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# Comparative Analysis by Frontal Affinity Chromatography of Oligosaccharide Specificity of GlcNAc-Binding Lectins, *Griffonia simplicifolia* Lectin-II (GSL-II) and *Boletopsis leucomelas* Lectin (BLL)

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**Lectin-based structural glycomics requires a search for useful lectins and their biochemical characterization to profile complex features of glycans. In this paper, two GlcNAc-binding lectins are reported with their detailed oligosaccharide specificity. One is a classic plant lectin, *Griffonia simplicifolia* lectin-II (GSL-II), and the other is a novel fungal lectin, *Boletopsis leucomelas* lectin (BLL). Their sugar-binding specificity was analyzed by frontal affinity chromatography using 146 glycans (125 pyridylaminated and 21 *p*-nitrophenyl saccharides). As a result, it was found that both GSL-II and BLL showed significant affinity toward complex-type *N*-glycans, which are either partially or completely agalactosylated. However, their branch-specific features differed significantly: GSL-II strongly bound to agalacto-type, tri- or tetra-antennary *N*-glycans with its primary recognition of a GlcNAc residue transferred by GlcNAc-transferase IV, while BLL preferred *N*-glycans with fewer branches. In fact, the presence of a GlcNAc residue transferred by GlcNAc-transferase V abolishes the binding of BLL. Thus, GSL-II and BLL forms a pair of complementally probes to profile a series of agalacto-type *N*-glycans.**

**Key words: carbohydrate binding specificity, frontal affinity chromatography, GlcNAc, lectin, *N*-linked glycans.**

Abbreviations: BLL, *Boletopsis leucomelas* lectin; GSL-II, *Griffonia simplicifolia* lectin-II; FAC, frontal affinity chromatography; PA, pyridylaminated; pNP, *p*-nitrophenyl.

Lectins, with their diverse sugar-binding specificities, are useful probes for the study of glycoconjugates in various biological materials. In fact, they have been used for only qualitative or semi-quantitative purposes. However, lectins are now being reconsidered as versatile tools for quantitative purposes, too, in the context of "lectin-based structural glycomics" (*1*). For this realization, a quantitative affinity technique, frontal affinity chromatography (FAC), was reinforced (*2–4*). More recently, an automated machine for high-throughput lectin-glycan interaction analysis, designated FAC-1, was developed (*1*, *5*).

To profile complex features of glycans, it is essential to prepare a range of useful lectins to cover diverse glycan structures. From a practical viewpoint, however, only few lectins presently available for agalacto-type *N*-glycans, which bear GlcNAc residue(s) at their non-reducing end(s). In this context, specific detection of non-reducing end GlcNAc residues in a branch-specific manner should be extremely valuable, because branching features of *N*-linked glycans are of much biological significance, such as in development and diseases including cancer metastasis (*6–8*). For their biosynthesis, a series of GlcNAc transferases are involved in mammals. So far, several plant lectins, which bind to chito-oligosaccharides, have been reported, but only few of them can recognize agalactosylated *N*-glycans in a specific manner. This is firstly because relatively few GlcNAc-binding lectins have been isolated so far, and secondly because no systematic analysis using complex glycans has been made from a quantitative viewpoint. Thus, it is essential to investigate the fine specificities of GlcNAc-binding lectins as potential tools for *N*-glycan profiling.

A leguminous plant lectin, *Griffonia* (*Bandeiraea*) *simplicifolia* lectin-II (GSL-II), is one of the most popular GlcNAc-binding lectins. Notably, GSL-II is distinct from other GlcNAc-binding lectins, represented by wheat germ agglutinin (WGA) and potato lectin (STL): the former specifically binds to non-reducing terminal GlcNAc residues, while the latter two recognize even internal GlcNAc residues (*9*, *10*). Therefore, GSL-II has been extensively used to detect non-reducing terminal GlcNAc residues (*11–13*). Another GlcNAc-binding lectin from the edible mushroom *Psathyrella venlutina* (PVL) is also used for this purpose (*14*). To date, however, no quantitative data using a panel of complex type *N*-glycans are available for

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them. More recently, a new GlcNAc-binding lectin (BLL) was isolated from another edible mushroom, *Kurokawa* (*Boletopsis leucomelas*) (*15*). Preliminary experiments suggested that BLL has significant affinity for agalacto-type *N*-glycans, but not for those lacking non-reducing terminal GlcNAc (*16*).

In order to develop unique GlcNAc-binding lectins to profile *N*-glycans, we attempted systematic analysis of detailed sugar-binding specificities of two such potential lectins, GSL-II and BLL by FAC using 146 glycans (125 pyridylaminated and 21 *p*-nitrophenyl oligosaccharides). To make comparison of the results easier, a new representation format designated "GRYP" code of branched *N*-glycan structures is also proposed.

## MATERIALS AND METHODS

*Oligosaccharides*—*p*-Nitrophenyl (pNP) glycosides of Galβ, GalNAcβ, Manα, GlcNAcα, Fucα and Galβ1-4Glcβ were purchased from SIGMA (St Louis, MO, USA); Galα, GalNAcα, Manβ, Galβ1-4GlcNAcβ, Galβ1-3GalNAc α (Core1), Galβ1-3(GlcNAcβ1-6)GalNAcα (Core2), GlcNAcβ1-3GalNAcα (Core3) and GlcNAcβ1-6GalNAcα (Core6) were from Toronto Research Chemicals, Inc. (North York, Canada); Glcα was from Calbiochem (San Diego, CA, USA). Other pNP-glycosides [Glcβ, GlcNAcβ and $(GlcNAcβ1–4)_{1–4}$GlcNAcα-pNP] were obtained from Seikagaku Co. (Tokyo, Japan).

Pyridylaminated (PA), *N*-linked glycans used in this study are listed in Fig. 1. Glycans numbered **002–014**, **103**, **105**, **107**, **108**, **307**, **313**, **314**, **323**, **405**, **410**, **418–420**, and **503** were purchased from Takara Bio Inc. (Kyoto, Japan), while the others were from Seikagaku Co. Non-labeled glycans, **906** and **907**, were obtained from Seikagaku Co., and were pyridylaminated with GlycoTag (Takara Bio). Glycolipid-type glycans and others, which lack non-reducing end GlcNAc, were prepared as described previously (for detailed structures, see fig. 2 in Ref. *5*).

*Lectins*—*Griffonia simplicifolia* lectin-II (GSL-II)-agarose was obtained from Vector Laboratories (Burlingame, CA). *Boletopsis leucomelas* lectin (BLL) was purified from the fruit body of the edible mushroom "Kurokawa" as previously described (*15*).

*Preparation of Lectin Columns*—BLL was dissolved in 0.2 M $NaHCO_3$ buffer, pH 8.3, containing 0.5 M NaCl and coupled to NHS-activated Sepharose (Amersham Bioscience, Uppsala, Sweden) according to the manufacturer's instructions. Lectin-Sepharose was suspended in 10 mM Tris-HCl buffer, pH 7.4, containing 0.8% NaCl

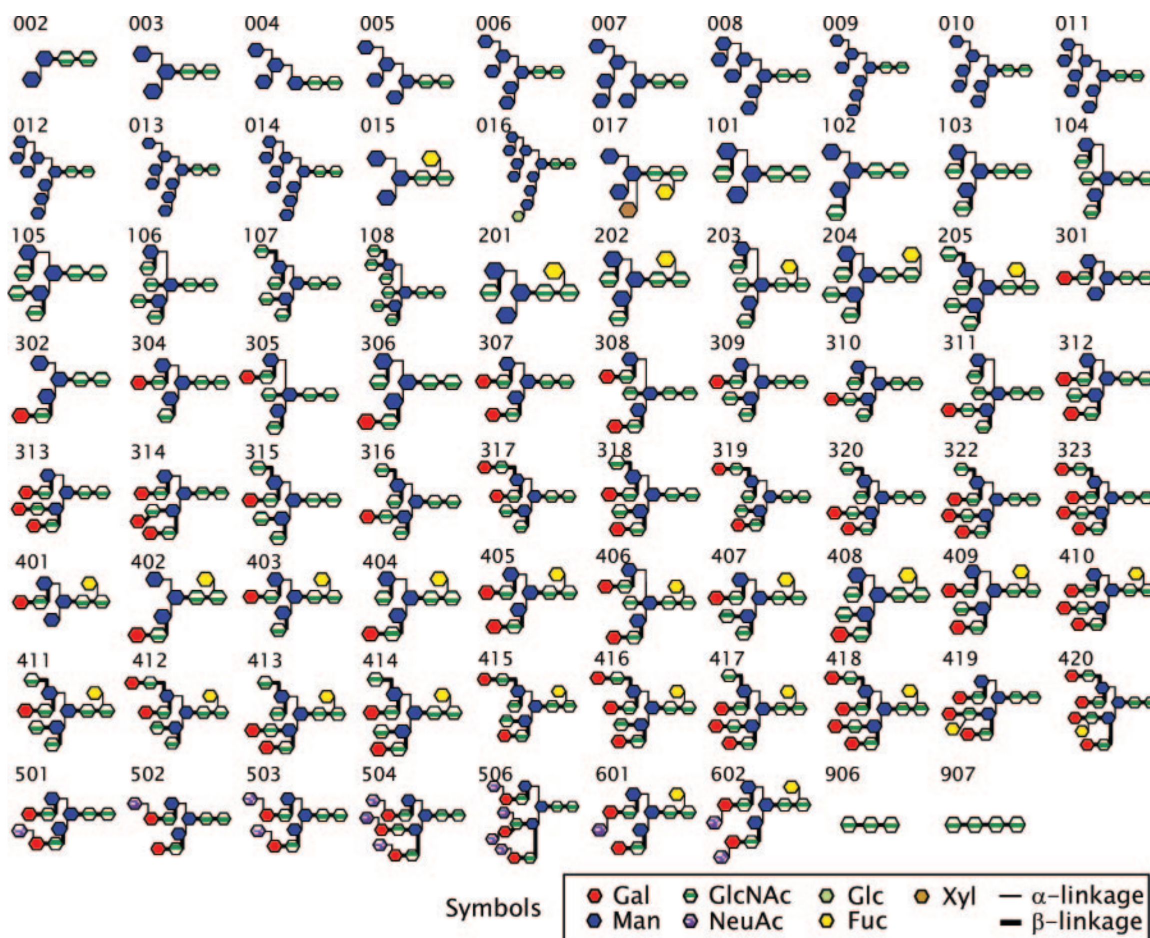


Fig. 1. **Schematic representation of PA-oligosaccharides used in this study.** The reducing terminal is pyridylaminated. Symbols used to represent pyranose rings of monosaccharides are shown in the box at the bottom of the figure.

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(TBS) and packed into a miniature column ( 2 mm × 10 mm, 31.4 μl) as previously described (*5*).

*Frontal Affinity Chromatography (FAC)*—Frontal affinity chromatography was performed with an automated system for FAC (FAC-1) as described previously (*1*, *5*). Briefly, lectin-columns prepared above were slotted into a stainless holder, and connected to the FAC-1 machine. The flow rate and the column temperature were kept at 0.125 ml/min and 25°C, respectively. After equilibration with TBS, an excess volume (0.5–0.8 ml) of pNP- (5 μM) and PA-glycans (2.5 or 5.0 nM) was successively injected into the columns by an auto-sampling system. Elution of pNP- and PA-glycans was monitored by measuring UV (280 nm) and fluorescence (excitation and emission wave lengths, 310 and 380 nm, respectively). The elution front relative to that of an appropriate standard oligosaccharide (*i.e.*, PA-lactose), *i.e.*, $V$-$V_0$, was then determined (*3*). Dissociation constants ($K_d$) were obtained from $V$-$V_0$ and $B_t$, according to the basic equation of FAC (*2–5*).

*Concentration-Dependence Analysis*—For the determination of effective ligand content, $B_t$, concentration-dependence analysis was performed as described previously (*3*). Various concentrations ($[A]_0$) of GlcNAcα-pNP for GSL-II and Galβ1-3GalNAcα-pNP for BLL were successively injected into the columns, and $V$-$V_0$ values were calculated essentially according to the method developed by Arata *et al.* (*17*). Woolf-Hofstee-type plots, *i.e.*, ($V$-$V_0$) *vs.* ($V$-$V_0$)$[A]_0$, were made, and $B_t$ and $K_d$ values were determined from the intercept and the slope, respectively, of the fitted curves.

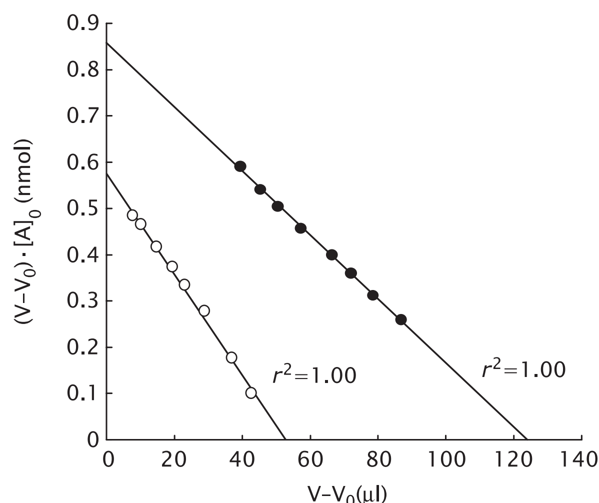


Fig. 2. **Determination of $B_t$ values**. Concentration-dependence analysis and subsequent Woolf-Hofstee type plot were performed. Various concentrations (2.5 to 70 μM) of GlcNAcα-pNP and Galβ1-3GalNAcα-pNP were applied to GSL-II (closed circle) and BLL (open circle) columns, respectively, and Woolf-Hofstee-type plots were made for the obtained $V$-$V_0$ values. $B_t$ and $K_d$ values were obtained from the intercept and the slope, respectively.

## RESULTS AND DISCUSSION

*Evaluation of the Lectin Columns Using pNP-Oligosaccharides*—In our preliminary experiments, BLL showed extremely high affinity for non-reducing terminal GlcNAc residues in both partially- and completely-agalactosylated *N*-glycans (*16*), while GSL-II showed moderate affinity to these *N*-glycans. Therefore, BLL-agarose and GSL-II-agarose were prepared at concentrations of 0.1 mg protein/ml gel and 3 mg protein/ml gel, respectively. For evaluation of the columns, so-called "concentration-dependence analysis" was conducted with appropriate *p*-nitrophenyl (pNP)-derivatives. For GSL-II, various concentrations (2.5 to 70 μM) of GlcNAcα-pNP were prepared, and were successively injected into the column. The resulting $B_t$ and $K_d$ values were 0.86 nmol and $6.9 \times 10^{-6}$ M, respectively (Fig. 2).

On the other hand, none of the GlcNAc-containing pNP derivertives tested, *i.e.* GlcNAcα-pNP, GlcNAcβ-pNP and $(\text{GlcNAc}\beta1\text{-}4)_{1\text{–}4}$GlcNAcα-pNP, showed significant affinity for BLL, at least under the employed conditions, despite the fact that the lectin was purified by affinity chromatography using GlcNAc-agarose (*15*). BLL was recently shown to possess sequence homology to another mushroom lectin, *Agaricus bisporus* agglutinin (ABA) (*15*), which is known to bind Galβ1-3GalNAc (T-antigen) with high affinity (*18*, *19*). However, a recent X-ray crystallographic study revealed that ABA possesses two carbohydrate-binding sites per subunit: one accommodates Galβ1-3GalNAc (T-antigen) and the other, GlcNAc (*20*). This finding implies that BLL can also recognize T-antigen as well as non-reducing terminal GlcNAc through distinct binding sites in a stoichiometric fashion. Thus, we attempted to determine whether or not BLL could bind to T-antigen. As a result, Galβ1-3GalNAcα-pNP was found to have significant affinity for BLL, and $B_t$ and $K_d$ values were determined to be 0.56 nmol and $1.1 \times 10^{-5}$ M, respectively (Fig. 2). Hence, the following discussion is made on the assumption that BLL has two equivalent sugar-binding sites, *i.e.*, for Galβ1-3GalNAc and for GlcNAc as ABA (*21*).

*Analysis Using* p*-Nitrophenyl (pNP) Derivatives*—Among the pNP-glycosides tested, GSL-II showed the strongest affinity for GlcNAcα-pNP, followed by chito-oligosaccharides, GlcNAcβ1-4GlcNAcα-pNP, $(\text{GlcNAc}\beta1\text{-}4)_2$GlcNAcα-pNP, $(\text{GlcNAc}\beta1\text{-}4)_3$GlcNAcα-pNP and $(\text{GlcNAc}\beta1\text{-}4)_4$GlcNAcα-pNP (Fig. 3A, left) in the order of reducing affinity. This result indicates that polymerization of GlcNAc has rather a detrimental effect on binding. GSL-II also showed significant affinity for GlcNAcβ-pNP, but its binding was 7.1 times weaker than for the α-anomer. This observation is consistent with previous reports (*9*, *10*). Although GSL-II showed a trace binding to *O*-linked glycans containing a non-reducing terminal GlcNAc (*i.e.*, Core2, Core3 and Core6α-pNP), the $V$-$V_0$ values were close to the error in the present FAC system (*i.e.*, 2 μl),

As addressed above, BLL possessed no detectable (*i.e.*, $V$-$V_0$ < 2 μl, $K_a < 2.3 \times 10^3$ $M^{-1}$) affinity for GlcNAcα/β-pNP and chito-oligosaccharides (Fig. 3A, right). Since BLL showed significant affinity for Galβ1-3GalNAcα-pNP (Core1, $K_d = 1.1 \times 10^{-5}$ M), this implies another binding site clearly distinct from the GlcNAc-binding site originally assumed for this lectin. In fact, binding of Galβ1-3GalNAcα-pNP to BLL was not inhibited at all, even by the presence of a high-concentration (0.25 M) of GlcNAc (data not shown). In ABA, the T-antigen binding site can hardly accommodate GalNAc (*20*), since its affinity was 2,000-fold less than that of T-antigen (*18*). Consistently no apparent affinity was detected for



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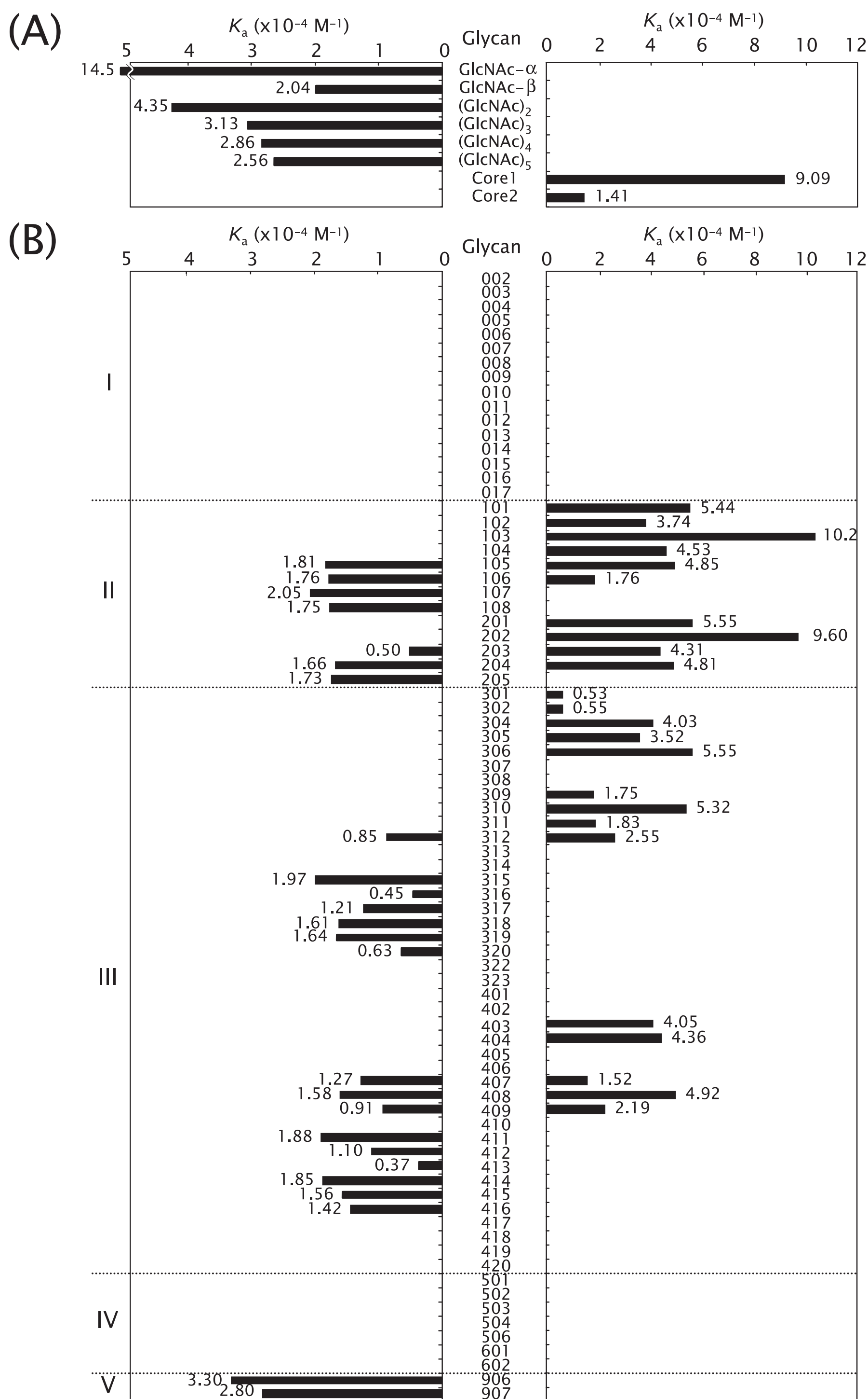


Fig. 3. **Bar graph representation of affinity constants ($K_a$) of pNP- (A) and PA-sugars (B) for GSL-II (left) and BLL (right).** The small Arabic figures in the center correspond to sugar numbers indicated in Fig. 1; large Roman figures on the left side of graphs represent types of glycans: high-mannose-type (I), agalacto-type (II), galactosylated-type (III) and sialylated-type (IV) *N*-linked glycans and chito-oligosaccharides (V).

GalNAcα/β-pNP in BLL. Notably, BLL also showed reduced but significant affinity for Galβ1-3(GlcNAcβ1-6) GalNAcα-pNP (Core2), while it had no affinity for GlcNAcβ1-3GalNAcα-pNP (Core3) and GlcNAcβ1-6GalNAcα-pNP (Core6). Therefore, non-reducing-end Gal is essential for BLL recognition, while modification at 6-OH of the reducing-end GalNAc drastically (*i.e.*, by approximately 85%) reduced the affinity.

*Overall Features of GSL-II and BLL Binding Specificity to PA-Oligosaccharides*—Both GSL-II and BLL showed substantial affinity for partially- and completely-agalactosylated complex-type glycans, while showing no apparent affinity to high-mannose-type glycans. It is therefore evident that none of these lectins recognize the reducing-end chitobiose of *N*-glycans. Moreover, neither BLL nor GSL-II showed apparent affinity for glycolipid-type glycans, which lack non-reducing end GlcNAc (data not shown; for detailed structures, see fig. 2 in Ref. *5*).

A distinguishing feature of GSL-II is its marked affinity for chito-oligosaccharides. As described above, GSL-II,



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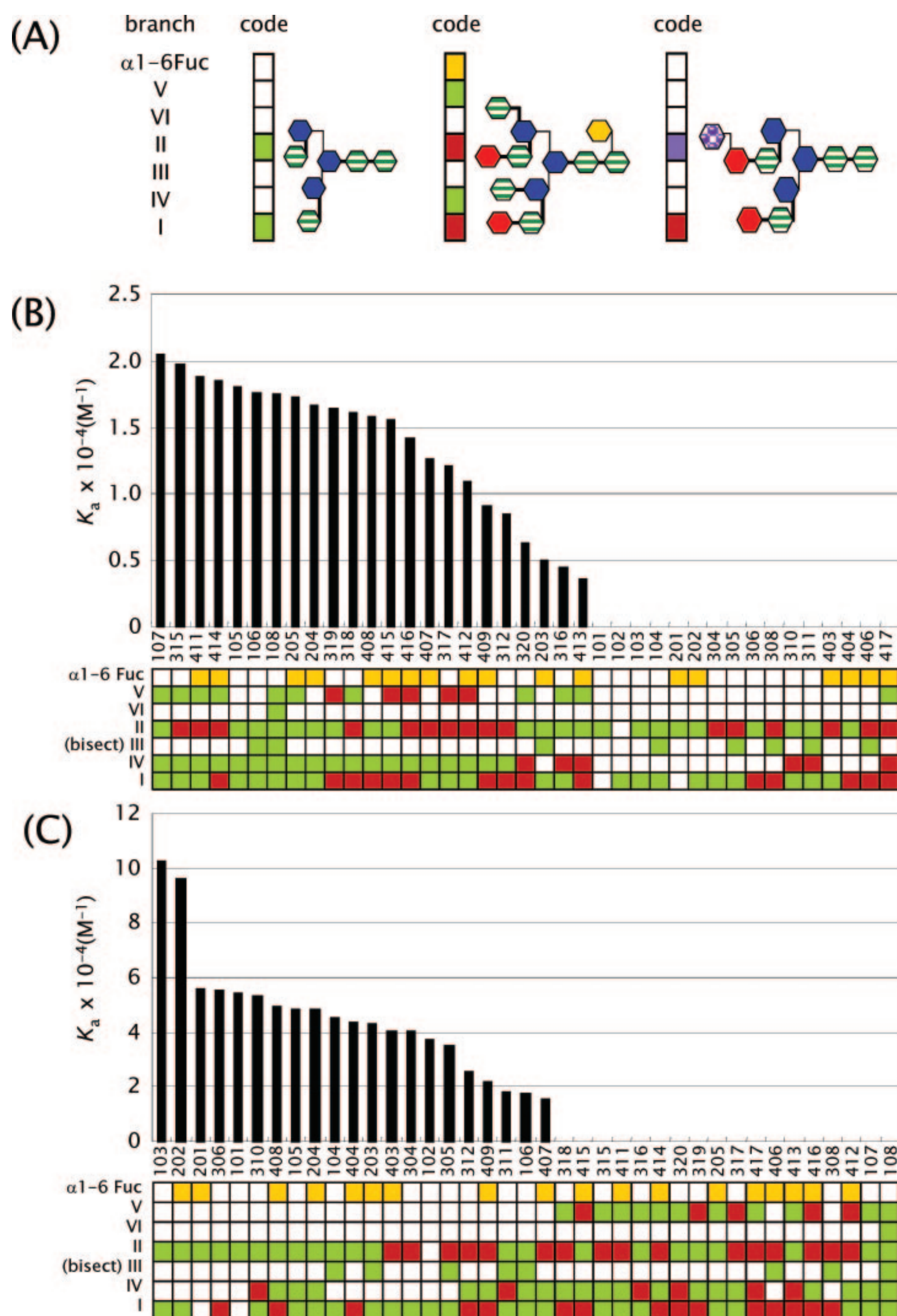


Fig. 4. **(A) Definition of "GRYP" code to represent branch positions and non-reducing end saccharides.** Each branch is numbered from I to VI corresponding to GlcNAc-transferases. Examples of glycan codes expressed by this system. Boxes are arranged along with the glycan structure drawn as described in Fig. 1. Non-reducing end sugar is shown in color: green, GlcNAc; red, galactose; purple, NeuAc; yellow, α1-6 (core) fucose; white, mannose (no-elongation). (B, C) Bar graph representation of affinity constants ($K_a$) of GSL-II (B) and BLL (C) toward completely/partially agalactosylated *N*-linked glycans in the order of affinity strength. Numbers at the bottom of the bar graphs correspond to sugar numbers indicated in Fig. 1. Corresponding GRYP codes are shown under the graphs.

but not BLL, showed substantial affinity for **906** and **907** (Fig. 3B). $K_a$ values determined for these PA-oligosaccharides correlated well with those obtained for corresponding pNP-glycosides (Fig. 3A, left).

While the global features of GSL-II and BLL are similar, detailed oligosaccharide specificities differ significantly. Here, to avoid a complicated discussion, we developed a new representation method for *N*-glycan structures, designated the "GRYP" code (Fig. 4A). Essential points are: (i) branch positions of *N*-glycans are numbered from I to VI according to the nomenclature system applied for mammalian GlcNAc-transferases. (ii) Non-reducing end sugars are shown in different colors; *i.e.*, GlcNAc (green), Gal (red), NeuAc (purple), whereas the presence of core fucose (α1-6Fuc) is expressed with another box colored in yellow.

*Fine Specificity of GSL-II*—Among completely agalactosylated *N*-glycans (**101**–**205**), GSL-II showed significant affinity for **105**–**108** and **203**–**205**, while demonstrating no apparent affinity for **101**–**104** and **201**–**202** (Fig. 3B, left). For galactosylated complex-type *N*-glycans (**301**–**420**), strong affinity was observed for **312**, **314**–**320**, **407**–**409** and **411**–**416**. In order to clarify a structural element required for GSL-II recognition, $K_a$ values determined by FAC were arranged in order of strength in a vertical bar graph together with the GRYP codes (Fig. 4B).



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Evidently, GSL-II strongly bound to tri- and tetra-antennary glycans, but had no, or if any, only trace affinity for mono- and bi-antennary ones. Notably, GSL-II showed remarkable affinity ($K_a > 8.5 \times 10^3$ M$^{-1}$) for a restricted set of glycans having non-reducing terminal GlcNAc at "branch-IV." For example, by comparison between **107** and **316** (Fig. 4B), it is clear that the addition of Gal (red-colored in branch-IV box) abolished the affinity. Although an increase in the number of non-reducing terminal GlcNAc residues somewhat enhanced the affinity (**105** *vs.* **107**), the essential feature of GSL-II binding is the presence of the intact GlcNAc residue at branch-IV.

There was no substantial effect whether GlcNAc residues at branches-I, -II, -III and -V were present or not, whether they were galactosylated or not, and whether the core chitobiose structure was α1–6 fucosylated or not. However, there is a significant loss of affinity, if all other branches are galactosylated (**312** and **409**). In the cases where branch-IV GlcNAc is galactosylated (**320**, **316** and **413**), the presence of branch-V GlcNAc compensated to some extent for the loss of affinity to branch-IV GlcNAc.

*Fine Specificity of BLL*—Among agalactosylated *N*-glycans (**101–205**), **101–106** and **201–204**, were recognized with relatively high affinity, while **107**, **108** and **205** were not recognized at all (Fig. 3B, right). Notably, such preference is almost opposite to the case of GSL-II. Among galactosylated complex-type *N*-glycans (**301–420**), strong affinity was observed for **304–306**, **309–312**, **403**, **404** and **407–409**. Again, this is opposite to the case of GSL-II, except for **407–409**.

As is evident from Fig. 4C, BLL showed the strongest affinity for bi-antennary, agalactosylated *N*-glycans (**103** and **202**). Binding to **103** was specifically inhibited by 100 mM GlcNAc, whereas that of Core1-pNP was not. This observation strongly support the above mentioned idea that BLL has dual sugar-binding sites (data not shown).

Mono-antennary, agalactosylated glycans are also good ligands for BLL, but with significant branch-specificity: *i.e.*. branch-II (*e.g.*, GlcNAcβ1-2Manα1-6Manβ in **306**) is 1.4 times more preferred than branch-I (GlcNAcβ1-2Manα1-3Manβ in **304**). Tri-antennary, agalactosylated glycans (**105** and **204**) are also good ligands, but the affinity is almost the same as for mono-antennary glycans (**101** and **201**). In other words, the GlcNAc residue at branch-IV has a rather detrimental effect for BLL recognition (compare **103** *vs.* **105**), in contrast to the case of GSL-II. This explains why these lectins show compensating features of agalacto-type *N*-glycans. The affinity of BLL was also reduced by the presence of bisecting GlcNAc (**103** *vs.* **104** and **202** *vs.* **203**). Importantly to note, none of the glycans having the branch-V (GlcNAcβ1-6Manα1-6Manβ, Fig. 4C) showed significant affinity to BLL. Since the presence of branch-V causes drastic structural change in the complex-type *N*-glycans, BLL recognition may require an intact conformation of agalacto, bi-antennary *N*-glycans.

*Summary of FAC Analysis*—Sugar-binding properties of GSL-II and BLL are summarized as follows: (i) both GSL-II and BLL show affinity for completely and partially agalactosylated, *N*-linked glycans, while their fine specificities are markedly different. (ii) The non-modified GlcNAc residue transferred by GlcNAc transferase-IV (GlcNAcβ1-4Manα1-3Manβ) is essential for GSL-II–binding. Thus, agalactosylated, tri- or tetra-antennary *N*-glycans containing this branch-IV are the best ligands for GSL-II. (iii) BLL shows high affinity for mono-, bi- and tri-antennary, agalactosylated glycans, while its affinity is drastically reduced by the presence of the GlcNAc residue transferred by GlcNAc transferase-V, and to a lesser extent, by those transferred by GlcNAc transferases-III and IV.

In order to profile *N*-glycan structures efficiently, a primary requisite is to know their frame structures consisting of five GlcNAc residues, each of which is closely associated with the biological functions of glycoproteins (*8*, *22–25*). In this study, we demonstrated that GSL-II and BLL can bind to agalactosylated glycans in a branch-specific and complementary manner. These findings as well as a novel expression format, the GRYP code, should contribute to the development of highly useful glycan profilers in the emerging field of glycomics (*26*).

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REFERENCES


1. Hirabayashi, J. (2004) Lectin-based structural glycomics: glycoproteomics and glycan profiling. *Glycoconj J.* **21**, 35–40
2. Hirabayashi, J., Arata, Y., and Kasai, K. (2000) Reinforcement of frontal affinity chromatography for effective analysis of lectin-oligosaccharide interactions. *J. Chromatogr. A* **890**, 261–271
3. Hirabayashi, J., Arata, Y., and Kasai K. (2003) Frontal affinity chromatography as a tool for elucidation of sugar recognition properties of lectins. *Methods Enzymol.* **362**, 353–368
4. Hirabayashi, J., Hashidate, T., Arata, Y., Nishi, N., Nakamura, T., Hirashima, M., Urashima, T., Oka, T., Futai, M., Muller, W.E., Yagi, F., and Kasai, K. (2002) Oligosaccharide specificity of galectins: a search by frontal affinity chromatography. *Biocim. Biophys. Acta* **1572**, 232–254
5. Nakamura, S., Yagi, F., Totani, K., Ito, Y., and Hirabayashi, J. (2005) Comparative analysis of carbohydrate-binding properties of two tandem repeat-type Jacalin-related lectins, *Castanea crenata* agglutinin and *Cycas revoluta* leaf lectin. *FEBS J.* **272**, 2784–2799
6. Demetriou, M., Granovsky, M., Quaggin, S., and Dennis, J.W. (2001) Negative regulation of T-cell activation and autoimmunity by Mgat5 *N*-glycosylation. *Nature* **409**, 733–739
7. Partridge, E.A., Le, Roy, C., Di, Guglielmo, G.M., Pawling, J., Cheung, P., Granovsky, M., Nabi, I.R., Wrana, J.L., and Dennis, J.W. (2004) Regulation of cytokine receptors by Golgi *N*-glycan processing and endocytosis. *Science* **306**, 120–124
8. Ohtsubo, K., Takamatsu, S., Minowa, M.T., Yoshida, A., Takeuchi, M., and Marth, J.D. (2005) Dietary and genetic control of glucose transporter 2 glycosylation promotes insulin secretion in suppressing diabetes. *Cell* **123**, 1307–1321
9. Shankar Iyer, P.N., Wilkinson, K.D., and Goldstein, I.J. (1976) An *N*-acetyl-D-glucosamine binding lectin from *Bandeiraea simplicifolia* seeds. *Arch. Biochem. Biophys.* **177**, 330–333
10. Ebisu, S., Shankar Iyer, P.N., and Goldstein, I.J. (1978) Equibrium dialysis and carbohydrate-binding studies on the 2-acetamido-2-deoxy-D-glucopyranosyl-binding lectin from *Bandeiraea simplicifolia* seeds. *Carbohydr. Res.* **61**, 129–138
11. Cho, E.Y., Choi, H.L., and Chan, F.L. (2002) Expression pattern of glycoconjugates in rat retina as analyzed by lectin histochemistry. *Histochem. J.* **34**, 589–600


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12. Aoki, K., Ishida, N., and Kawakita, M. (2001) Substrate recognition by UDP-galactose and CMP-sialic acid transporters. Different sets of transmembrane helices are utilized for the specific recognition of UDP-galactose and CMP-sialic acid. *J. Biol. Chem.* **276**, 21555–21561
13. Nagano, M., Stubiger, G., Marchetti, M., Gmeiner, G., Allmaier, G., and Reichel, C. (2005) Detection of isoforms of recombinant human erythropoietin by various plant lectins after isoelectric focusing. *Electrophoresis* **26**, 1633–1645
14. Kitamura, N., Ikekita, M., Hayakawa, S., Funahashi, H., and Furukawa, K. (2004) Suppression of proliferation and neurite extension of human neuroblastoma SH-SY5Y cells on immobilized *Psathyrella velutina* lectin. *J. Neurosci. Res.* **75**, 384–390
15. Koyama, Y., Katsuno, Y., Miyoshi, N., Hayakawa, S., Mita, T., Muto, H., Isemura, S., Aoyagi, Y., and Isemura M. (2002) Apoptosis induction by lectin isolated from the mushroom *Boletopsis leucomelas* in U937 cells. *Biosci. Biotechnol. Biochem.* **66**, 784–789
16. Koyama, Y., Suzuki, T., Odani, S., Nakamura, S., Kominami, J., Hirabayashi, J., and Isemura, M. (2006) Carbohydrate specificity of lectins from *Boletopsis leucomelas* and *Aralia cordate*. *Biosci. Biotechnol. Biochem.* **70**, 542–545
17. Arata, Y., Hirabayashi, J., and Kasai, K. (2001) Application of reinforced frontal affinity chromatography and advanced processing procedure to the study of the binding property of a *Caenorhabditis elegans* galectin. *J. Chromatogr. A* **905**, 337–43
18. Irazoqui, F.J., Vides, M.A., and Nores, G.A. (1999) Structural requirement of carbohydrate to bind *Agaricus bisporus* lectin. *Glycobiology* **9**, 59–64
19. Wu, A.M., Wu, J.H., Herp, A., and Liu J.H. (2003) Effect of polyvalencis of glycotopes on the binding of a lectin from the edible mushroom. *Agaricus bisporus*. *Biochem. J.* **371**, 311–320
20. Carrizo, M.E., Capaldi, S., Perduca, M., Irazoqui, F.J., Nores, G.A., and Monaco, H.L. (2005) The antineoplastic lectin of the common edible mushroom (*Agaricus bisporus*) has two binding sites, each specific for a different configuration at a single epimeric hydroxyl. *J. Biol. Chem.* **280**, 10614–10623
21. Nakamura-Tsuruta, S., Kominami, J., Kuno, A., and Hirabayashi, J. (2006) Evidence that *Agaricus bisporus* agglutinin (ABA) has dual sugar-binding specificities. *Biochem. Biophys. Res. Commun.* in press
22. Ioffe, E., Liu, Y., and Stanley, P. (1996) Essential role for complex *N*-glycans in forming an organized layer of bronchial epithelium. *Proc Natl Acad Sci.USA* **93**, 11041–11046
23. Wang, Y., Tan, J., Sutton-Smith, M., Ditto, D., Panico, M., Campbell, R.M., Varki, N.M., Long, J.M., Jaeken, J., Levinson, S.R., Wynshaw-Boris, A., Morris, H.R., Le, D., Dell, A., Schachter, H., and Marth J.D. (2001) Modeling human congenital disorder of glycosylation type IIa in the mouse: conservation of asparagine-linked glycan-dependent functions in mammalian physiology and insights into disease pathogenesis. *Glycobiology* **11**, 1051–1070
24. Yoshimura, M., Nishikawa, A., Ihara, Y., Taniguchi, S., and Taniguchi, N. (1995) Suppression of lung metastasis of B16 mouse melanoma by *N*-acetylglucosaminyltransferase III gene transfection. *Proc. Natl. Acad. Sci. USA* **92**, 8754–8758
25. Yamamoto, H., Swoger, J., Greene, S., Saito, T., Hurh, J., Sweeley, C., Leestma, J., Mkrdichian, E., Cerullo, L., Nishikawa, A., Ihara, Y., Taniguchi, N., and Moskal, J.R. (2000) Beta1,6-*N*-acetylglucosamine-bearing *N*-glycans in human gliomas: implications for a role in regulating invasivity. *Cancer Res.* **60**, 134–142
26. Kuno, A., Uchiyama, N., Koseki-Kuno, S., Ebe, Y., Takashima, S., Yamada M., and Hirabayashi, J. (2005) Evanescent-field fluorescence-assisted lectin microarray: a new strategy for glycan profiling. *Nature Methods* **2**, 851–856

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