Bandeiraea (Griffonia) simplicifolia lectin-I, isolectin A_4, reacting with Tn (GalNAcα1 → Ser/Thr) or galabiose (Galα1 → 4Gal) containing ligands

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Abstract ***Bandeiraea (Griffonia) simplicifolia*** **lectin-I, isolectin A_4(GS I-A_4) reacting with the Tn(GalNAcα1 → Ser/Thr) sequence or human blood group P^k active disaccharide (E, Galα1 → 4Gal, galabiose) was studied by quantitative precipitin (QPA) and precipitin-inhibition assays. When human blood group P_1 or Tn active glycoproteins were tested by QPA, GS I-A_4 reacted strongly with both the Tn active glycoproteins purified from asialo porcine, ovine and armadillo submandibular glands and a P_1 active glycoprotein isolated from sheep hydatid fluid. They precipitated over 80% of the lectin nitrogen added. The asialo porcine salivary glycoprotein-GS I-A_4 interaction was inhibited by both Tn containing glycopeptides and Galα1 → 4Gal indicating that GS I-A_4 not only reacts with human blood group A(GalNAcα1 → 3Gal) and B(Galα1 → 3Gal) active disaccharides, but also recognizes the Tn sequence and the E(Galα1 → 4-Gal) ligand. From these results, the carbohydrate specificity of GS I-A_4 can be defined as A, Tn ≥ B and E.**

Key words: *Bandeiraea (Griffonia) simplicifolia* lectin-I A_4; Tn-active glycoprotein; Sheep hydatid cyst glycoprotein; Binding property

1. Introduction

The *Bandeiraea (Griffonia) simplicifolia* lectin-I (GS I) is one of two lectin families extracted from the seeds of *B. (Griffonia) simplicifolia* (GS) [1]. It agglutinates human blood group A and B cells and is specific for GalNAcα1 → and Galα1 → at different strengths [2]. The GS I family is a glycoprotein of M_r 114000 consisting of four subunits [2]. This family is composed of five tetrameric isolectins with different binding specificities (A_4, A_3B, A_2B_2, AB_3 and B_4) [3], which result from the combination of two different glycoprotein (A and B) subunits. GS I-A_4 is the member that contains only A subunits. This lectin is most specific for GalNAc, but also reacts with Galα1 → [4]. However, little is known about the interaction of GS I-A_4 with the **Tn**(GalNAcα1 → Ser/Thr) sequence and the galabiose (**E**, Galα1 → 4Gal) ligand. The **Tn** determinant is one of the simplest carbohydrate chains in which GalNAcα1 → is linked to the Ser/Thr of the protein core. It has been proposed as a marker for cancerous tissues [5–9]. At the surface of the red cell membrane, the **Tn** transformation indicates an acquired disorder characterized by the exposure of normally cryptic GalNAc residues α → linked to the hydroxyl group of Ser or Thr of membrane sialoglycoproteins [10,11]. The **Tn** antigen can also be detected at the cell surface of erythrocytes, granulocytes, platelets, and B and T lymphocytes of patients presenting the **Tn** syndrome [12]. Galabiose is the isomer of blood group B active disaccharide (Galα1 → 3Gal), the structural difference being only in the linkage of carbon-3 and carbon-4, respectively, of the second galactose residue. The Galα1 → 4Gal sequence is frequently found in the carbohydrate chains of many glycosphingolipids located at the surface of mammalian cell membranes, such as intestinal and red blood cells and is a receptor for the uropathogenic *E. coli* ligand and for toxin attachment [13,14]. Recently, we found that this sequence also functions as an important receptor for toxic lectins, such as abrin-a and mistletoe toxic lectin-I [14,15]. Therefore, it should be useful to define the affinity of GS I-A_4 for these two ligands, **Tn** and **E**.

In this study, the binding properties of GS I-A_4 with the **Tn** determinant and the galabiose ligand were characterized by quantitative precipitin and precipitin inhibition assays. The results showed that GS I-A_4 bound strongly with **Tn**(GalNAcα1 → Ser/Thr) containing glycoproteins and **E**(Galα1 → 4-Gal) sequences at their terminal non-reducing ends and these reactions were strongly inhibited by **Tn** glycopeptides and Galα1 → 4Gal, indicating that GS I-A_4 is not only reacting with blood group **A** and **B** disaccharides but also recognizes the **Tn** determinant and the **E** sequence.

2. Materials and methods

2.1. Lectin

The *B. simplicifolia* lectin-I isolectin A_4(GS I-A_4) was purchased from Sigma Chemical Co. (L-3019), St Louis, MO, USA.

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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; GS I-A_4 (BS I-A_4), *Griffonia (Bandeiraea) simplicifolia* lectin-I A_4; GP, glycoprotein; THGP, Tamm-Horsfall glycoprotein; MSM, one of human blood group A active glycoproteins purified from human ovarian cyst fluid [26]; OSM, ovine submandibular glycoprotein-major; ASG-A, armadillo submandibular glycoprotein, fraction A [17] which is one of the sialic acid containing glycoproteins in armadillo submandibular glands; native ASG-Tn [18], a naturally occurring Tn glycoprotein isolated from the extract of armadillo submandibular glands in 0.01 M PBS, pH 6.8 after removal of ASG-A; PSM, porcine salivary GP-major; BSM, bovine submandibular GP-major; QPA, quantitative precipitin assay; QPIA, quantitative precipitin-inhibition assay. Lectin determinants that are used to classify applied lectins [14,30,31] are expressed in bold: **A**(GalNAcα1 → 3Gal); **B**(Galα1 → 3Gal); **Tn**(GalNAcα1 → Ser/Thr); **E**(galabiose, Galα1 → 4Gal) [30,31], the human blood group P^k(Galα1 → 4Galβ1 → 4Glc-Cer) active disaccharide or part of P_1 determinant structure (Galα1 → 4Galβ1 → 4GlcNAcβ1 → 3-Galβ1 → 4Glc-Cer) at the terminal non-reducing end

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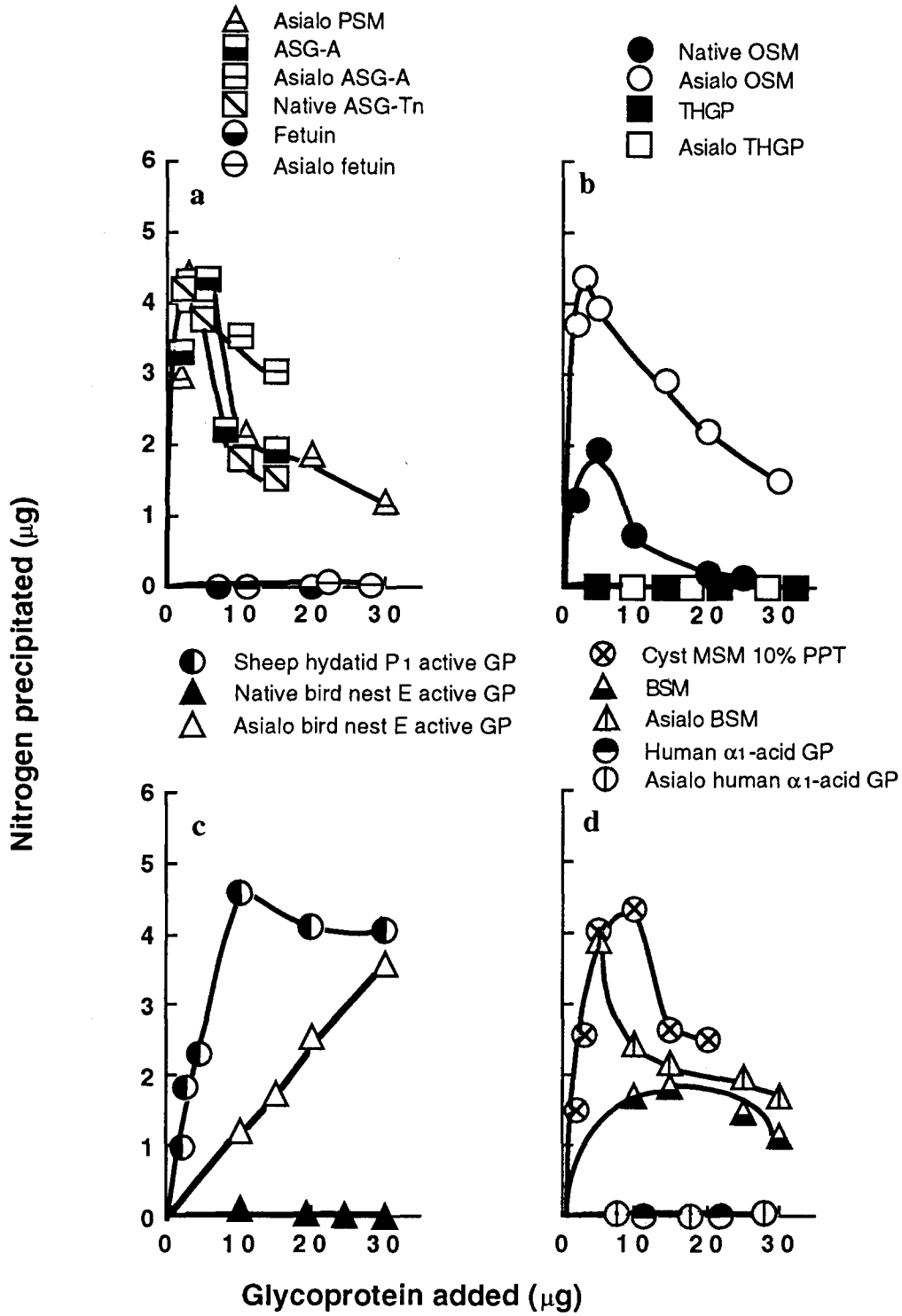

Fig. 1. Quantitative precipitin curves of GS I-A_4 with glycoproteins. Conditions: 5.0 μg GS I-A_4 nitrogen; total volume 300 μl.

2.2. Sialoglycoproteins

The major fraction of ovine (OSM), bovine (BSM), and porcine (PSM) submandibular glycoproteins, as well as fraction A of armadillo (ASG-A) submandibular glycoprotein and native ASG-**Tn** were prepared according to the method of Tettamanti and Pigman [16] or its modification [17–20]. Their chemical composition was analyzed as described previously [16,17].

Human blood group P_1-active glycoprotein [21,22] and Tamm-Horsfall glycoprotein Sd(a+) [23,24] were kindly provided by Dr. W.M. Watkins, University of London, Royal Postgraduate Medical School Hammersmith Hospital, London, UK. The P_1-active glycoprotein was isolated from sheep hydatid cyst by extraction with 95% w/v phenol. The fraction was phenol insoluble and precipitated between 37 and 40% ethanol [21,22]. The human blood group A glycoprotein (MSM) was purified from human ovarian cyst fluid [25,26]. The crude salivary gland mucus glycoprotein (native BN GP) of the Chinese swiftlet (genus *Collocalia*) was extracted with 66°C distilled H_2O for 20 min from commercial bird nest (BN) [27]. Fetuin and human α_1-acid glycoprotein were purchased from Sigma Chemical Co., St. Louis, MO. USA.

For desialylation, a sample of the native glycoprotein, dissolved in 0.01 M HCl at 4 mg/ml was hydrolyzed at 80°C for 90 min and

Table 1
Comparison of precipitating activities of GS I-A_4 with various glycoproteins[a]

Curve in Fig. 1	Glycoprotein (GP)	Maximum precipitated (μg N)	Glycoprotein giving 50% ppt (μg)
a	Asialo porcine salivary GP-major (PSM) (**A**, A_h, **T**, **Tn**)[b]	4.4 (88%)[c]	1.0
	Armadillo submandibular GP (ASG-A) (**Tn** only)	4.3 (86%)	1.0
	Asialo ASG-A (**Tn** only)	4.0 (80%)	1.0
	Native ASG-Tn (**Tn**)	4.2 (84%)	1.0
	Fetuin	0	–
	Asialo fetuin (**II**, **T**)	0	–
b	Ovine salivary GP-major (OSM)	1.9 (38%)	–
	Asialo OSM (**Tn**)	4.4 (88%)	1.0
	Tamm-Horsfall glycoprotein (THGP)	0	–
	Asialo THGP	0	–
c	Sheep hydatid P_1 active GP (**E**)	4.6 (92%)	5.0
	Native bird nest P_1 active GP (cryptic **E**)	0.1 (2%)	–
	Asialo bird nest P_1 active GP (**E**, **F**)	3.6 (72%)	23.0
d	Cyst MSM 10% ppt (**A**)	4.3 (86%)	2.0
	Bovine salivary GP-major (BSM)	1.8 (36%)	–
	Asialo BSM	3.9 (78%)	1.5
	Human α_1-acid GP	0	–
	Asialo human α_1-acid GP (**II**)	0	–

[a]Abbreviations: GP, glycoproteins.
[b]The symbol in parentheses indicates the human blood group activity and/or lectin determinants (A_h, **A**, **E**, **F**, **II**, **Tn**, **T**).
[c]The value in parentheses indicates the % of μg N precipitated at maximum when the amount of lectin added is expressed as 100% (= 5.0 μg N).

dialyzed for 48 h against frequently changed water. The non-dialyzable material was centrifuged to remove insoluble particles and freeze-dried [16,17].

2.3. Sugar inhibitors

Tn glycopeptide preparation: The OSM **Tn** glycopeptides were prepared from asialo-OSM, which was dissolved in 15 mM phosphate buffered saline (PBS, pH 7.5), and digested with proteinase K (Sigma Chemical Co., St. Louis, MO; 10 mg glycoprotein/mg enzyme) at 37°C for 6 h, then at 65°C overnight. The precipitate was removed by centrifugation at 13000 rpm for 30 min. The supernatant was redigested by adding half the amount of proteinase K. Digestion was repeated three times. The glycopeptides were harvested and filtered through a molecular mass cut off (MMCO) 3000 membrane (Amicon Co., MA, USA). Tn glycopeptides used for this study were mixtures of Tn containing glycopeptides in the filtrable fraction (MMCO < 3000). The size distributions of Tn containing glycopeptides were examined by Bio-Gel P-2 (400 mesh) column chromatography using ddH_2O as eluant and dextran, raffinose (trisaccharide) and glucose as references.

Gal, *p*-NO_2-phenylαGalNAc, Galα1→3Galα1→methyl(**B**), Galα1→4Gal(**E**) and Galβ1→4Glc(**L**) were from Sigma Chemical Co., St. Louis, MO, USA.

2.4. Lectinochemical (binding) assays

Quantitative precipitin and precipitin-inhibition assays were performed using a microprecipitin technique [28] employing 5.0 μg of lectin nitrogen (N) mixed with varying amounts of glycoprotein. The mixture was incubated at 37°C for 1 h and kept at 4°C for 1 week. Total N in the washed precipitates was estimated by the ninhydrin method [29].

3. Results and discussion

In this paper, we expand the scope of this study and characterize the binding properties of GS I-A_4 with **Tn**(GalNAcα1→Ser/Thr) and galabiose ligand (Galα1→4Gal) by both quantitative precipitin (QPA) and precipitin-inhibition (QPIA) assays. During the past 2 decades, these analytical systems have been successfully used as valuable tools for characterizing saccharide-lectin interactions [14,15,19,30,31], as such studies can provide insight into the specificities and size parameters of lectin-glycan interactions. When the glycoforms, including human blood group **A**, P_1 and **Tn** active

Table 2
Inhibition of asialo porcine salivary glycoprotein-GS I-A_4 interactions by sugar inhibitors[a]

Glycoprotein	Inhibition (%)[b]					
	0.28 μmol Galα1→3Galα1→methyl (**B**) added	0.35 μmol Galα1→4Gal (**E**) added	0.13 μmol **Tn** mixture added[c]	0.10 μmol *p*-NO_2-phenyl-α-GalNAc added	2.2 μmol Gal added	1.2 μmol Galβ1→4Glc (**L**) added
Asialo porcine salivary GP	81.8	78.5	99.4	100	86.7	1.8

[a]5.0 μg N of lectin in a 3.0 ml glass centrifuge tube was mixed with or without (control) sugar inhibitor, 2.2 μmol Gal, 0.28 μmol Galα1→3Galα1→methyl, 0.35 μmol Galα1→4Gal, 1.2 μmol Galβ1→4Glc, 0.10 μmol *p*-NO_2-phenyl-α-GalNAc, 0.13 μmol **Tn** containing glycopeptide mixture, respectively, as inhibitors. After incubation at 37°C for 30 min, 7.5 μg of the corresponding 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.
[c]μmol GalNAc in **Tn** containing glycopeptides.

glycoproteins were tested by QPA, GS I-A_4 reacted strongly with both **Tn** containing glycoproteins purified from armadillo (Fig. 1a) and asialo ovine (Fig. 1b) submandibular glycoproteins, a P_1 active glycoprotein (Fig. 1c) isolated from sheep hydatid fluid [21,22], asialo porcine salivary glycoprotein (Fig. 1a), and a blood group A active glycoprotein MSM (Fig. 1d) isolated from human ovarian cyst fluid [26]. They precipitated over 80% of the lectin nitrogen added (Table 1) and 1.0–5.0 μg of each glycoprotein was required to achieve 50% precipitation. GS I-A_4 also bound well with the asialo bird nest glycoprotein (BN) from the salivary gland of the Chinese swiftlet which contains Galα1→4Gal residues at the non-reducing end of the carbohydrate chains [27] and precipitated about 72% of lectin nitrogen at the point of 30 μg asialo BN glycoprotein added (Fig. 1c and Table 1). GS I-A_4 did not precipitate fetuin (Fig. 1a), human α_1-acid glycoprotein, (Fig. 1d), native THGP Sd(a+) (Fig. 1b) and their asialo products that contain neither **Tn** nor **E** determinants [23,24,30]. Bovine and ovine submandibular glycoproteins were about one-half as reactive as their corresponding asialo products (Fig. 1b and 1d), because their **Tn** determinants are shielded by sialic acids. Native bird nest glycoprotein (Fig. 1c), in which most of the Galα1→4Gal sequences are masked by sialic acids [27], was inactive.

To establish that the asialo porcine salivary glycoprotein-GS I-A_4 interaction occurs through lectin determinants rather than being non-specific, three disaccharide determinants (Galα1→4Gal, Galα1→3Galα→methyl and Galβ1→4Glc), a mixture of **Tn** containing glycopeptides from asialo OSM (mainly of GalNAcα1→Ser/Thr peptides eluting near raffinose on BioGel P-2 column chromatography), *p*-NO_2-phenylα GalNAc and Gal were used to inhibit the lectin-asialo porcine salivary glycoprotein association. By inhibition of 5.0 μg N of lectin, 79–100% of the precipitation was inhibited by 0.28, 0.35, 0.13, 0.10 and 2.2 μmol of Galα1→3Galα→methyl, Galα1→4Gal, **Tn** glycopeptide mixtures, *p*-NO_2-phenylαGalNAc and Gal, respectively, but not at all or insignificantly with 1.2 μmol Galβ1→4Glc as a control (Table 2).

From the above results, it can be concluded that the **Tn** sequence (GalNAcα1→Ser/Thr) and the Galα1→4Gal disaccharide, in addition to blood group **A**(GalNAcα1→3Gal) and **B**(Galα1→3Gal), exhibit affinity for GS I-A_4.

Most plant tissues contain a single lectin, but many seeds contain two or more lectins that differ in their sugar specificities and other properties [32]. In the seeds of *G. simplicifolia*, several lectins are present for GalNAc, Gal or GlcNAc. At present, little knowledge is available concerning their physiological function. However, the results on the binding properties of GS I-A_4 suggest that this lectin might be useful as a reagent for detecting the presence of **A**, **Tn**, **B** and **E** determinants in glycoforms.

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