Jacalin interacts with Asn-linked glycopeptides containing multi-antennary oligosaccharide structure with terminal α-linked galactose

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Abstract The carbohydrate binding properties of jacalin lectin were examined using RAF9 cell-derived D-[6-^{3}H]glucosamine-radiolabeled total glycopeptides containing N-linked and O-linked oligosaccharides. The binding of N-linked glycopeptides to jacalin was abolished by treatment of α-galactosidase whereas O-linked glycopeptides were still bound lectin after this treatment. The removal of O-linked oligosaccharides by mild alkaline/borohydride treatment completely eliminated the lectin binding of α-galactosidase treated glycopeptides. These results demonstrate that jacalin interacts with cellular glycopeptides containing N-linked oligosaccharides with terminal α-galactose residues as well as glycopeptides containing O-linked oligosaccharides.

Key words: Jacalin lectin; RAF9 cell-derived N-linked glycopeptide; Terminal α-galactose residue; α-Galactosidase

1. Introduction

Jacalin lectin from jackfruit seeds, *Artocarpus integrifolia* which belongs to Moraceae plant family, consists of two polypeptide chains, a heavy chain of α and a light chain of β [1,2]. The cDNA analysis of jacalin revealed that lectin is synthesized as a preproprotein and processed into functional two-chain molecule by proteolytic cleavage [3]. The recent analysis of X-ray crystal structure of jacalin showed that lectin has tetrameric structure of heterodimer subunit (α/β), and each subunit contains a unique carbohydrate binding site [4].

The carbohydrate binding property of Jacalin lectin has been known to be highly specific to T-antigenic disaccharide, galβ1,3GalNAc-*O*-αSer/Thr [5,6]. Due to its binding specificity toward O-linked oligosaccharide, jacalin has been used in many applications of O-linked glycoprotein and glycopeptide isolations [7–10]. However, there has been little information about the interaction between jacalin and N-linked oligosaccharides. In this study, we investigated the carbohydrate binding specificities of jacalin using cellular N-linked glycopeptides. Our results demonstrate that glycopeptides containing multi-antennary N-linked oligosaccharides with terminal α-galactose residues can bind jacalin with high affinity.

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Abbreviations: CHO, Chinese hamster ovary; N-linked, asparagine-linked; O-linked, serine/threonine-linked; Con A, concanavalin A; GlcNAc, *N*-acetylglucosamine; GalNAc, *N*-acetylgalactosamine; a-m-gal, α-methylgalactopyranoside; a-m-glc, α-methylglucoside; a-m-man, α-methylmannoside; BSA, bovine serum albumin

2. Materials and methods

2.1. Materials

Jacalin lectin and jacalin agarose were purchased from Vector Laboratories (Burlingame, CA). Con A-Sepharose and CNBr-activated Sepharose 4B were obtained from Pharmacia LKB Biotechnology Inc. Sephadex G-25, α-methylgalactopyranoside, melibiose, green coffee bean α-galactosidase, jack bean β1,4-galactosidase and β1,4- *N*-acetylhexosaminidase were obtained from Sigma Chemical Co., St. Louis, MO. α1,3/1,4-fucosidase was purchased from Takara Shuzo Co. All-trans-retinoic acid was purchased from Eastman Kodak Co. Neuraminidase (*Arthrobacter ureafaciens*) was obtained from Boehringer Mannheim Co. Radioisotope D-[6-^{3}H]glucosamine (25 Ci/mmole) was purchased from ICN Pharmaceuticals, Irvine, CA. The radioactive standard monosaccharides, *N*-acetyl[^{14}C]galactosamine/*N*-acetyl[^{3}H]glucosamine, and radioactive standard sugar alcohols, *N*-acetyl[^{3}H]glucosaminitol, *N*-acetyl[^{14}C]galactosaminitol, and Galβ1,3GalNAcO[^{3}H] were prepared as described previously [11].

2.2. Cell culture and metabolic radiolabeling of cellular glycoproteins

Mouse teratocarcinoma F9 cells were obtained from American Type Culture Collection and monolayer cells were cultured in Dulbecco's modified Eagle's medium supplemented with 15% fetal bovine serum and antibiotics (100 units/ml of penicillin and 100 mg/ml of streptomycin). F9 cells were induced to differentiate into RAF9 cells by treating retinoic acid (10^{-7} M) for three days [12] and cells were metabolically radiolabeled with D-[6-^{3}H]glucosamine. F9 cells were grown for 3 days in the medium supplemented with and without retinoic acid and the cultures were fed with medium containing 200 μCi/ml D-[6-^{3}H]glucosamine (25 Ci/mmole) for overnight. The radiolabeled cells were solubilized with mild sonications in PBS containing 0.1% Nonidet P-40 and protease inhibitors, and total glycoproteins were prepared by 10% TCA precipitation as previously described [13]. The radiolabeled glycopeptides were prepared by Pronase treatment (10 mg/ml) of total glycoproteins in digestion buffer (0.1 M Tris-Cl containing 1 mM $CaCl_2$, pH 8.0) under toluene atmosphere [11].

2.3. Column chromatography and descending paper chromatography

Con A-Sepharose lectin column chromatography was carried out as previously described [11]. The radioactive glycopeptides were fractionated on a 2 ml column of Con A-Sepharose (0.7×5 cm) with 2 ml collection of each fraction. The Jacalin-Sepharose 4B was prepared to 4 mg/ml of coupling density by manufacturer's instructions using CNBr-activated gel and jacalin lectin column chromatography was performed as previously described [10]. The chromatography of glycopeptides on Sephadex G-25 was conducted on a column (1×68 cm) equilibrated in 0.1 M pyridine-acetate buffer (pH 5.6). The descending paper chromatography on Whatman No. 1 filter paper of monosaccharides and oligosaccharides was conducted in solvent A (*n*-butylalcohol/pyridine/water, 6:4:3) for composition analysis, and in solvent B (ethyl acetate/pyridine/acetic acid/water, 5:5:1:3) for size separation, respectively [13]. The radioactivity on paper was determined by cutting strip into 1 cm sections followed by measuring in a Beckman liquid scintillation counter.

2.4. β-elimination and exoglycosidase treatment

For mild alkaline borohydride treatment, radiolabeled glycopeptides were treated with 50 mM NaOH containing 1 M $NaBH_4$ at 45°C for 16 h as described [11]. The reaction was terminated by neutralization with 4 N glacial acetic acid. The α-galactosidase treatment was

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performed in either 50 mM sodium-acetate buffer (pH 6.0) or 10 mM HEPES (pH 6.5) containing 150 mM NaCl and 5 mM $CaCl_2$ at 37°C with 100 milliunits of enzyme, and other treatments of exoglycosidases (β1,4-galactosidase, β-hexosaminidase, neuraminidase, and α1,3/1,4-fucosidase) were conducted as previously described [13–15].

3. Results

In the present study, we describe our finding that jacalin has carbohydrate binding specificity to terminal α-galactose residues present on cellular N-linked glycopeptides. Total glycopeptides prepared from metabolically D-[6-^{3}H]glucosamine-radiolabeled F9 and RAF9 cells were applied on a column of jacalin lectin immobilized on Sepharose 4B and the bound glycopeptides were isolated by elution with 100 mM α-methylgalactopyranoside (Fig. 1). As shown in Fig. 1A and B, 19–20% of total radioactive glycopeptides from RAF9 cells were bound jacalin whereas 6–7% were bound in case of F9 cell-derived glycopeptides. Compared with other cell-derived glycopeptides, such as CHO and K562 cells, the glycopeptides from RAF9 cells have been consistently shown to have a 3–4-fold increase in jacalin binding (data not shown). It has been well known that RAF9 cells exhibit several-fold increased expression of terminal α1,3-galactose on cellular glycoproteins compared with F9 cells [18,19], and therefore we investigated the possible interaction between jacalin and cellular glycopeptides containing terminal α-galactose residues.

The jacalin-bound RAF9 glycopeptides (Fig. 1A) were applied to a column of Sephadex G-25 for molecular sizing and desalting as shown in Fig. 1C. The large sized glycopeptides in the region of V_o of Sephadex G-25 column were pooled and then applied to a column of Con A-Sepharose (Fig. 1D).

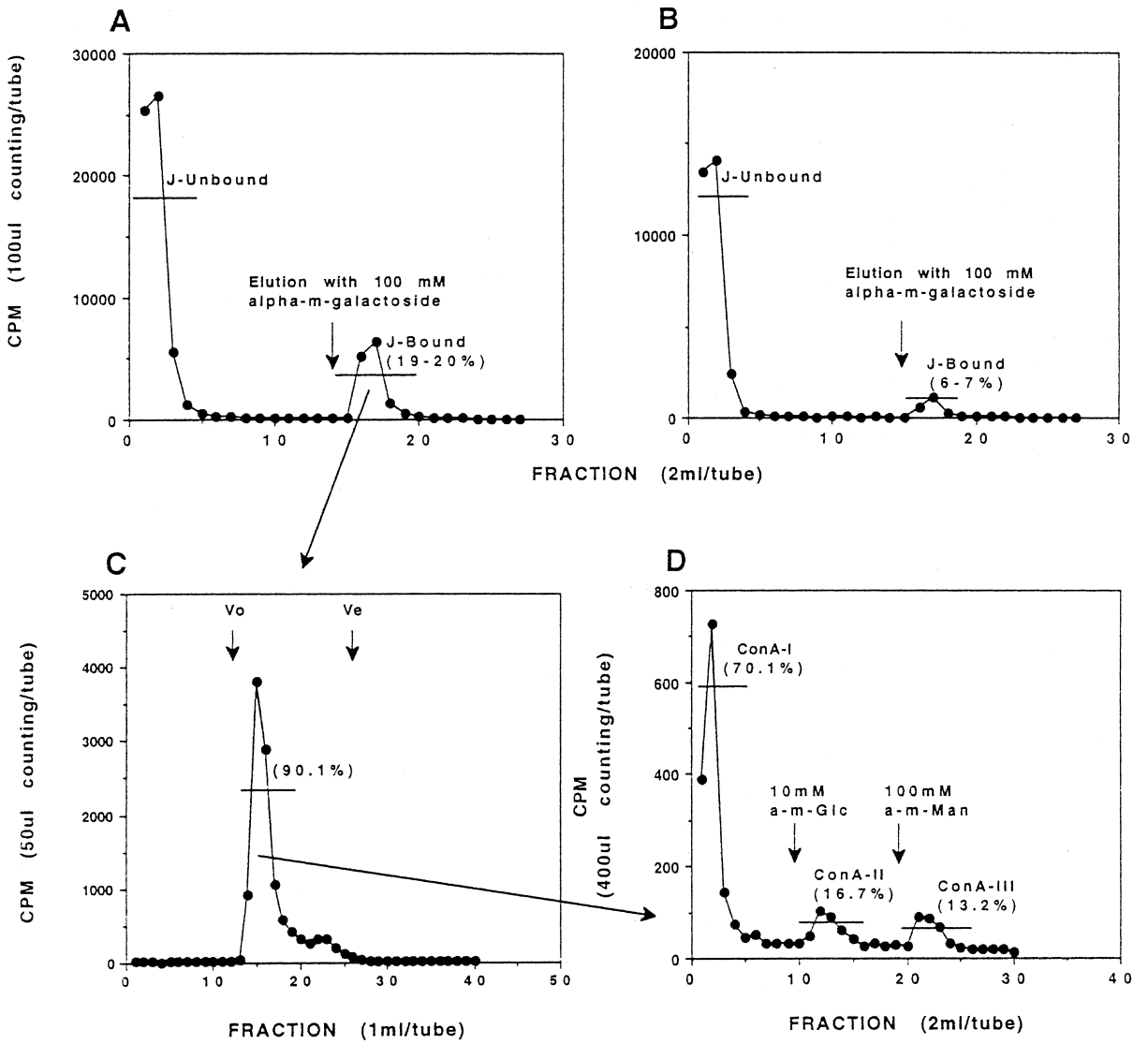

Fig. 1. Serial column chromatography of cellular glycopeptides. D-[6-^{3}H]glucosamine-radiolabeled total glycopeptides from RAF9 cells (A) and F9 cells (B) were applied to a column of Jacalin-Sepharose 4B. The unbound glycopeptides (J-Unbound) and bound glycopeptides (J-Bound) were fractionated by elution with 100 mM α-methyl-galactopyranoside (a-m-gal). The J-Bound glycopeptides from RAF9 cells were pooled as indicated by horizontal bar and applied to a column of Sephadex G-25 for molecular sizing (C). The apparant V_o and V_e were pre-determined with BSA and α-methylmannoside, respectively. The large sized glycopeptides were pooled as indicated and applied to a column of Con A-Sepharose (D). The Con A-bound glycopeptides were eluted sequentially with 10 mM α-methylglucoside (a-m-glc) and 100 mM α-methylmannoside (a-m-man). The fractions were pooled and designated as Con A-I (unbound), Con A-II (bound and eluted with 10 mM a-m-glc) and Con A-III (bound and eluted with 100 mM a-m-man).

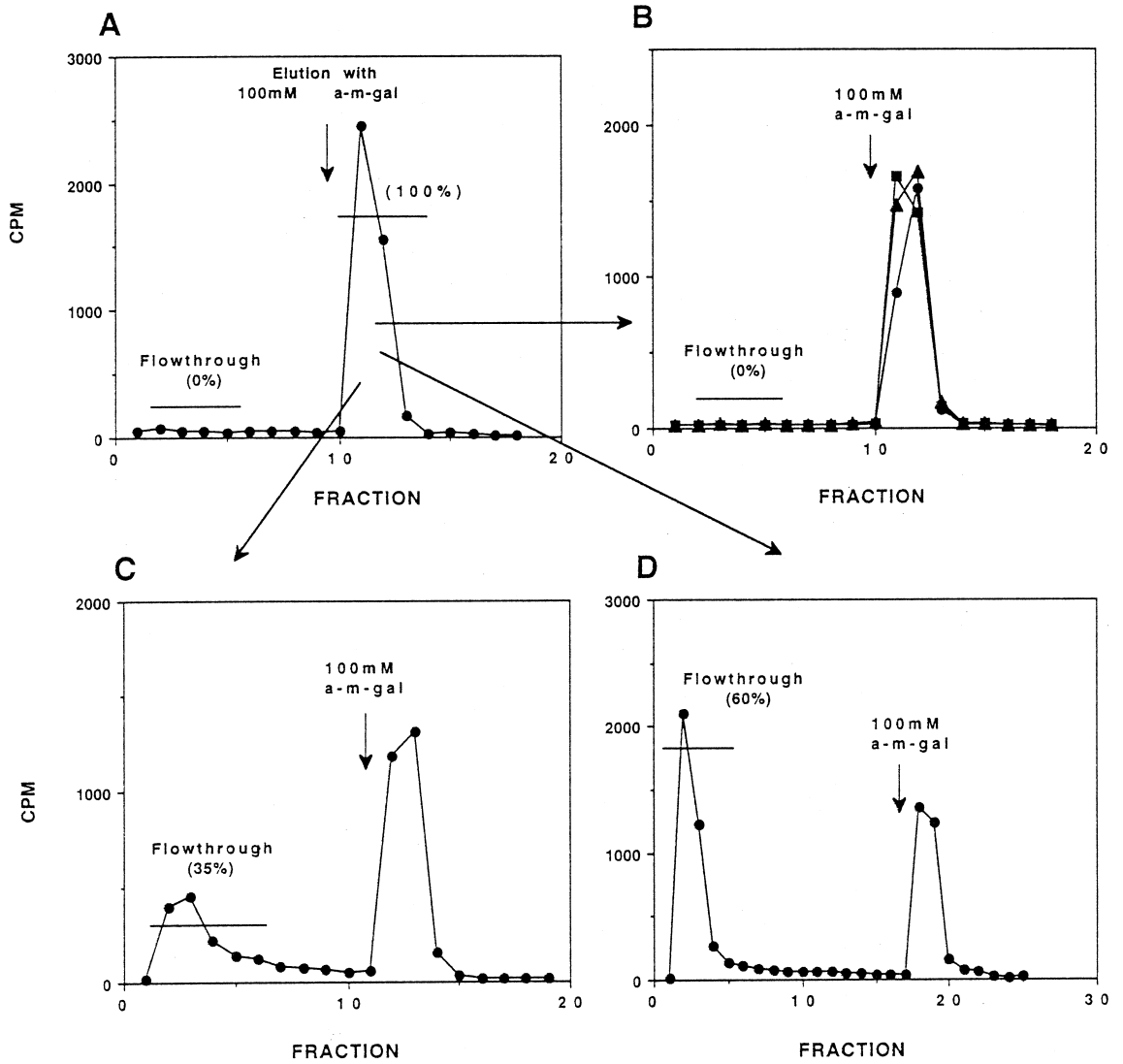

Fig. 2. Jacalin-Sepharose 4B column chromatography of Con A-I glycopeptides before and after exoglycosidase treatments. The intact Con A-I glycopeptides were reapplied to Jacalin-Sepharose 4B and eluted with 100 mM α-methyl-galactopyranoside (a-m-gal) (A). The Con A-I glycopeptides were treated with heat-inactivated α-galactosidase (circle), α-1,3/1,4-fucosidase (triangle) and β-1,4-galactosidase (square) and reapplied to a column of Jacalin-Sepharose 4B (B). The Con A-I glycopeptides were treated with α-galactosidase for overnight (C) or for 3 days (D) and the treated samples were subsequently reapplied to lectin column.

Previous studies have shown that Con A-I fraction contains both complex type N-linked oligosaccharides (tri-antennary, tetra-antennary, and more branched) and O-linked oligosaccharides [20]. However, complex type bi-antennary N-linked oligosaccharides are contained in Con A-II, and high mannose type N-linked oligosaccharides are in Con A-III fraction [20]. As shown in Fig. 1D, 70% of radioactivity designated as Con A-I glycopeptides, 17% of radioactivity as Con A-II, and 13% of radioactivity as Con A-III were recovered, respectively. Since the Con A-I glycopeptides (Fig. 1D) were considered to contain multi-antennary N-linked glycans and O-linked glycans, these glycopeptides were pooled and used to test further jacalin binding.

First, Con A-I glycopeptides were reapplied to Jacalin-Sepharose 4B, to confirm the lectin binding capacity. As expected, these glycopeptides were 100% rebound to jacalin (Fig. 2A). Second, to explore whether the terminal α-galactosylation on these glycopeptides could influence jacalin binding, these Con A-I glycopeptides were treated with α-galactosidase for overnight up to three days for complete cleavage of terminal α-galactose residues, and the treated samples were applied to column of Jacalin-Sepharose 4B. As shown in Fig. 2C and D, 35%, and 60% of applied radioactive samples were passed through the jacalin column without binding after overnight and after 3 day treatment, respectively. As a control, treatment of Con A-I glycopeptides by heat-inactivated α-galactosidase, or by other exoglycosidases such as α-1,3/1,4-fucosidase and β-1,4-galactosidase has no effect on jacalin binding (Fig. 2B). In parallel with this, the binding properties of Con A-II and Con A-III glycopeptides (Fig. 1D) to jacalin were examined and turned out to be not affected by α-galactosidase treatment (data not shown). These data indicate that removal of terminal α-galactose residues from Con A-I glycopeptides resulted in significant abolishment of jacalin binding and terminal α-galactose residues contained in Con A-I glycopeptides could directly participate in lectin binding.

The composition analysis of *N*-acetylglucosamine and *N*-acetylgalactosamine of jacalin-unbound glycopeptides (flow-

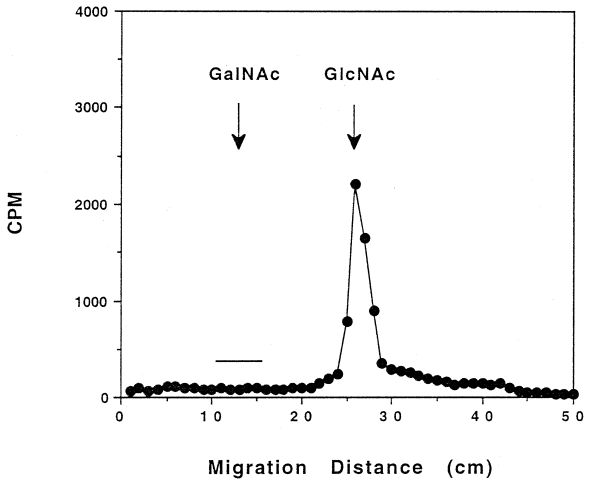

Fig. 3. Descending paper chromatography of jacalin-unbound glycopeptides (from Fig. 2D) after treatment with 4 N HCl and subsequent reacetylation. The strong acid hydrolyzed samples were analyzed by descending paper chromatography for 50 h in solvent system A. The arrow indicates the migration position of standard monosaccharide of *N*-acetyl[^{3}H]glucosamine (GlcNAc) and *N*-acetyl[^{14}C]galactosamine (GalNAc).

through fractions in Fig. 2D) showed almost complete lack of *N*-acetylgalactosamine, suggesting that these glycopeptides contain mainly N-linked glycans, not O-linked glycans (Fig. 3). Third, to further investigate the binding properties of jacalin-bound glycopeptides after 3 day treatment of α-galactosidase (Fig. 2D), the samples were further treated with mild alkaline/borohydride to effect β-elimination of O-linked oligosaccharides. The treated samples were then applied to a column of Jacalin-Sepharose 4B and as shown in Fig. 4, almost all of the applied samples did not bind to lectin. These data demonstrate that the residual lectin binding of glycopeptides after exhaustive treatment of α-galactosidase (Fig. 2D) was turned out to be due to interaction between jacalin and O-linked glycans present on glycopeptides.

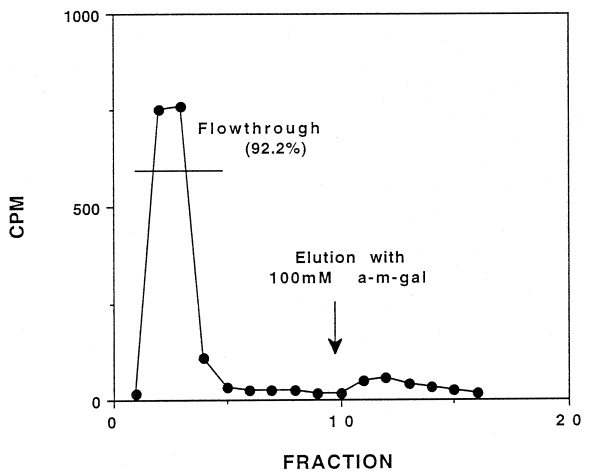

Fig. 4. Jacalin-Sepharose 4B column chromatography of jacalin-bound glycopeptides (from Fig. 2D) after mild alkaline/borohydride treatment for β-elimination. The β-eliminated samples were reapplied to lectin column and the bound materials were eluted with 100 mM α-methyl-galactopyranoside (a-m-gal).

4. Discussion

Previous binding studies of various glycans to jacalin have shown that jacalin selectively interacts with Thomsen-Friedenreich antigen (T-antigenic disaccharide, Galβ1,3GalNAc-*O*-Ser/Thr) [5]. It should be noted that GalNAc must be in α-anomeric configuration for high affinity binding of T-antigenic disaccharide to jacalin since lectin has been known to poorly interact with same disaccharide containing β-anomeric configuration [6]. It has been also shown that α-linked, not β-linked galactopyranoside, such as α-methylgalactopyranoside or melibiose can be used as hapten sugar for elution of jacalin agarose column [7,9]. These observations indicate that jacalin may have high binding specificity toward α-anomers of galactopyranosides and 2-acetamido-2-deoxygalactopyranosides.

However, there is also report that jacalin has very poor affinity to terminal α-anomeric glycans, such as galα1,3-α-methylgalactopyranoside, galα1,4-gal, A- and B-blood group trisaccharides [6]. Consistent with this, it was found that the synthetic trisaccharide, galα1,3-lactose or galα1,3-*N*-acetyllactosamine has no significant affinity to jacalin lectin [22]. Although the molecular basis for these observations has not been clearly understood, our results suggest some possibility that multiple orientation of terminal α-galactose residues might be important for jacalin binding with high affinity.

In addition, some previous studies have shown that glycoproteins containing only N-linked oligosaccharides did not bind to jacalin [9]. Based on our results, however, it seems likely that the structure of N-linked oligosaccharides in those glycoproteins may lack terminal α-linked galactose residues. Therefore, in particular, the approaches to define jacalin binding using cell-derived glycoproteins or glycopeptides needs to be cautious since many glycoproteins synthesized from mammalian species except human and old world monkeys may contain some possible N-linked oligosaccharide structures with terminal α-galactose residues in addition to O-linked oligosaccharides [21].

Results of the present study demonstrate that RAF9 cell-derived N-linked glycopeptides bind to jacalin and this interaction is mediated by terminal α-galactose residues present on multi-antennary structure of N-linked oligosaccharides. Although the spacial conformation of terminal α-galactose residues on multi-antennary oligosaccharides needs to be determined, it is very likely that multiple residues of α-linked galactose may be necessary for strong interaction with tetrameric subunit structure of jacalin lectin [4], and may enhance the lectin binding with high avidity.

5. Additional references

[16,17]

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