

Polyvalency of Tn (GalNAc α -1-Ser/Thr) glycotopes as a critical factor for *Vicia villosa* B₄ and glycoprotein interactions

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Abstract *Vicia villosa* B₄ (VVL-B₄) is an important lectin for detecting exposed Tn (GalNAc α -1-Ser/Thr) determinants on cancer cells. In order to elucidate the binding factors involved in VVL-B₄ and glycotope interaction, the binding properties of this lectin were analyzed by enzyme-linked lectinosorbent and inhibition assays. From the results, it is concluded that the most critical factor affecting VVL-B₄ binding is polyvalency at the α anomer of Gal with –NH CH₃CO at carbon-2 (Tn epitope), which enhances the reactivity by 3.3×10^5 times over monovalent Gal. The reactivities of glycotopes can be ranked as follows: high density Tn cluster \gg Tn glycopeptides (MW $< 3.0 \times 10^3$) \gg monomeric Tn to tri- Tn glycopeptides \gg other GalNAc α / β -related structural units $>$ Gal and Gal α - or β -linked ligands, demonstrating the essential role of the polyvalency of Tn glycotopes in the enhancement of the binding.
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Key words: Polyvalency; Multivalent effect; Carbohydrate specificity; Glycoprotein binding; Lectin; *Vicia villosa* B₄

1. Introduction

The Tn determinant (GalNAc α -O-Ser/Thr), normally a cryptic structure in the peptide core of O-glycoproteins (gps), is expressed in an unmasked form in about 90% of human carcinomas [1–3]. A direct link has been shown to exist between carcinoma aggressiveness and the density of this antigen, including extent of tissue spread and vessel invasion [4].

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Abbreviations: Sugars: Gal, D-galactopyranose; Glc, D-glucopyranose; D-Fuc, D-fucopyranose; L-Ara, L-arabinose; GalNAc, 2-acetamido-2-deoxy-D-galactopyranose; GlcNAc, 2-acetamido-2-deoxy-D-glucopyranose; Man, D-mannopyranose; NeuNAc/Sialic acid, N-acetylneuraminic acid; Glycoproteins (gp): HSM, hamster submaxillary sialyl Tn gp; ASG-Tn, native Tn glycoprotein from armadillo salivary gland; OSM, ovine submandibular glycoprotein-major; BSM, bovine submandibular glycoprotein-major; HOC, human ovarian cyst fluid; PSM, porcine salivary glycoprotein; THGP, Tamm-Horsfall glycoprotein; Lectin: VVL-B₄, Tn-specific lectin from seeds of *Vicia villosa*; The mammalian carbohydrate structural units in glycans used to define binding properties of VVL-B₄ and expressed in parentheses after glycoproteins are listed in Table 2; II, Gal β 1 \rightarrow 4GlcNAc; mII, poly (multi) valent II; ELLSA, enzyme-linked lectinosorbent assay; Reagent: TBS-T, Tris-HCl-buffered saline containing 0.05% Tween 20

Therefore, the Tn determinant (GalNAc α -O-Ser/Thr) has been considered to be the most specific human tumor-associated structure [4]. The Tn antigen has been characterized using both monoclonal antibodies and plant lectins [5–7]. Among these, a plant lectin from the seeds of *Vicia villosa* (VVL-B₄) has been recognized as one of the most useful probes. This lectin is a tetramer composed of two different subunits. Three related isolectins, composed of different amounts of the two subunits, have been purified [8]. The A₄ isolectin (composed of four A subunits) is responsible for the anti-A₁ blood group activity. The B₄ isolectin, which is composed of four B subunits, does not agglutinate normal human erythrocytes, but does agglutinate Tn erythrocytes. VVL-B₄ also agglutinates erythrocytes expressing the Cad determinant [9,10]. The Tn antigen is a terminal O-linked α -GalNAc residue, while the Cad determinant is an O-linked chain with a sialylated β -GalNAc. Many papers have described the binding properties of VVL-B₄ [11–16]. However, these studies were limited to the interaction/inhibition of GalNAc-related saccharides [11,12], Tn monomer to tri-Tn synthetic glycopeptides [13], simple sugar crystal lectin binding [16], and histochemical detection [17]. The recognition profiles with natural glycotope clusters in macromolecules and binding factors [18,19] have not been extensively examined. Therefore, we characterized the binding properties of this lectin by enzyme-linked lectinosorbent (ELLSA) and inhibition assays using our library of ligands [20]. From the results, it is concluded that five important factors are involved in VVL-B₄ binding, with the configuration of Gal being the most essential; the cluster form of Gal with –NH CH₃CO at carbon-2 (high density Tn cluster) enhanced the reactivity by more than 3.3×10^5 times over monovalent Gal. Apart from the Tn glycotope, all other mammalian glycotopes reacted weakly with VVL-B₄, and the reactivities of glycotope clusters and monomeric glycotopes can be ranked in decreasing order as: polyvalent Tn \gg Tn glycopeptides \gg monomeric Tn to tri-Tn glycopeptides $>$ GalNAc \gg Gal, thus giving an excellent example of affinity enhancement by glycotope polyvalencies relevant to carbohydrate–protein recognition processes.

2. Materials and methods

2.1. Glycoproteins

Tn and sialyl-Tn containing ovine, bovine, armadillo, and porcine submandibular gps were purified according to the procedure of Tettamanti and Pigman [21] and its modifications [22]. Native ASG-Tn, a salivary gp of the nine-banded armadillo containing only Tn as carbohydrate side chains, was isolated as described by the method of Wu et al. [23].

Glycophorin A, a sialyl T_α-containing gp, was prepared from the membranes of human blood group O erythrocytes [24,25]. Asialo-glycophorin (T_α glycophorin) was prepared by mild acid hydrolysis [21,22]. Tn-glycophorin was prepared from asialo-glycophorin by Smith degradation [20,24].

ABH blood group active gps (e.g. Cyst MSS, Beach and Tighe) were prepared from human ovarian cyst fluid [26–29]. Human α₁-acid gp and fetuin, which contain multi-antennary II containing N-glycans, were purchased from Sigma Chemical (St. Louis, MO, USA).

Desialylation of sialo gps was performed by mild acid hydrolysis in 0.01 N HCl at 80°C for 90 min and dialyzed against distilled H₂O for 2 days to remove small fragments [21,22].

2.2. Sugars used for inhibition studies

Monosaccharides and oligosaccharides were purchased from Sigma Chemical and Dextra (Berkshire, UK). Tn glycopeptides (MW < 3.0 × 10³) and tri-antennary Galβ1 → 4GlcNAc glycopeptides were prepared from asialo fetuin and asialo OSM, respectively [30,31].

2.3. Lectin

VVL-B₄ was purchased from Sigma Chemical. Biotinylation of lectin was performed as described by Duk et al. [20].

2.4. Lectinochemical assays

The assay was performed according to the procedures described by

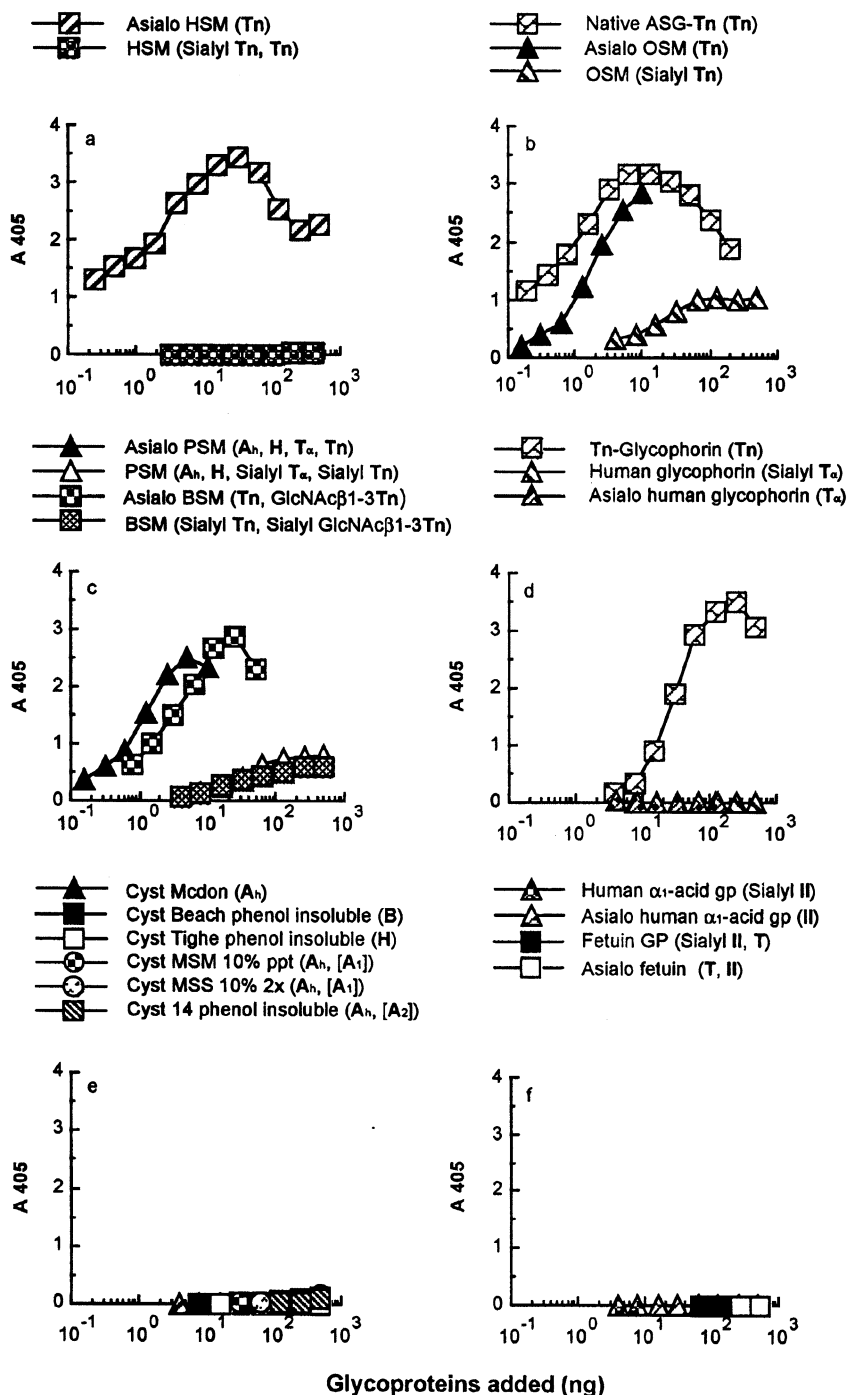


Fig. 1. Binding of VVL-B₄ to microtiter plates coated with serially diluted human blood group ABH and Tn active gps, sialo and asialo gps. The amount of lectin used was 5 ng/well. Total volume of the assay was 50 μl. A₄₀₅ was recorded after 2 h incubation.

Duk et al. [20]. The volume of each reagent applied to the plate was 50 μ l/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 VVL-B₄. The control lectin sample was diluted twofold with TBS-T. After 30 min at room temperature, the samples were tested by the binding assay, as described by Duk et al. [20]. The inhibitory activity was estimated from the inhibition curve and is expressed as the amount of inhibitor (nmol/well in Table 2 and ng in Table 3) giving 50% inhibition of the control lectin binding.

3. Results

3.1. VVL-B₄-glycoform interaction

The interaction profiles of VVL-B₄ with various gps, as studied by a microtiter plate ELLSA, are illustrated in Fig. 1 and the maximum A_{405} value and the amount required for 1.5 (A_{405}) units are shown in Table 1. Among the ligands tested, VVL-B₄ bound most strongly to polyvalent **Tn** containing gps (asialo hamster, porcine, ovine, and bovine submaxillary mucin in Fig. 1a–c and native armadillo submandibular **Tn** gps in Fig. 1b) [18,19]. These reached absorbance values of over 2.5 (A_{405}) within 2 h and required less than 3.1 ng gp for 1.5 units of absorbance. VVL-B₄ also reacted well with the human **Tn**-glycophorin (Fig. 1d) but required 20.0 ng of gp to reach 1.5 units of absorbance. VVL-B₄ reacted poorly with sialylated **Tn** containing gps (OSM, PSM, BSM, HSM in Fig. 1a–c), **T α** gp (asialo human glycophorin, Fig. 1d), ABH blood group gps (Cyst Mcdon (**A_h**), Cyst Beach phenol-in-

soluble (**B_h**), Cyst Tighe phenol-insoluble (**H**); Fig. 1e) as well as native and asialo fetuin/human α_1 -acid gp (**mII**). These results indicate that the unmasked polyvalent **Tn** glycotopes, but not the other mammalian structures (**I/II**, **ABH** and **T**) are the essential factors for VVL-B₄ binding.

3.2. Inhibition of VVL-B₄-glycoform interaction by mono- and oligosaccharides

The ability of various sugar ligands to inhibit the binding of VVL-B₄ with a **Tn**-containing gp (asialo OSM) as measured by ELLSA is shown in Fig. 2 and the nanomoles of ligands required for 50% inhibition of the lectin–glycan interaction are listed in Table 2. Among the monosaccharides and oligosaccharides studied, the **Tn** glycopeptide fraction (MW < 3.0 $\times 10^3$) from ovine submandibular gp was the best inhibitor. It was about 1.2 $\times 10^3$, 13.3 and 3.3 times more active than Gal, GalNAc and GalNAc α 1 \rightarrow Ser/Thr (**Tn**), respectively. This fraction was about 5–28 times more active than other GalNAc-related ligands – **P α** (GalNAc β 1-3Gal α 1-O-Me), **S** (GalNAc β 1-4Gal), **A** (GalNAc α 1-3Gal), **P** (GalNAc β 1-3Gal) and **F** (GalNAc α 1-3GalNAc) – while **L** (Gal β 1-4Glc), **I** (Gal β 1-3GlcNAc), **II** (Gal β 1-4GlcNAc), **E** (Gal α 1-4Gal), **T α** (Gal β 1-3GalNAc α 1-benzyl), **T** (Gal β 1-3GalNAc), **B** (Gal α 1-3Gal) and **T β** (Gal β 1-3GalNAc β 1-O-Me) (curves 24–31) were poor inhibitors.

3.3. Inhibition of VVL-B₄-glycoform interaction by various glycoproteins

The abilities of various gps to inhibit the binding of VVL-B₄ with asialo OSM were analyzed by ELLSA and are shown

Table 1
Binding of VVL-B₄ (5 ng/well) to human blood group A, B, H, P₁ Le^a and Le^b active gps, sialo and asialo gps by ELLSA^a

Graph in Fig. 1	Glycoprotein (lectin determinants ^b ; blood group specificity)	1.5 (A_{405}) unit (ng)	Maximum A_{405}	
			Absorbance reading ^c	Binding intensity ^c
Exposed polyvalent Tn gps				
a	Asialo HSM (Tn)	0.4	3.4	+++++
b	Native ASG- Tn (Tn)	0.5	3.2	+++++
c	Asialo PSM (Tn , Tα , A_h , H)	1.1	2.5	++++
b	Asialo OSM (Tn)	1.7	2.9	+++++
c	Asialo BSM (Tn , GlcNAc β 1-3 Tn)	3.1	2.9	+++++
d	Tn -glycophorin (Tn)	20.0	3.5	+++++
Cryptic Tn and other gps				
b	OSM (sialyl Tn , Tn)	–	1.0	++
c	PSM (A_h , H , sialyl Tα , sialyl Tn)	–	0.8	+
c	BSM (sialyl Tn , sialyl GlcNAc β 1-3 Tn)	–	0.6	+
e	Cyst MSM 10% ppt (A_h , [A₁])	–	0.2	±
e	Cyst MSS 10% 2 \times (A_h , [A₁])	–	0.1	–
e	Cyst 14 phenol-insoluble (A_h , [A₂])	–	0.1	–
e	Cyst Mcdon (A_h)	–	0.1	–
e	Cyst Beach phenol-insoluble (B)	–	0.1	–
a	HSM (Sialyl Tn , Tn)	–	0	–
d	Human glycophorin (sialyl Tα)	–	0	–
d	Asialo human glycophorin (Tα)	–	0	–
f	Human α_1 -acid gp (sialyl II)	–	0	–
f	Asialo human α_1 -acid gp (II)	–	0	–
e	Cyst Tighe phenol-insoluble (H)	–	0	–
f	Fetuin gp (sialyl II , T)	–	0	–
f	Asialo fetuin (T , II)	–	0	–

^a5 ng of biotinylated lectin was added to various gp concentrations ranging from 0.001 ng to 5 μ g.

^bThe symbol in parentheses indicates the human blood group activity and/or lectin determinants [19]. Expressed in bold are: **A** (GalNAc α 1-3Gal); **A_h** (GalNAc α 1-3[LFCu α 1-2]Gal); **B** (Gal α 1-3Gal); **H** (LFCu α 1-2Gal); **T** (Gal β 1-3GalNAc); **Tn** (GalNAc α 1-Ser/Thr); **I/II** (Gal β 1-3/4GlcNAc).

^cThe results were interpreted according to the measured A_{405} after 2 h incubation as follows: +++++ (OD \geq 2.5), ++++ (2.5 > OD \geq 2.0), +++ (2.0 > OD \geq 1.5), ++ (1.5 > OD \geq 1.0), + (1.0 > OD \geq 0.5), ± (0.5 > OD \geq 0.2), and – (OD < 0.2).

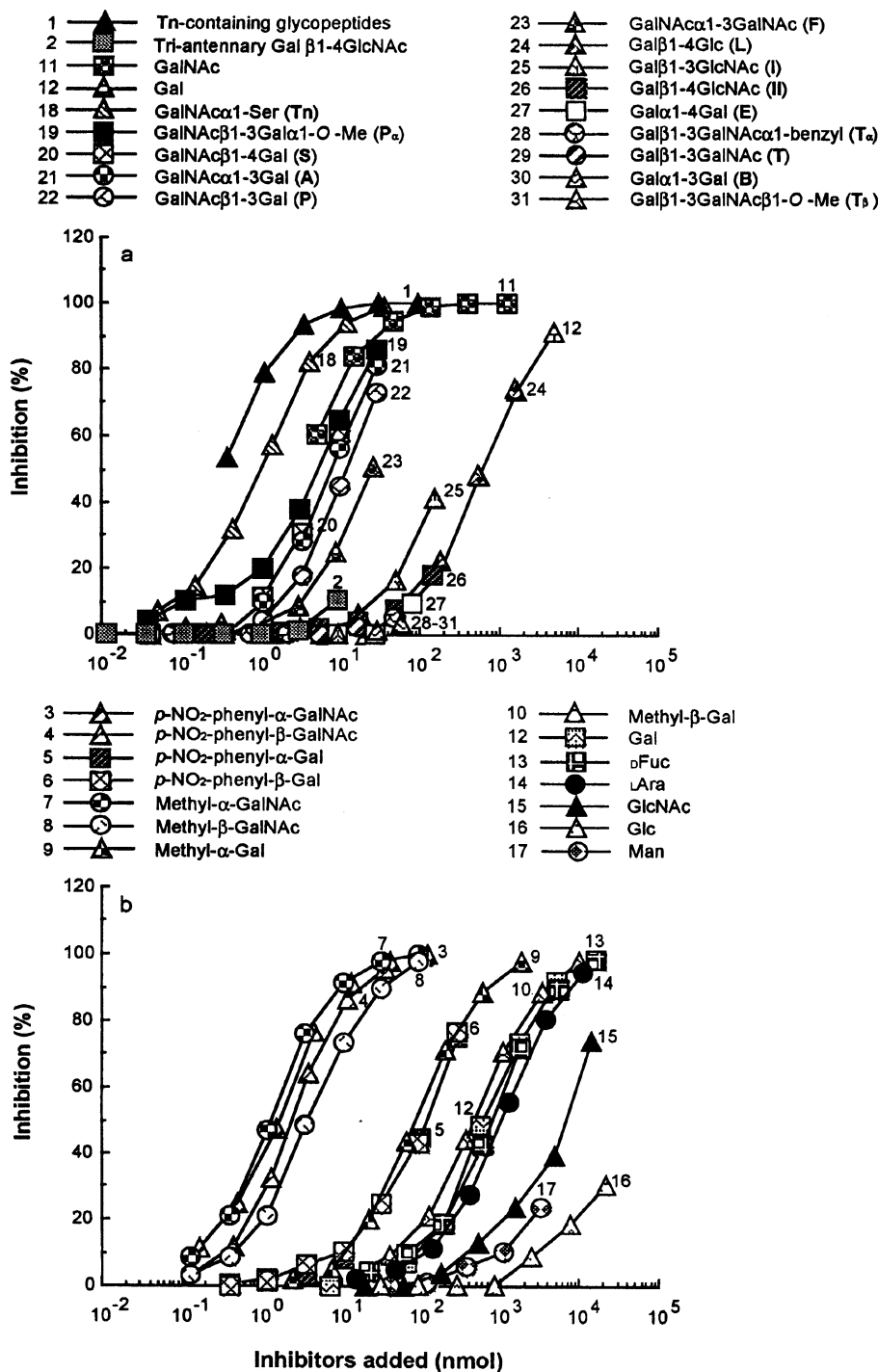


Fig. 2. Inhibition of VVL-B₄ binding to a Tn-containing gp (asialo OSM)-coated ELLSA plate by various saccharides. The amount of gp in the coating solution was 10.0 ng/well. The lectin (10.0 ng/well) was pre-incubated with an equal volume of serially diluted inhibitor. The final VVL-B₄ content was 5.0 ng/well. The total volume was 50 μ l. A₄₀₅ was recorded after 2 h incubation. a: Inhibition profile of mammalian structural units. b: Inhibition profile of monosaccharides and their derivatives.

in Fig. 3. The amounts of gp required for 50% inhibition are listed in Table 3. Among the gps tested for inhibition of interaction, three Tn-containing gps (asialo OSM, native ASG-Tn and asialo BSM) were the best (Fig. 3), requiring less than 0.6 ng to inhibit 50% of the interaction. They were up to 3.3×10^5 , 4.4×10^3 and 1.5×10^3 times more active than Gal, GalNAc, and Tn monomer, respectively, while the Tn glycopeptide fraction (MW < 3.0×10^3) was only 5.2 times more

active than Tn monomer (curves 1–4 vs. 10 and 11). These results establish that polyvalency of Tn plays an important role in VVL-B₄ and gp binding. Proline-rich Tn gp (asialo HSM, curve 4), and a low density of Tn gp (curve 5, Tn-glycophorin, containing less than 15 Tn residues/mol) reduced the reactivity significantly. Apart from asialo OSM and asialo BSM, most crypto-Tn glycans and I/II-containing gps were inactive.

Table 2
Amount of various saccharides giving 50% inhibition of binding of VVL-B₄ (5 ng/50 μl) by asialo OSM (2 ng/50 μl)^a

Curve No.	Curve in Fig. 2	Saccharide	Quantity giving 50% inhibition (nmol)	Relative potency ^b
Simple Tn cluster				
1	a	Tn-containing glycopeptides (MW < 3.0 × 10 ³)	0.3	1200.0
2	a	Tri-antennary Galβ1 → 4GlcNAc	> 8.9 (10.0%)	–
Monosaccharides and their derivatives				
3	b	<i>p</i> -NO ₂ -phenyl-α-GalNAc	1.9	189.5
4	b	<i>p</i> -NO ₂ -phenyl-β-GalNAc	2.3	156.5
5	b	<i>p</i> -NO ₂ -phenyl-α-Gal	102.0	3.5
6	b	<i>p</i> -NO ₂ -phenyl-β-Gal	102.0	3.5
7	b	Methyl-α-GalNAc	1.2	300.0
8	b	Methyl-β-GalNAc	3.5	102.9
9	b	Methyl-α-Gal	80.0	4.5
10	b	Methyl-β-Gal	410.0	0.9
11	a	GalNAc	4.0	90.0
12	a, b	Gal	360.0	1.0
13	b	D-Fuc	700.0	0.5
14	b	L-Ara	910.0	0.4
15	b	GlcNAc	7.0 × 10 ³	0.051
16	b	Glc	> 2.0 × 10 ⁴ (30.0%) ^c	–
17	b	Man	> 3.0 × 10 ³ (23.7%)	–
Mammalian carbohydrate structural units				
18	a	GalNAcα1-Ser (Tn)	1.0	360.0
19	a	GalNAcβ1-3Galα1-O-Me (P _α)	5.0	72.0
20	a	GalNAcβ1-4Gal (S)	6.0	60.0
21	a	GalNAcα1-3Gal (A)	8.0	45.0
22	a	GalNAcβ1-3Gal (P)	11.0	32.7
23	a	GalNAcα1-3GalNAc (F)	28.0	12.9
24	a	Galβ1-4Glc (L)	540.0	0.7
25	a	Galβ1-3GlcNAc (I)	> 148.3 (41.1%)	–
26	a	Galβ1-4GlcNAc (II)	> 145 (17.9%)	–
27	a	Galα1-4Gal (E)	> 31.1 (9.3%)	–
28	a	Galβ1-3GalNAcα1-benzyl (T _α)	> 46.9 (4.6%)	–
29	a	Galβ1-3GalNAc (T)	> 57.8 (3.4%)	–
30	a	Galα1-3Gal (B)	> 31.1 (1.4%)	–
31	a	Galβ1-3GalNAcβ1-O-Me (T _β)	> 27.8 (0.8%)	–

^aThe inhibitory activity was estimated from the inhibition curve in Fig. 2 and is expressed as the amount of inhibitor giving 50% inhibition. Total volume 50 μl.

^bRelative potency = quantity of Gal (curve 12) required for 50% inhibition taken as 1.0/quantity of sample required for 50% inhibition.

^cThe inhibitory potency of inactive saccharides is expressed as the maximum amount of sugar tested that yielded inhibition (in parentheses) below 50%.

4. Discussion

During the past two decades, it has been established that many multi-branched oligosaccharides exhibit a significant increment in lectin binding reactivity as compared to their linear counterparts [32], especially ‘the glycoside cluster effect’ in mammalian hepatic asialo gp receptors [33,34]. Based on the results of this study, the concept of glycoside cluster effect can be classified into two groups: (a) the ‘*multi-antennary* or simple glycoside cluster effect’ as in galactosides with hepatic lectin [33,34] and tri-antennary II sequences as in a galectin from chicken liver (CG-16) [35] or Tn glycopeptides (curve 7 in Table 3). The molecular sizes of these ligands are usually less than 1.0 × 10⁴; and (b) the ‘*high-density polyvalent* or complex glycoside cluster effect’, such as polyvalent Tn in asialo OSM which generates an enhancement in affinity with VVL-B₄ by about 3.3 × 10⁵ times over Gal (curves 1 vs. 11 in Table 3) and is about 1000 times more active than monomeric Tn. A similar phenomenon was also observed with some animal lectins [36]. This peculiar effect could be explained by a remarkably extended sugar binding surface area formed by the planar array of four sugar binding sites of VVL-B₄ tetramers, based on the model of another structurally well-studied polyvalent ligand binding protein, the mammalian mannose binding protein A [37]. However, the effect of poly-

valencies of glycotopes on the carbohydrate–protein binding does not always make such an important contribution. For example, the potency in the interaction of *Pseudomonas aeruginosa* II lectin with L-Fucα1 polyvalent glycans is about as strong as or weaker than the incremental increase by carbohydrate specificity of monomers [38] (unpublished data). Therefore, to obtain a solid description of the carbohydrate specificities of a lectin in order to elucidate its functional roles and biomedical applications, the following information should be given: (i) monosaccharide specificity (Gal, GalNAc, GlcNAc, and/or Man), (ii) expression of reactivities toward mammalian disaccharides and Tn structural units (in decreasing order) [32], (iii) the most active ligand, (iv) simple multi-valent or cluster effect of carbohydrate structured unit such as Tn glycopeptides and multi-antennary glycotopes to inhibit binding, and (v) complex multivalent or cluster effects present in macromolecules with known glycotopes.

Based on these guidelines, we demonstrated the effects of various factors on VVL-B₄ binding as follows: (a) the configuration of Gal is essential (Table 3), (b) –NH CH₃CO at carbon-2 of GalNAc enhances the reactivity by more than 73 times (Table 3), (c) the α-anomer of GalNAc (Tn) is more potent than the β-anomer (GalNAcβ1 →) and is the most active ligand, (d) a simple Tn cluster (Tn glycopeptide; MW < 3.0 × 10³; Table 3) can increase the reactivity up to

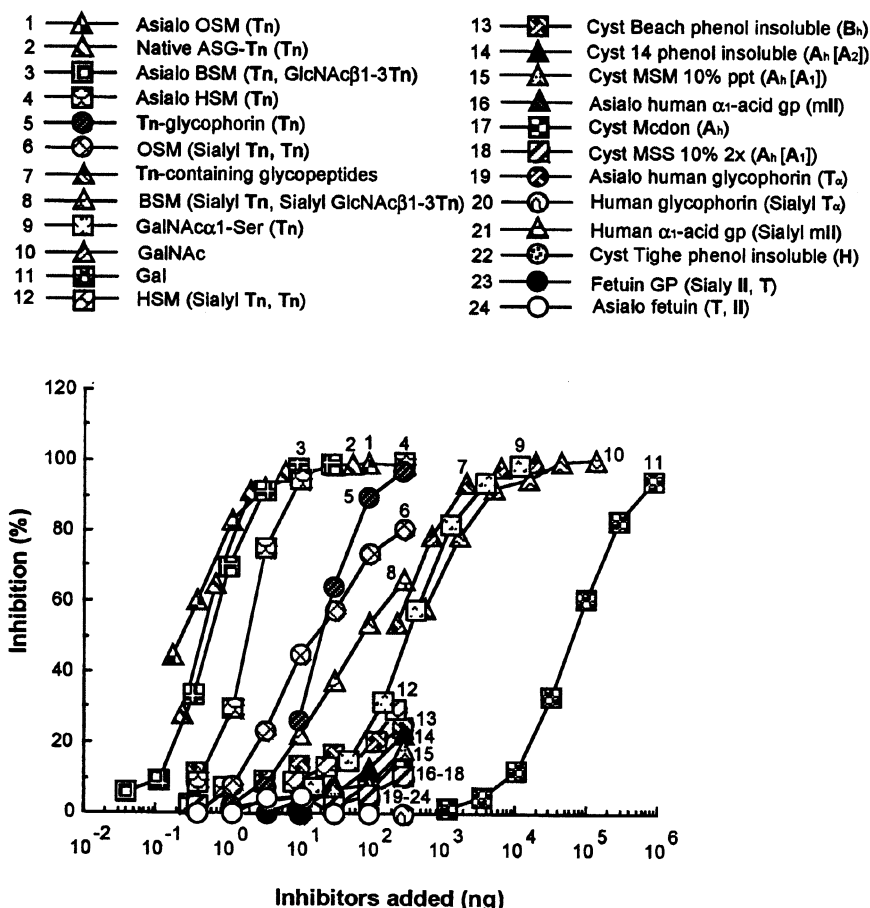


Fig. 3. Inhibition of VVL-B₄ binding to a Tn-containing gp (asialo OSM)-coated ELLSA plate with various gps. The quantity of gp in the coating solution was 10.0 ng/well. The quantity of lectin used for the inhibition assay was 5 ng/well. The total volume was 50 μ l. A_{405} was recorded after 2 h incubation. The amount (ng) of gp required to induce 50% inhibition was determined.

1.1×10^3 times (curve 7 in Table 3), and (e) high density of Tn is $> 5.0 \times 10^3$ times more active than Tn monomer.

In previous studies [13,39], the binding of VVL-B₄ was shown to be independent of the density of Tn structures. Their conclusions were based on the results of three small synthetic Tns (one to three Tn residues) containing the glycopeptides used as inhibitors. Their results, thus, were fitting within an independent zone of the Tn density as compared with the known profile. In this study, the unrevealed part of VVL-B₄-gp binding was explored by using our developed method and a wider range (four to 100 residues) of Tn polyvalent-containing glycopeptides and gps from natural sources. As a result, the effect of the polyvalency on binding was detected. Combining the results of previous reports and this study, it is concluded that the concept of 'polyvalency of Tn (GalNAc α 1 \rightarrow Ser/Thr) glycoepitope as a critical factor for *V. villosa* B₄ and gp interactions' becomes more understandable and valuable.

It was reported that VVL-B₄ has been used to differentiate between Sd.(a⁺) and Sd.(a⁻) Tamm-Horsfall gps (THGP) [40]. The disaccharide structural unit (GalNAc β 1 \rightarrow 4Gal residue) of Sd.(a⁺) THGP was a poor inhibitor for VVL-B₄ binding. However, the binding forces of THGP Sd.(a⁺) and VVL-B₄ interaction can be derived from the following two factors: (a) the amount of lectin added for the interaction was increased 20 times and (b) the polyvalency effect of Gal-

NAc β 1 \rightarrow 4Gal enhanced the reactivity. These two factors may also explain the interaction of VVL-B₄ with Cad-carrying erythrocytes [9,10]. In order to establish a reliable differential binding system, it is suggested to quantify the amount of lectin required for detecting Tn, Sd.(a⁺), and Cad epitopes. However, this is hampered by the lack of availability of related glycans and ligands.

In summary, the binding profile of VVL-B₄ can be expressed in decreasing order as follows: high density of polyvalent Tn macromolecules ($MW > 1.0 \times 10^5$) \gg Tn glycopeptides ($MW < 3.0 \times 10^3$) \gg monomeric Tn to tri-Tn glycopeptides $>$ GalNAc $>$ GalNAc β 1-3Gal α 1-O-Me (P α), GalNAc β 1-4Gal (S), GalNAc α 1-3Gal (A), GalNAc β 1-3Gal (P), GalNAc α 1-3GalNAc (F) $>$ Gal \gg Gal β 1-4Glc (L), Gal β 1-3GlcNAc (I), Gal β 1-4GlcNAc (II), Gal α 1-4Gal (E), Gal β 1-3GalNAc α 1-benzyl (T α), Gal β 1-3GalNAc (T), Gal α 1-3Gal (B), Gal β 1-3GalNAc β 1-O-Me (T β) (weak or inactive), where polyvalency of Tn clusters plays a critical role in binding. Glycoconjugates on the cell surface occur frequently in clustered and polyvalent forms. Among them, many weak ligands, such as the binding ability of GalNAc β 1 \rightarrow 4Gal, can be enhanced to reach a biologically significant level by polyvalency effects [40]. Therefore, this binding profile has to be updated whenever new cluster effects on lectin binding are identified. The information obtained from this study not only facilitates understanding of VVL-B₄ interaction with nat-

Table 3

Amounts of different gps giving 50% inhibition of binding of VVL-B₄ (5 ng/50 μl) by asialo OSM (2 ng/50 μl)^a

Curve No.	Glycoprotein/glycan	Quantity giving 50% inhibition (ng)	Relative potency ^b
1	Asialo OSM (Tn)	0.2	3.3 × 10 ⁵
2	Native ASG- Tn (Tn)	0.4	1.6 × 10 ⁵
3	Asialo BSM (Tn , GlcNAcβ1-3 Tn)	0.6	1.1 × 10 ⁵
4	Asialo HSM (Tn)	2.0	3.2 × 10 ⁴
5	Tn -glycophorin (Tn)	20.0	3.2 × 10 ³
6	OSM (sialyl Tn , Tn)	30.0	2.2 × 10 ³
7	Tn -containing glycopeptides (MW < 3.0 × 10 ³)	60.9	1.1 × 10 ³
8	BSM (sialyl Tn , sialyl GlcNAcβ1-3 Tn)	100.0	648.0
9	GalNAcα1-Ser (Tn)	308.0	210.4
10	GalNAc	884.8	73.2
11	Gal	6.5 × 10 ⁴	1.0
12	HSM (sialyl Tn , Tn)	> 277.8 (29.5%) ^c	–
13	Cyst Beach phenol-insoluble (B)	> 277.8 (24.3%)	–
14	Cyst 14 phenol-insoluble (A_h [A₂])	> 277.8 (22.7%)	–
15	Cyst MSM 10% ppt (A_h [A₂])	> 277.8 (18.2%)	–
16	Asialo human α ₁ -acid gp (mII)	> 277.8 (16.3%)	–
17	Cyst Mcdon (A_h)	> 277.8 (12.3%)	–
18	Cyst MSS 10% 2 × (A_h [A₁])	> 277.8 (10.5%)	–
19	Asialo human glycophorin (T_α)	> 277.8 (4.1%)	–
20	Human glycophorin (sialyl T_α)	> 277.8 (3.8%)	–
21	Human α ₁ -acid gp (sialyl mII)	> 277.8 (3.1%)	–
22	Cyst Tighe phenol-insoluble (H)	> 277.8 (0%)	–
23	Fetuin gp (sialyl II , T)	> 277.8 (0%)	–
24	Asialo fetuin (T , II)	> 277.8 (0%)	–

^aThe inhibitory activity was estimated from the inhibition curve in Fig. 3 and is expressed as the amount of inhibitor giving 50% inhibition. Total volume 50 μl.

^bRelative potency = quantity of Gal (curve 11) required for 50% inhibition taken as 1.0/quantity of sample required for 50% inhibition.

^cThe inhibitory potency of inactive saccharides is expressed as the maximum amount of sugar tested that yielded inhibition (in parentheses) below 50%.

ural cellular (polyvalent) ligands, but also explains the various biological activities of VVL-B₄ with respect to lectin–receptor recognition processes. These special VVL-B₄ binding features also support the concept that every lectin with a unique amino acid sequence has its own binding characteristics [19].

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