].

To determine the shielding effect of sialic acid on the terminal sugar residues of the carbohydrate side chains at the nonreducing ends, the native glycoprotein was subjected to mild acid hydrolysis at pH 2.0, 80°C for 90 min [11,12]. The mild acid treated product is defined as asialo-THGP [13].

2.1. Sugar inhibitors

p-NitrophenylαGalNAc, *p*-nitrophenylβGalNAc, Galβ1→4Glc, Galβ1 → 4GlcNAc, GlcNAcβ1→4GlcNAc, *N*-acetylneuraminyl-lactose (*N*-acetylneuraminylα2→3/α2→6Galβ1→4Glc) and GlcNAc were from Sigma Chemical Co., St.Louis, MO, USA.

2.2. Lectins

Triticum vulgaris (Wheat Germ) and ricin were purchased from

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Abbreviations: Gal, D-galactopyranose; Glc, D-glucopyranose; GalNAc, 2-acetamido-2-deoxy-D-galactopyranose; GlcNAc, 2-acetamido-2-deoxy-D-glucopyranose; NeuAc, *N*-acetylneuraminic acid; WGA, *Triticum vulgaris* (wheat germ) agglutinin; ML-I, mistletoe toxic lectin-I; THGP, Tamm–Horsfall glycoprotein; asialo THGP, asialo Tamm–Horsfall glycoprotein.

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Sigma Chemical Co. The mistletoe lectin-I (ML-I) provided by Dr. Uwe Pfüller, Universität Witten/Herdecke, Institute of Phytochemistry, Witten, Germany, 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 [14]. Abrin-a was purified from the seeds of *Abrus precatorius* (jequirity bean) by Sepharose-4B and DEAE-cellulose column chromatographies [15].

2.3. Lectinochemical assays

Quantitative precipitin and precipitin inhibition assays were performed by a microprecipitation technique [16] using 5.1 to 6.3 μg of lectin nitrogen (N) mixed with varying amounts of glycoprotein. The mixture was incubated at 37°C for 1h and kept at 4°C for 1 week. The total N in the washed precipitates was estimated by the ninhydrin method [17].

3. Results and discussion

In our previous work, we found that native and asialo-Tamm-Horsfall glycoproteins Sd(a+) are important ligands for the detection of GalNAcβ1→and Galβ1→4GlcNAc active lectins [13]. In this study, the binding properties of THGP and asialo-THGP with three toxic lectins(abrin-a, ricin and ML-I) and with WGA were characterized by quantitative precipitin (QPA) and precipitin-inhibition (QPIA) assays.

During the past two decades, this analytical system has been successfully used as a valuable tool to characterize the saccharide-binding affinity of lectins [13,18–26], as such studies can provide insight into the specificities and size parameters of the lectin-glycan interactions. From the results of the QPA (Table 1 and Fig. 1a), it was found that both glycoproteins reacted strongly with abrin-a and precipitated over 80% of the lectin nitrogen added (Fig. 1a). THGP also bound well with mistletoe toxic lectin-I and precipitated 86% of the lectin tested (Fig. 1b), while the precipitability of its asialo product showed a 28% decrease. The native glycoprotein completely precipitated the WGA added (Fig. 1c), but its reactivity was reduced dramatically after desialylation. In contrast, the poorly reactive THGP with ricin exhibited substantially increased activity after removal of sialic acid and completely precipitated the added lectin (Fig. 1d). To confirm that the Sd(a+) THGP-lectin interaction occurs through lectin determinants rather than being nonspecific, seven inhibitors: *p*-NO_2-phenylαGalNAc, *p*-NO_2-phenylβGalNAc, Galβ1→4GlcNAc, Galβ1→4Glc, GlcNAcβ1→4GlcNAc, *N*-acetylneuraminyl-lactose and GlcNAc were chosen to inhibit its lectin association. When the inhibition assay was performed in the range of 4.8 to 6.0 μg N of lectins, and 20 μg of THGP or asialo-THGP (Table 2), 63 to 100% of the precipitations of ML-I, abrin-a, ricin with native

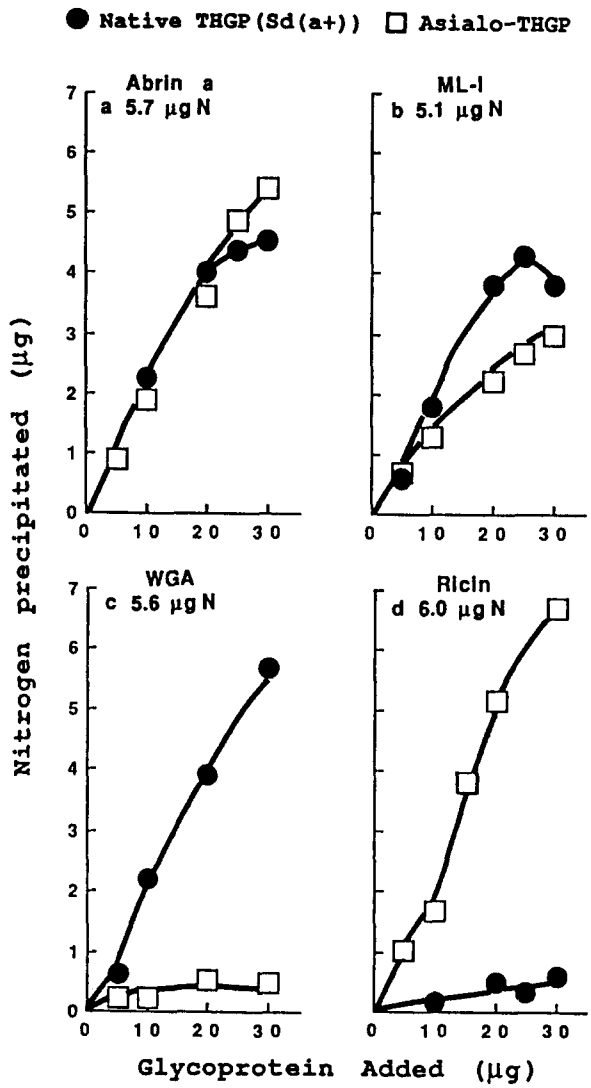

Fig. I. Quantitative precipitin curves of native and asialo-Tamm–Horsfall glycoproteins with three toxic lectins (abrin-a, ricin and mistletoe toxic lectin-I, ML-I) and WGA. The amount of lectin nitrogen added ranged from 5.1 to 6.0 μg. Total volume: 300 μl.

THGP and/or asialo THGP were inhibited by 0.95 μmoles of *p*-NO_2-phenylβGalNAc, 3.6 μmoles Galβ1→4Glc, 2.6 μmoles Galβ1→4GlcNAc and over 95% inhibition of the precipitation of WGA with native THGP occurred in the presence of

Table 1
Comparative precipitation activities of Tamm–Horsfall glycoprotein, blood type Sd(a+) with three toxic lectins and *T. vulgaris* (WGA) agglutinin

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	Abrin a	**E** and **II**	5.7	4.6 (81%)	5.4 (95%)	13.5	15.5
2	Mistletoe toxic lectin-I(ML-I)	**E, L, T, I/II**	5.1	4.4 (86%)	3.0 (58%)	14.5	23.5
3	*T. vulgaris* wheat germ, WGA)	$\mathbf{C_3}$, $\mathbf{C_4} > \mathbf{C_2}$	5.6	5.7 (102%)	0.5 (8.9%)	12.5	–
4	Ricin	**T, I/II > L > E&B**	6.0	0.6 (10%)	6.7 (112%)	–	15.5

[a] Carbohydrate specificity of lectins as expressed by lectin determinants **B**, Galα1→3Gal; **E**, Galα1→4Gal; **I/II**, Galβ1→3/4GlcNAc; **L**, Galβ1→4Glc; **T**, Galβ1→3GalNAc; **C**, GlcNAcβ1→4GlcNAc (chitin disaccharide); $\mathbf{C_3}$, $(GlcNAc\beta1\rightarrow4)_3$; $\mathbf{C_4}$, $(GlcNAc\beta1\rightarrow4)_4$.
[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%.

Table 2
Inhibition of Tamm–Horsfall glycoprotein Sd(a+)-toxic lectins and WGA by sugar inhibition[a]

Lectin tested	Amount of Lectin	Inhibition (%)[b]						
		1.20 μmole *p*-NO_2-phenyl αDGalNAc added	0.95 μmole *p*-NO_2-phenyl βDGalNAc added	3.6 μmole Galβ1→4Glc (L) added	2.6 μmole Galβ1→4Glc NAc (II) added	2.3 μmole GlcNAc added	1.6 μmole NeuAcα2→3 /α2→6 Galβ1→4Glc added	11.2 μmole GlcNAcβ1→4 GlcNAc added
(THGP)								
ML-I	4.8 μgN	49.7	86.4	96.8	88.8	2.8	98.1	0
Abrin a	6.0 μgN	84.0	62.2	92.2	88.3	38.7	58.3	0
WGA-I	5.0 μgN	31.3	18.5	0	53.1	100	36.5	94.5
(Desialized THGP)								
Abrin a	6.0 μgN	92.2	69.5	97.1	90.4	25.0	12.4	2.4
Ricin	6.0 μgN	90.8	96.5	98.9	97.4	0	29.0	4.8

[a]A range from 4.8 to 6.0 μg N of lectins in 3.0 ml glass centrifuge tube was mixed with or without (control) 1.2 μmole *p*-nitrophenyl-α-DGalNAc,0.95 μmole *p*-nitrophenyl-b-DGalNAc, 3.6 μmole Galβ1→4Glc, 2.6 μmole Galβ1→4GlcNAc, 1.6 μmole SA2,3SA2,6 Galβ1→4Glc, 1.2 μmole chitin and 2.3 μmole GlcNAc, respectively, as inhibitors. After incubation at 37°C for 30 min, 20 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 × 100.

2.3 μmoles GlcNAc and 1.2 μmoles GlcNAcβ1 → 4GlcNAc. From the above results, it can be concluded that native and/or asialo Tamm–Horsfall glycoproteins contain the important receptors for WGA and the three toxic lectins. The results imply that sialic acid and the configuration it imposes on the oligosaccharide chains is of major importance for precipitation of WGA because its precipitability was lost after removal of sialic acid.

Toxic lectins, in general, contain two kinds of polypeptide chains – A and B chains. The A chain is a catalytic or toxic unit, and the B chain contains combining sites for special carbohydrates. Thus, in comparison with most of the other lectins (agglutinins), the toxic lectins tested possess only one half of the combining sites. But, they still precipitated strongly with THGP and/or asialo THGP (Table 1 and Fig. 1) indicating that these two glycoproteins contain highly potent ligands for these three toxic lectins. These results also imply that these two glycoproteins can function as cleaning agents to absorb such toxic biomolecules as ricin and abrin-a from the uro-renal system.

Recently, we described that the Galα1→4Galβ1→4GlcNAc trisaccharide contains important ligands (**E**, Galα1→4Gal and crypto **II**, Galβ1→4GlcNAc) for these three toxic lectins [26]. In this study, it is demonstrated that the blood group **II** sequence (Galβ1→4GlcNAc) alone in the carbohydrate moiety of THGP and/or asialo THGP, as shown in Fig. 1, Tables 1 and 2, can be the major receptor for these toxic lectins, as Galα1 → 4Gal (E) sequence is absent from these glycoproteins [4].

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References

[1] Muchmore, A. (1990) Kidney International 37, 1395–1401.
[2] Lambert, C., Steele, B.J. and Rook, G.A.W. (1993) Immunology 79, 203–210.
[3] Rindler, M.J., Naik, S.S., Li, N. and Hoops, T.C. (1990) J. Biol. Chem. 265, 20784–20789.
[4] Hård, K., Van Zadelhoff, G., Moonen, P., Kamerling, J.P. and Vliegenthart, J.F.G. (1992) Eur. J. Biochem. 209, 895–915.
[5] Donald, A.S.R., Yates, A.D., Soh, C.P.C., Morgan, W.T.J. and Watkins, W.M. (1983) Biochem. Biophys. Res. Commun. 115, 625–631.
[6] Donald, A.S.R., Soh, C.P.C., Watkins, W.M. and Morgan, W.T.J. (1982) Biochem. Biophys. Res. Commun. 104, 58–65.
[7] Soh, C.P.C., Morgan, W.T.J., Watkins, W.M. and Donald, A.S.R. (1980) Biochem. Biophys. Res. Commun. 93, 1132–1139.
[8] Fletcher, A.P., Neuberger, A., Ratcliffe, W.A. (1970) Biochem. J. 120, 417–424.
[9] Fletcher, A.P., Neuberger, A., Ratcliffe, W.A. (1970) Biochem. J. 120, 425–432.
[10] Tamm, I. and Horsfall, F.L. (1950) Proc. Soc. Exp. Biol. Med. 74, 108–114.
[11] Tettamanti, G. and Pigman, W. (1968) Arch. Biochem. Biophys. 124, 41–50.
[12] Wu, A.M. and Pigman, W. (1977) Biochem. J. 161, 37–47.
[13] Wu, A.M., Watkins, W.M., Song, S.C., Herp, A. and Wu, J.H. (1995) Biochem. Biophys. Res. Commun. 209, 103–110.
[14] Franz, H., Ziska, P. and Kindt, A. (1981) Biochem. J. 195, 481–484.
[15] Lin, J.Y., Lee, T.C., Hu, S.T. and Tung, T.C. (1981) Toxicon 19, 41–45.
[16] Kabat, E.A. (1961) Kabat and Mayer's Experimental Immunochemnistry, 2nd Edn., C.C. Thomas, Springfield, IL.
[17] Schiffman, G., Kabat, E.A. and Thompson, W. (1964) Biochemistry 3, 113–120.
[18] Wu, A.M. and Sugii, S. (1988) Adv. Exp. Med. Biol. 228, 205–263.
[19] Wu, A.M. and Sugii, S. (1991) Carbohydr. Res. 213, 127–143.
[20] Wu, A.M., Wu, J.H. and Shen, F.S. (1994) Biochem. Biophys. Res. Commun. 178, 251–256.
[21] Wu, A.M., Shen, F.S., Herp, A. and Wu, J.H. (1994) Mol. Immunol. 31, 485–490.
[22] Wu, A.M., Lin, S.R., Chin, L.K., Chow, L.P. and Lin, J.Y. (1992) J. Biol. Chem. 267, 19130–19139.
[23] Wu, A.M., Sugii, S., Gruezo, F.G. and Kabat, E.A. (1988). Carbohydr. Res. 178, 243–257.
[24] Wu, J.H., Herp, A. and Wu, A.M. (1993) Mol. Immunol. 30 333–339.
[25] Wu, A.M., Kabat, E.A., Gruezo, F.G. and Allen, H.J. (19 Arch. Biochem. Biophys. 204, 622–639.
[26] Wu, A.M., Song, S.C., Wu, J.H., Pfüller, U., Chou, L.P. and Y.J. (1995) Biochim. Biophys. Acta 1243, 124–128.