Biochimie 83 (2001) 653–658

S0300908401013025/FLA

Analysis of the interaction between lectins and tetra- and tri-saccharide mimics of the Sd^a determinant by surface plasmon resonance detection

José L. Jiménez Blanco, Simon R. Haseley, Johannis P. Kamerling, Johannes F.G. Vliegenthart*

Bijvoet Center, Department of Bio-Organic Chemistry, Utrecht University, P.O. Box 80075, 3508 TB Utrecht, the Netherlands

(Received 10 April 2001; accepted 14 May 2001)

Abstract — The binding properties of a spacer-linked synthetic Sd^a tetrasaccharide β-D-Gal*p*NAc-(1→4)-α-Neu5Ac-(2→3)]-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→O)-$(CH_2)_5$-NH_2 (**1**), two tetrasaccharide mimics β-D-Gal*p*-(1→4)-α-Neu5Ac-(2→3)]-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→O)-$(CH_2)_5$-NH_2 (**2**) and β-D-Glc*p*NAc-(1→4)-α-Neu5Ac-(2→3)]-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→O)-$(CH_2)_5$-NH_2 (**3**), and two trisaccharide mimics β-D-Gal*p*NAc-(1→4)-3-*O*-(SO_3H)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→O)-$(CH_2)_5$-NH_2 (**4**) and β-D-Gal*p*NAc-(1→4)-3-*O*-(CH_2COOH)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→O)-$(CH_2)_5$-NH_2 (**5**) with lectins from *Dolichos biflorus* (DBL), *Maackia amurensis* (MAL), *Phaseolus limensis* (PLL), *Ptilota plumosa* (PPL), *Ricinus communis* 120 (RCL120) and *Triticum vulgaris* (wheat germ agglutinin, WGA) have been investigated by surface plasmon resonance (SPR) detection. MAL, PPL, RCL120 and WGA did not display any binding activity with compounds **1–5**. However, DBL and PLL, both exhibiting GalNAc-specificity, showed strong binding activity with compounds **1**, **4** and **5**, and **1**, **3**, **4** and **5**, respectively. The results demonstrate that SPR is a very useful analysis system for identifying biologically relevant oligosaccharide mimics of the Sd^a determinant. © 2001 Société française de biochimie et biologie moléculaire / Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

carbohydrate-lectin binding / Sd^a determinant / surface plasmon resonance / Tamm-Horsfall glycoprotein

1. Introduction

In view of the important roles glycan chains play in various cell surface phenomena such as cell-cell interactions, cell adhesion and tumor metastasis, our attention has focussed on the Sd^a determinant β-D-Gal*p*NAc-(1→4)-α-Neu5Ac-(2→3)]-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→R [1]. Initially discovered on human erythrocytes [2], and consequently termed a blood group antigen, Sd^a activity is also abundant in stomach, kidney, and colon tissues [1], and has been found as a terminal sequence in N-glycans of human Tamm-Horsfall glycoprotein (TH-gp) [3–7]. TH-gp binds to neutrophils and a role as a specific ligand for neutrophil integrins has been suggested [8]. Furthermore, TH-gp has been proposed to be involved in the prevention of urinary tract and urinary bladder infection by the inhibitory action of its glycans towards fimbriae-mediated adherence of *Escherichia coli* to uro-epithelial cells [9, 10].

Human anti-Sd^a serum and *Dolichos biflorus* lectin (DBL) both strongly react with Sd^a (+) red blood cells. Oligosaccharide fragments from Sd^a (+) TH-gp and biosynthesised tetrasaccharides structurally related to the Sd^a determinant [11] were able to inhibit this reaction. Recently, a differential binding study of human blood group Sd^a (+) and Sd^a (-) TH-gp, by microprecipitation techniques using GalNAc-specific agglutinins, demonstrated that terminal sialic acid of TH-gp from Sd^a (+) individuals is involved in the interaction with DBL [12].

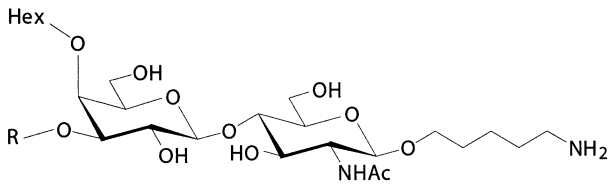
In order to generate information about the structural requirements of the Sd^a determinant needed for its biological activity, synthetic Sd^a mimics are excellent probes for investigation. In this context, binding studies with lectins can be used to identify positively reacting mimics. For this purpose a biosensor based on surface plasmon resonance (SPR) detection was utilised [13]. In this system, substrate biomolecules can be coupled to a carboxymethylated dextran surface of a sensor chip, and subsequently the interaction with potent analyte biomolecules can be analysed. In the present study, several lectins were immobilised to the sensor chip in order to investigate the lectin-carbohydrate interaction of a synthetic Sd^a determinant tetrasaccharide (**1**), two tetrasaccharide mimics (**2** and **3**) and two trisaccharide mimics (**4** and **5**). The methodology applied has previously been developed in our group [14].

2. Materials and methods

2.1. Materials and instrumentation

Compounds **1–5** were synthesised [15, 16] (*figure 1*) and provided by Dr. P.B. van Seeventer (Bijvoet Center,

*Correspondence and reprints.
E-mail address: j.f.g.vliegenthart@chem.uu.nl (J.F.G. Vliegenthart).



Compound	Hex	R
1	β-D-GalNAc	α-Neu5Ac
2	β-D-Gal	α-Neu5Ac
3	β-D-GlcNAc	α-Neu5Ac
4	β-D-GalNAc	SO_3H
5	β-D-GalNAc	$HOOC\text{-}CH_2$
1'	β-D-GalNAc	H

Figure 1. Synthetic analogue of the Sd^a tetrasaccharide determinant **1**, the desialylated trisaccharide **1'** and mimic compounds **2–5**.

Utrecht University, Netherlands). The sialic acid-free compound **1'** was obtained by treatment of **1** with 0.2 M H_2SO_4 for 2 h at 80 °C [3]. *Dolichos biflorus* (DBL), *Maackia amurensis* (MAL), *Phaseolus limensis* (PLL), *Ptilota plumosa* (PPL), *Ricinus communis* 120 (RCL120) and *Triticum vulgaris* (wheat germ agglutinin, WGA) agglutinins were supplied by Sigma (St. Louis, USA). BIAcore 2000 instrumentation, BIAevaluation software 3.0 and sensor chip CM5 were obtained from Pharmacia Biosensor AB (Uppsala, Sweden). SPR experiments were performed at 25 °C in 10 mM Tris-buffered saline (pH 7.5) containing 150 mM NaCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, and 0.02% (w/v) NaN_3.

2.2. Preparation of sensor surfaces

After equilibration of the sensor surface with Tris buffer (used throughout the immobilisation) the sensor surface was activated at a flow rate of 5 μL/min with a 10 min pulse of a 1:1 mixture of freshly prepared 50 mM *N*-hydroxysuccinimide and 200 mM *N*-ethyl-*N'*-(dimethylaminopropyl)-carbodiimide. Lectins were bound to the activated surface during several pulses of the lectin solution (200 μg/mL in 10 mM sodium acetate buffer, pH 5.0): one lectin to channels 1 and 3 and another lectin to channels 2 and 4. Blockage of the remaining *N*-hydroxysuccinimide esters was performed by addition of 1.0 M ethanolamine hydrochloride, pH 8.5, for 10 min. The lectins bound to channels 3 and 4 were denatured by flowing 6 M guanidinium chloride, pH 1.0, (2×4 min) and 0.5% SDS (4 min) across the channels. The denatured lectin channels were used to measure the level of non-specific binding and to serve as blank channels for analysis of the data [14].

2.3. Experimental set-up

All interaction analyses were performed at a flow rate of 5 μL/min using Tris buffer as eluent. Injection times were 7 min followed by 10 min of dissociation. Regeneration was performed using a 2-min pulse of sodium acetate, pH 4.3, for removal of glycans.

2.4. Data analysis

Association and dissociation rate constants were calculated by non-linear fitting of the primary sensorgram data using BIAevaluation 3.0 software. The closeness of fit for each estimated parameter is described by the statistical χ^2. The equilibrium association rate constant (K_A) was calculated experimentally from the equilibrium steady-state responses. Samples were appropriately diluted in Tris buffer to give concentrations between 1 mM and 10 μM of oligosaccharide.

3. Results

*3.1. Binding properties of oligosaccharides **1–5** with different lectins*

The lectins utilised in this study [17] were chosen for their carbohydrate specificities (*table I*), in order to compare the binding properties of oligosaccharides **1–5** (*figure 1*). Solutions (20 mM) of **1–5** in Tris-buffered saline (pH 7.5) were flowed across the different lectin surfaces and the binding results are described in *table I*. Only DBL and PLL bound to a number of the synthetic oligosaccharides.

The GalNAc specific DBL showed strong affinity for Sd^a determinant **1** (*figure 2*) and the trisaccharide mimics **4** and **5**. Compound **5** bound to DBL stronger than compound **1** indicating that this mimic trisaccharide could resemble the binding properties of the Sd^a determinant. Compound **3** and the sialic acid-free compound **1'** displayed low DBL affinity.

MAL did not show any binding with compounds **1–3** (*table I*) despite that in each case the trisaccharide sequence α-Neu5Ac-(2→3)-β-D-Gal-(1→4)-β-D-GlcNAc [18] is present. The lack of binding may be due to the presence of the branching Hex residue (*figure 1*). Also compounds **4** and **5** did not bind.

PLL bound strongly with compounds **1**, **3**, **4** and **5** (*table I, figure 3*). Although PLL is a D-GalNAc specific lectin [19, 20], compound **3** containing terminal β-D-GlcNAc was shown to be able to bind to this lectin (*figure 3*). This may result from the similar three-dimensional structures adopted in aqueous solution by **1** and **3**, which is due to intramolecular hydrogen bonding between the carboxyl group of Neu5Ac and the NAc group of Hex [21].

Table I. Lectin-binding properties of compounds **1–5** resulting from BIAcore experiments.

Lectin	*Monosaccharide specificity*	*References*	*Active oligosaccharides*
DBL	αGalNAc>βGalNAc	[11, 12, 30]	**5>1>4>>3>1'**
MAL	Neu5Ac(α2-3)Gal(β1-4)GlcNAc	[18, 29]	–
PLL	αGalNAc>βGalNAc>Gal	[19, 20, 30]	**5>1>3>4>>2**
PPL	αGal	[22]	–
RCL120	βGal>αGal>>GalNAc	[23, 24]	–
WGA	$(GlcNAc\beta1\text{-}4)_3$>βGlcNAc>Neu5Ac	[25–28, 30]	–

–, no binding

PPL, an α-D-Gal specific lectin, was used as a blank lectin. The absence of binding of compounds **1–5** with PPL (*table I*) is in agreement with its reported specificity [22].

RCL120 does not show any affinity for compounds **1–5** (*table I*). This lectin reacts strongly with oligosaccharides containing terminal D-Gal [23, 24]. It is likely that in the case of compound **2**, Neu5Ac interferes with the binding of this lectin.

Although WGA is a D-GlcNAc and Neu5Ac specific lectin [25–28], it did not bind to compounds **1** and **2**, both having a terminal Neu5Ac residue, and to compound **3** with a terminal Neu5Ac and a terminal GlcNAc residue (*table I*). It should be noted that the indicated compounds have Neu5Ac (**1–3**) or GlcNAc (**3**) in a branched rather than in a linear situation. The branching feature seems to be responsible for the non-binding.

3.2. Quantitative analysis of the binding properties of DBL and PLL with active oligosaccharide ligands

DBL and PLL were used for the measurement and comparison of association and dissociation kinetics of the different active compounds (*figure 1*). The binding of oligosaccharides **1**, **4**, **5** and **1'** with DBL produced similar sensorgrams (see *figure 2A* for **1**). The same held for oligosaccharides **1**, **3**, **4** and **5** with PLL (see *figure 3A* for **3**). The equilibrium association constants (K_A) were calculated by using BIAevaluation software 3.0 from experiments with concentrations of oligosaccharides from 200 μM to 25 μM for DBL and from 1 mM to 31.25 μM for PLL, following a set of accurately two-fold dilutions of the more concentrated samples. Injection of buffer was used as an internal 'zero' standard to compensate for any small differences (usually ≤ 5 RU) between the native and

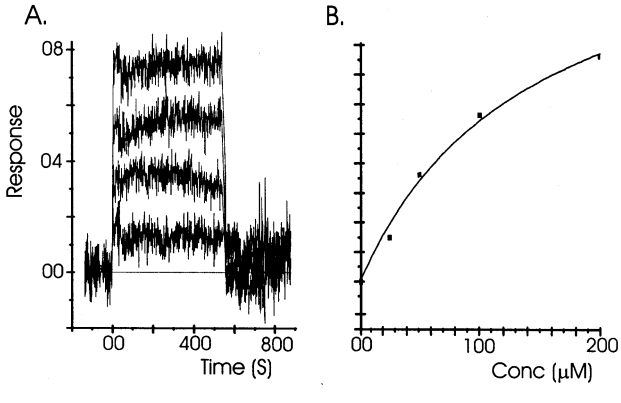


Figure 2. A. Relative response (RU) versus time for the binding of compound **1** to immobilised DBL at different concentrations of oligosaccharide. Concentration of oligosaccharide (from top to bottom): 200, 100, 50 and 25 μM. **B.** A plot of equilibrium response against concentration (steady-state analysis). The interaction K_A was derived from this plot by using BIAevaluation software 3.0.

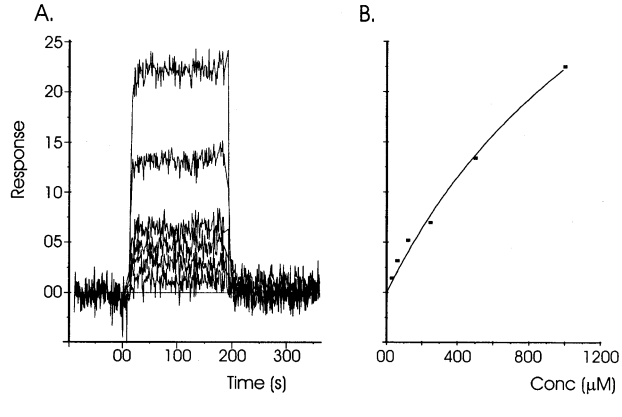


Figure 3. A. Relative response (RU) versus time for the binding of compound **3** to immobilised PLL at different concentrations of oligosaccharide. Concentration of oligosaccharide (from top to bottom): 1000, 500, 250, 125, 62.5 and 31.25 μM. **B.** A plot of equilibrium response against concentration (steady-state analysis). The interaction K_A was derived from this plot by using BIAevaluation software 3.0.

Table II. Kinetics data for binding of compounds **1–5** to *Dolichos biflorus* lectin (DBL).

Oligosaccharide	K_A (M^{-1})[a]	χ^2
1	9.92×10^3	0.004
1'	6.50×10^2	0.080
2	–	–
3	–	–
4	3.07×10^3	0.099
5	2.22×10^4	0.011

[a] Average values from three experiments. The closeness of fit is indicated by the value of χ^2.

denatured lectin surfaces. To compensate for non-specific binding, the results at the denatured lectin surface were subtracted from those at the native lectin surface. An overview of the results for DBL and PLL is given in *tables II* and *III*, respectively.

The kinetic constants of DBL (*table II*) revealed that this lectin is highly specific for the Sd^a determinant tetrasaccharide and the trisaccharide mimics **4** and **5**. Compound **5** shows the highest affinity for DBL ($K_A = 2.22 \times 10^4$ M^{-1}). The higher affinity of **5**, when compared with **1**, can be explained by taking into account that the carboxymethyl group at O-3 of Gal resembles the stereo-electronic properties of the carboxyl function of Neu5Ac in the Sd^a determinant, but that it is less voluminous and probably experiences less steric hindrance at the lectin binding site. The low value of K_A of the desialylated derivative **1'** indicated that the presence of a charged functional group at O-3 of Gal is an important structural feature for DBL recognition.

The kinetic data for the binding of PLL show a high specificity for compounds **1**, **3**, **4** and **5** (*table III*). Again, the carboxylated trisaccharide **5** had the highest value, $K_A = 2.16 \times 10^4$ M^{-1}. This suggests that the carboxyl group of the Neu5Ac residue in Sd^a determinant tetrasaccharide **1** is involved in the interaction with PLL.

Table III. Kinetics data for binding of compounds **1–5** to *Phaseolus limensis* lectin (PLL).

Oligosaccharide	K_A (M^{-1})[a]	χ^2
1	1.37×10^4	1.11
2	–	–
3	5.65×10^3	0.067
4	3.63×10^3	0.308
5	2.16×10^4	0.003

[a] Average values from three experiments. The closeness of fit is indicated by the value of χ^2.

4. Discussion

In this study, we have characterised the binding properties of synthetic compounds **1–5** with a set of lectins. Reliable and reproducible data were obtained for the glycan-DBL and glycan-PLL interactions even though the SPR response was very low (≤ 30 RU, *figures 2A* and *3A*). The calculated K_A values were in the range of those expected for lectin-carbohydrate binding (10^3–10^4 M^{-1}) [14]. The lectins MAL, RCL120 and WGA did not show any binding with the tetrasaccharides **1–3** although they could have interacted according to their monosaccharide affinities. This lack of interaction can be due to the branched structure of compounds **1–3**, which may have hindered the interaction of the terminal monosaccharide at the binding site of the lectin. Until now, only linear oligosaccharides have been tested for glycan-lectin binding studies of MAL [14, 18, 29], RCL120 [23, 24, 30], and WGA [25–28, 30].

DBL showed affinity for the Sd^a tetrasaccharide (**1**) and the two mimic trisaccharides **4** and **5**. Early studies with this lectin described it to be specific for terminal α-D-GalNAc of the blood group A oligosaccharides [31]. Later it was reported [11] that terminal non-reducing β-linked D-GalNAc of oligosaccharides from degradation products of Sd^a (+) TH-gp showed also binding with DBL. In addition, differential binding studies of human blood group Sd^a (+) TH-gp with DBL revealed that removal of sialic acid from Sd^a (+) TH-gp decreased the efficiency of its interaction with this lectin [12]. Thus, the strong affinity of DBL for **1** can be explained by the presence of the terminal β-D-Gal*p*NAc-(1→4)-α-Neu5Ac-(2→3)]-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc fragment. The low K_A value of the non-3-substituted derivative **1'** (*table II*) supports the earlier finding that removal of the sialic acid residue lowers the affinity for DBL. The binding of DBL with the 3-*O*-sulfated (**4**) and 3-*O*-carboxymethylated (**5**) mimics suggest that these polar substituents can efficiently resemble the stereo-electronic properties of the carboxyl group of Neu5Ac. Compound **5** has a higher K_A than **4** (*table II*), which probably means that the carboxymethyl group better mimics the charge and disposition of the carboxyl group of Neu5Ac in the Sd^a determinant. In fact, conformational analysis of compound **5** shows that the carboxyl group adopts a conformation in anti disposition with respect to C-3 of Gal, similar to that adopted by the carboxyl group of Neu5Ac in **1** [21] (unpublished results, *figure 4*).

Compounds **1**, **3**, **4** and **5** were recognised by PLL. Previous studies into the carbohydrate-specificity of PLL reported it to be a GalNAc binding lectin [19, 20] with high affinity for the blood group A trisaccharide α-D-Gal*p*NAc-(1→3)-α-L-Fuc*p*-(1→2)]-β-D-Gal*p*-(1→R)) [19, 30]. However, we have found that PLL also binds terminal β-D-Glc*p*NAc as in mimic **3**. Consequently, PLL appears to be less selective in its specificity than DBL.

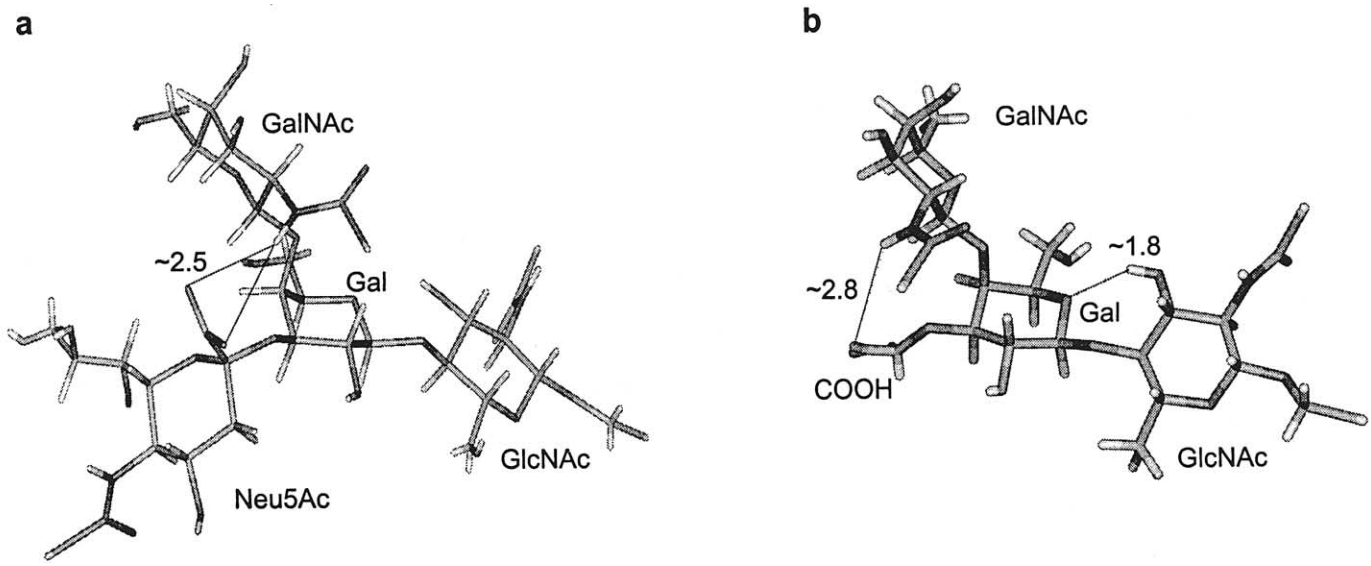


Figure 4. Average simulated models of the Sd[a] determinant **1** (**a**) and compound **5** (**b**).

The unexpected recognition of mimic **3** could be due to its conformational analogy with the Sd[a] tetrasaccharide **1** [21]. Compounds **1** and **3** adopt a similar three-dimensional structure in solution with a hydrogen bond between the NAc group of Hex and the carboxyl group of Neu5Ac [21]. The highest affinity with PLL (K_A 2.16×10^4 M^{-1}) was that of **5** and could again be explained by the conformational similarity between **5** and the Sd[a] determinant **1** as obtained from NMR experiments and molecular modelling studies (unpublished results, *figure 4*). These findings confirm the importance of the three-dimensional structure of oligosaccharides on carbohydrate-lectin recognition phenomena.

In conclusion, DBL, but also PLL, are suitable lectins for identifying synthetic mimics with possible Sd[a] (+) activity. From this study it would appear that the Neu5Ac residue can be replaced by CH_2COOH, or even by sulfate, and its Sd[a] (+) activity may well be conserved. Furthermore, binding assays of mimics with different lectins provide useful information about the structural and conformational requirements of carbohydrate-lectin recognition phenomena.

Acknowledgments

We thank Dr. P.B. van Seeventer for providing compounds **1-5**. J.L.J.B. and S.H. thank the European Commission DG-XII-E (Biotechnology program) for postdoctoral grants BIO4CT975071 and FAIRCT973142, respectively. We thank the Netherlands Organization for Scientific Research (NWO) for financing the purchase of a BIAcore 2000 instrument.

References

[1] Watkins W.M., Sd[a] and Cad antigens, in: Cartron J.P., Rouger P. (Eds.), Blood cell biochemistry: molecular basis of major human blood group antigens, vol. 6, Plenum Press, New York, 1995, pp. 351–375.

[2] Macvie S.I., Morton J.A., Pickles M.M., The reactions and inheritance of a new blood group antigen, Vox Sang. 13 (1967) 485–492.

[3] Donald A.S.R., Yates A.D., Soh C.P.C., Morgan W.T.J., Watkins W.M., A blood group Sd[a]-active pentasaccharide isolated from Tamm-Horsfall urinary glycoprotein, Biochem. Biophys. Res. Commun. 115 (1983) 625–631.

[4] Williams J., Marshall R.D., van Halbeek H., Vliegenthart J.F.G., Structural analysis of the carbohydrate moieties of human Tamm-Horsfall glycoprotein, Carbohydr. Res. 134 (1984) 141–155.

[5] Hård K., van Zadelhoff G., Moonen P., Kamerling J.P., Vliegenthart J.F.G., The Asn-linked carbohydrate chains of human Tamm-Horsfall glycoprotein of one male; novel sulfated and novel *N*-acetylgalactosamine-containing N-linked carbohydrate chains, Eur. J. Biochem. 209 (1992) 895–915.

[6] Van Rooijen J.J.M., Kamerling J.P., Vliegenthart J.F.G., The abundance of additional *N*-acetyllactosamine units in N-linked tetraantennary oligosaccharides of human Tamm-Horsfall glycoprotein is a donor-specific feature, Glycobiology 8 (1998) 1065–1075.

[7] Van Rooijen J.J.M., Voskamp A.F., Kamerling J.P., Vliegenthart J.F.G., Glycosylation sites and site-specific glycosylation in human Tamm-Horsfall glycoprotein, Glycobiology 9 (1999) 21–30.

[8] Toma G., Bates Jr J.M., Kumar S., Uromodulin (Tamm-Horsfall protein) is a leukocyte adhesion molecule, Biochem. Biophys. Res. Commun. 200 (1994) 275–282.

[9] Parkkinen J., Virkola R., Korhonen T.K., Identification of factors in human urine that inhibit the binding of *Escherichia coli* adhesins, Infect. Immun. 56 (1988) 2623–2630.

[10] Pak J., Pu Y., Zhang Z.T., Hasty D.L., Wu X.R., Tamm-Horsfall protein binds to type 1-fimbriated *E. coli* and prevents the *E. coli* from binding to uroplakin Ia and Ib receptors, J. Biol. Chem. (2001) in press.

[11] Soh C.P.C., Donald A.S.R., Feeney J., Morgan W.T.J., Watkins W.M., Enzymic synthesis, chemical characterisation and Sd^a activity of GalNAcß1-4[NeuAcα2-3]Galß1-4GlcNAc and GalNAcß1-4[NeuAcα2-3]Galß1-4Glc, Glyconjugate J. 6 (1989) 319–332.

[12] Wu A.M., Wu J.H., Watkins W.M., Chen C.P., Song S.C., Chen Y.Y., Differential binding of human blood group Sd(a+) and Sd(a-) Tamm-Horsfall glycoproteins with *Dolichos biflorus* and *Vicia villosa*-B_4 agglutinins, FEBS Lett. 429 (1998) 323–326.

[13] Jönsson U., Malmqvist M., Real time biospecific interaction analysis. The integration of surface plasmon resonance, Detection, general biospecific interface chemistry and microfluidics into one analytical system, Adv. Biosensors 2 (1992) 291–336.

[14] Haseley S.R., Talaga P., Kamerling J.P., Vliegenthart J.F.G., Characterization of the carbohydrate binding specificity and kinetic parameters of lectins by using surface plasmon resonance, Anal. Biochem. 274 (1999) 203–210.

[15] Van Seeventer P.B., Corsten M.A., Sanders M.P., Kamerling J.P., Vliegenthart J.F.G., Synthesis of two analogues of the Sd^a determinant. Replacement of the sialic acid residue by a sulfate or a carboxymethyl group, Carbohydr. Res. 299 (1997) 171–179.

[16] Van Seeventer P.B., Kamerling J.P., Vliegenthart J.F.G., Synthesis of the Sd^a determinant and two analogous tetrasaccharides, Carbohydr. Res. 299 (1997) 181–195.

[17] Goldstein I.J., Winter H.C., Poretz R.D., Plant lectins: tools for the study of complex carbohydrates, in: Montreuil J., Vliegenthart J.F.G., Schachter H. (Eds.), Glycoproteins II, New comprehensive Biochemistry, Vol. 29b, Elsevier, Amsterdam, 1997, pp. 403–474.

[18] Knibbs R.N., Goldstein I.J., Ratcliffe R.M., Shibuya N., Characterization of the carbohydrate binding specificity of the leukoagglutinating lectin from *Maackia amurensis*: comparison with other sialic acid-specific lectins, J. Biol. Chem. 266 (1991) 83–88.

[19] Roberts D.D., Etzler M.E., Goldstein I.J., Subunit heterogeneity in the lima bean lectin, J. Biol. Chem. 257 (1982) 9198–9204.

[20] Pandolfino E.R., Namen A.E., Munske G.R., Magnuson J.A., A comparison of the cell-binding characteristics of the mitogenic and nonmitogenic lectins from lima beans, J. Biol. Chem. 258 (1983) 9203–9207.

[21] Jiménez Blanco J.L., van Rooijen J.J.M., Erbel P.J.A., Leeflang B.R., Kamerling J.P., Vliegenthart J.F.G., Conformational analysis of the Sd^a determinant-containing tetrasaccharide and two mimics in aqueous solution by using ^{1}H NMR ROESY spectroscopy in combination with MD simulations, J. Biomol. NMR 16 (2000) 59–77.

[22] Rogers D.J., Blunden G., Evans P.R., Ptilota plumosa, a new source of a blood-group B specific lectin, Med. Lab. Sci. 34 (1977) 193–200.

[23] Nicolson G.L., Blaustein J., Etzler M.E., Characterization of two plant lectins from *Ricinus communis* and their quantitative interaction with a murine lymphoma, Biochemistry 13 (1974) 196–204.

[24] Lin T.T.S., Li S.S.L., Purification and physicochemical properties of ricins and agglutinins from *Ricinus communis*, Eur. J. Biochem. 105 (1980) 453–459.

[25] Nagata Y., Burger M.M., Wheat germ agglutinin; molecular characteristics and specificity for sugar binding, J. Biol. Chem. 249 (1974) 3116–3122.

[26] Peters B.P., Ebisu S., Goldstein I.J., Flashner M., Interaction of wheat germ agglutinin with sialic acid, Biochemistry 18 (1979) 5505–5511.

[27] Zeng X., Murata T., Kawagishi H., Usui T., Kobayashi K., Analysis of specific interactions of synthetic glycopolypeptides carrying *N*-acetyllactosamine and related compounds with lectins, Carbohydr. Res. 312 (1998) 209–217.

[28] Wright C.S., Crystal structure of a wheat germ agglutinin / glycophorin-sialoglycopeptide receptor complex; structural basis for cooperative lectin-cell binding, J. Biol. Chem. 267 (1992) 14345–14352.

[29] Kawaguchi T., Matsumoto I., Osawa T., Studies on hemagglutinins from *Maackia amurensis* seeds, J. Biol. Chem. 249 (1974) 2786–2789.

[30] Wu J.H., Watkins W.M., Chen C.P., Song S.C., Wu A.M., Interaction of a human blood group Sd(a-) Tamm-Horsfall glycoprotein with applied lectins, FEBS Lett. 384 (1996) 231–234.

[31] Etzler M.E., Kabat E.A., Purification and characterization of a lectin (plant hemagglutinin) with blood group A specificity from *Dolichos biflorus*, Biochemistry 9 (1970) 869–877.