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Anomer specificity of the 14 kDa galactose-binding lectin: A reappraisal

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Abstract. A β-anomer preference among galactosides has been attributed to the S-type 14 kDa galactose binding lectin. Here the anomeric preference of this lectin from bovine brain (BBL) is reexamined using inhibition of lectin-mediated haemagglutination, binding of the lectin to dot-blotted glycoproteins and affinity electrophoresis of the lectin through polysaccharide-containing gels. 1·0-methyl α-D-galactoside was 8 times better inhibitor of BBL than the corresponding ß-anomer. The terminal galactose in bovine thyroglobulin (exclusively. α-linked) were also nearly 8 times more inhibitory than those in asialofetuin (exclusively ß-linked). The terminal α-galactose-containing endogenous glycoproteins of bovine brain were nearly 4 times better inhibitors of BBL than laminin. Removal of terminal α-galactose units by α-galactosidase fully abolished the BBL binding of thyroglobulin and endogenous glycoproteins. BBL was also sugar-specifically retarded by polyacrylamide gel containing guar galactommannan which bears only α-linked galactose. Data indicated that α-galactosides were sometimes better than their β-anomers in binding to BBL. The significance of this observation to the physiological role of galactose-binding lectins is discussed.

Keywords. Galactose -binding lectin; α -galactoside; anomer specificity; bovine brain.

1. Introduction

Galactose-binding lectins having subunit molecular weight around 14 kDa (L-14) are the most ubiquitous among animal tissue lectins, having been detected in such tissues as brain, heart, placenta and muscle (Sharon and Lis 1989). Despite lacking a signal peptide during biosynthesis, this lectin is detectable on cell surface as well as in the extracellular matrix in addition to the cytoplasm (Hynes *et al* 1989). This extracellular location and galactose specificity predicted a role for L-14 in cell adhesion involving endogenous cognate glycoconjugates. Recognition of glycoconjugates by L-14 has been implicated in immunological events and in translocation of cancer cells (Raz *et al* 1986; Sharon and Lis 1989). However, information on endogenous L-14-binding glycoconjugates is still in infancy (Joubert *et al* 1992; Jaison and Appukuttan 1994; Kannan and Appukuttan 1993, 1994).

Early studies using extraneous sugars had attributed to L-14 a preference for β-anomer among galactosides (Waard *et al* 1976; Caron *et al* 1987). That the commonest of galactosidic disaccharides, lactose as well as the readily available

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Abbreviations used: L-14, 14 kDa galactose-binding lectin; BBL, bovine brain 14 kDa galactose binding lectin: PBS. 20mM potassium phosphate buffer, pH 7·4 containing 150mM NaCl; HRP, horse radish peroxidase; TAG, terminal α-linked galactose.

animal oligosaccharides were β-anomers might have prompted this conclusion. Comparisons of galactosides identical in structure except for anomeric linkage of terminal galactose were hardly done. However, laminin, the glycoprotein with an unusually high affinity for L-14 has α-linked galactose moiety as terminal sugar (Zhou and Cummings 1990). In addition, many tumour cell types as well as bacteria with which L-14 is supposed to interact (Castronovo *et al* 1989; Galili *et al* 1988a) are known to carry on their cell surface glycoconjugates having terminal α-galactosyl moiety. In the present work we reexamined the anomeric specificity of bovine brain L-14 (BBL) using anomers of simple galactosides, glycoproteins and polysaccharides employing dot blot and agglutination inhibition assays and affinity electrophoresis. Results indicated that α-galactosides are equally efficient ligands for BBL as β-galactosides, if not better.

2. Materials and methods

Bovine thyroglobulin, fetuin, paranitrophenyl α- and β-galactosides, l-0-methyl α- and β-galactosides, guar gum galactomannan, yeast mannan, coffee bean α-galactosidase and horse radish peroxidase (HRP) were obtained from Sigma Chemicals Co., St. Louis, Mo USA. Nitrocellulose paper was purchased from Schleicher and Schuell, Germany. Thyroglobulin and fetuin were desialylated by heating their solutions in 0·1 N H_2SO_4 at 80°C for 1 h, followed by dialysis against water or PBS at 4°C. BBL was prepared from bovine brain grey matter by affinity chromatography on laclose-Sepharose as described earlier (Jaison and Appukuttan 1994). BBL-HRP conjugate was prepared by glutaraldehyde conjugation in a solution of 1 mg BBL and 2 mg HRP in 1 ml PBS as described by Heyderman *et al* (1989). Laminin from bovine heart muscle was prepared as described by Timpl *et al* (1982) except that gel filtration was performed on Sepharrose –4B (2Cm×60cm) and laminin eluted in void volume was used. Jacalin was prepared as described earlier (Sureshkumar *et al* 1982) and jacalin-Sepharose 48 was prepared by the cyanogen bromide procedure (Lowe 1979) using 4 mg lectin per ml of Sepharose in the presence of 0·15 M galactose to protect the sugar-binding site of jacalin. Jacalin-binding bovine brain and bovine heart glycoproteins were prepared using jacalin-Sepharose 4B as described by Jaison *et al* (1993).

2·1 *Recognition by BBL of glycoproteins on dot blots*

PBS solutions of thyroglobulin, asialothyroglobulin or asialofetuin (2 µl containing 4 µg protein) were blotted on to duplicate 5 mm × 5 mm nitrocellulose strips. For comparing relative efficiencies of glycoproteins in binding BBL, serial two-fold dilutions of aisalofetuin, bovine thyroglobulin, bovine heart laminin, jacalin-binding bovine brain glycoproteins and jacailin binding bovine heart glycoproteins were each dotted on successive positions on a long strip of nitrocellulose. After air-drying the strips were blocked by keeping in 5% BSA in PBS overnight and then transferred to 150 µl solution of BBL-HRP (50µg BBL per ml) in PBS containing 1% BSA, incubated previously for 1 h with or without 100 mM lactose. The strips, after being in contact with the conjugate for 2 h, were washed thrice in PBS-1% BSA and binding of conjugate detected using a mixture of 1 mg 4-chloronaphthol in

0·35 ml anhydrous methanol, 2 µl 30% H_2O_2 and 1·65 ml PBS. To check the effect of α-galactosidase treatment of glycoproteins on their recognition by BBL, 1–2 mg/ml solution of each of the glycoproteins (bovine thyroglobulin, asialothyroglobulin, asialofetuin, bovine brain jacalin-binding glycoproteins and bovine heart jacalin binding glycoproteins) in 0·5 M citrate phosphate buffer, pH 5·2, was incubated at 37°C for 45 min with or without 0·4 units per ml coffee bean α-galactosidase. The samples were then blotted on nitrocellulose sheets and probed with BBL-HRP as described above.

2.2 *Inhibition of agglutination by BBL*

Inhibitory concentrations of galactosides and glycoproteins were determined as described earlier (Sureshkumar *et al* 1982), using serial two-fold dilutions of the inhibitors against twice the minimum haemagglutinating concentration of BBL in sample volumes of 250 µ1 containing 1% trypsinized rabbit erythrocytes in 8 mm diameter glass test tubes.

2.3 *Affinity electrophoresis in polysaccharide-containing polyacrylamide gel*

Polyacrylamide gels (10%) containing guar galactomannan (50% saturated) or yeast mannan (2 mg/ml) each with and without 100mM 1-0-methyl α-D-galactoside were prepared by dissolving appropriate amounts of the polysaccharide and/or galactoside in aqueous solution of polymerizing agent, before mixing with the acrylamide. BBL (10 µg) was run in each gel as described earlier (Kannan and Appukuttan 1993). The gels fixed with 12.5% trichloroacetic acid and stained with Coomassie brilliant blue.

3. Results

The complex type oligosaccharides of bovine thyroglobulin contain as their terminal non-reducing sugar, either an α (1→3) linked galactose residue or a sialic acid residue (Spiro and Bhoyroo 1984). The penultimate sugar in both cases is β-linked galactose, that forms part of an N-acetyl lactosamine structure. The terminal α-linked galactose (TAG) units account for 23% of the total galactose content of the glycoprotein (Spiro and Bhoyroo 1984). Inspite of terminal β-linked galactose units being absent or indetectably low in bovine thyroglobulin, this glycoprotein was readily recognized sugar-specifically by BBL (figure 1). Moreover, removal of TAG units by coffee bean α-galactosidase completely abolished the BBL binding to thyroglobulin, confirming that the lectin binding was exclusively due to α-galactosyl units. The α-anomer specificity of the coffee bean α-galactosidase was testified by the fact that enzyme treatment of asialofetuin did not affect BBL binding to this glycoprotein which is rich in terminal β-galactose units, but lacks TAG units (Green *et al* 1988). Notably, even though asialothyroglobulin contains some terminal β-linked galactose units, removal of its TAG units by α-galactosidase fully abolished its recognition by BBL (figure 1). Recognition of TAG units by BBL was further established by the observation that jacalin-binding glycoproteins from bovine brain grey matter, as well as from bovine heart muscle offered efficient ligands for BBL binding which was abolished upon α-galactosidase treatment of these glycoproteins

(Figure 2). Jacalin recognizes obligosaccharide groups terminating in either α-linked galactose or in the T-antigenic structure, GaI β 1→ 3 Gal NAc α (Sureshkumar *et al* 1982; Mahanta *et al* 1992).

A comparison of BBL-binding capacity of asialofetuin and thyroglobulin was made from intensities of dot blots of BBL-HRP binding to serial dilutions of both glycoproteins, starting with equal carbohydrate concentrations. Results (figure 3)

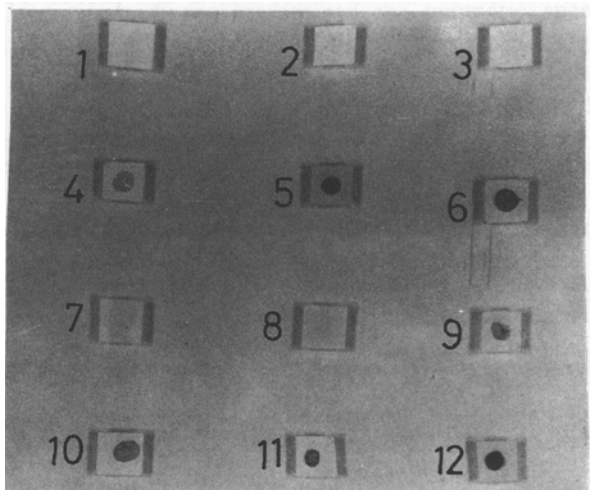

Figure 1. Effect of inhibition with sugar or pretreatment with α-galactosidase on recognition by BBL of bovine thyroglobulin (1,4,7,10), desialylated bovine thyroglobulin (2,5,8,11) and asialofetuin (3,6,9,12). Strips 1 to 6 were dotted with 4 μg proteins, blocked and probed with BBL-HRP in the presence (1,2,3) or absence (4,5,6) of 100 mM lactose. Strips 7 to 12 were dotted with 4 μg protein after incubation for 1 h in citrate phosphate buffer pH 5·2 in the presence (7,8,9) or absence (10,11,12) of coffee bean α-galactosidase. The dots were probed with BBL-HRP as described in the text.

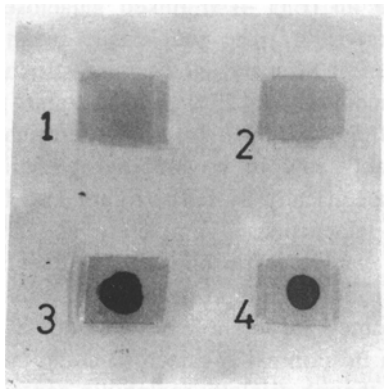

Figure 2. Effect of α-galactosidase treatment of jacalin binding glycoproteins from bovine brain (1.3) and bovine heart (2,4) on their recognition by BBL using BBL-HRP. Glycoproteins were incubated in citrate-phosphate buffer pH5·2 for 1 h in the presence (1,2) or absence (3,4) or coffee been α-galactosidase before dot-blotting on to nitrocellulose strips (2 μg per strip).

though semiquantitative due to two-fold serial dilutions, indicated that on the basis of carbohydrate content thyroglobulin was nearly eight times as efficient as asialofetuin in BBL binding. Similar comparisons among bovine heart laminin and jacalin-binding bovine brain and heart glycoproteins revealed that the latter glycoproteins were at least 4 times as effective as laminin in binding BBL (figure 3).

Study of inhibition of BBL-mediated haemagglutination (table 1) showed that l-0-methyl α-D-Gal is nearly eight times as efficient as its β-anomer as an inhibitor. Comparing bovine thyroglobulin and asialofetuin on the basis of carbohydrate content showed again that the exclusively α-galactoside-containing thyroglobulin is eight times better inhibitor of BBL than the exclusively ß-galactoside-containing asialofetuin. The difference between anomers of *p*-nitrophenyl galactoside was not

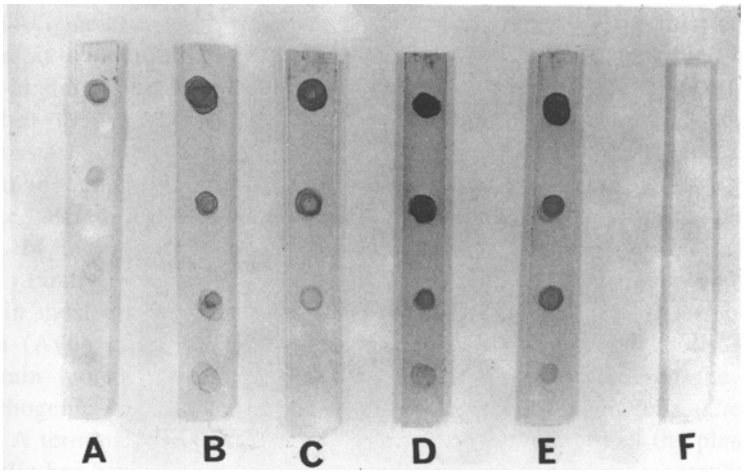

Figure 3. Relative capacities different glycoproteins to bind BBL. Each of strips A to E contained dots of serial two-foId dilutions of different glycoproteins Starting with the highest concentration on top Glycoproteins and their starting amounts were: A-asialofetuin. 0·03 µg; B-bovine thyroglobulin. 0·03 µg; C-bovine heart laminin, 0·24 µg, D jacalin-binding bovine brain glycoproteins, 0·24 µg; E-jacalin-binding bovine heart glycoproteins. 0·24 µg. Strip F contained five blots, one each of the highest concentrations in A to E respectively from top to bottom, probed with BBL-HRP containing 100mM lactose.

Table 1. Comparison of α- and β-galactose -containing sugars and glycoproteins in inhibition of BBL-mediated haemagglutination.

Sugar/glycoprotein	Minimum concentration required for inhibition of agglutination
1-O-methyl α-D-Gal	6·25 mM
1-O-methyl β-D-Gal	50·00 mM
P-nitrophenyl α-D-Gal	10·00 mM
P-nitrophenyl β-D-Gal	5·00 mM
Bovine thyroglobulin	2·24 µg/ml*
Asialofetuin	18·00 µg/ml*

*Concentration of neutral sugar in the glycoproteins.

marked probably due to the contribution of the hydrophobic nitrophenyl group towards lectin binding, reported earlier (Williams *et al* 1979). Though Caron *et al* (1987) reported a better inhibition of BBL-mediated agglutination of neuraminidase-treated erythrocytes by 1-0-methyl B-GaI than by its α-anomer, our data on agglutination inhibition is in line with that on glycoprotein recognition by BBL on dot blots.

Affinity electrophoresis of BBL in Polyacrylamide gel (figure 4) also demonstrated that guar galactomannan that contained only the α-anomer of galactose could retard the lectin mobility and that 1-0-methyl α-Gal could fully reverse this binding. On the other hand, yeast mannan containing no galactose moiety did not retain the lectin.

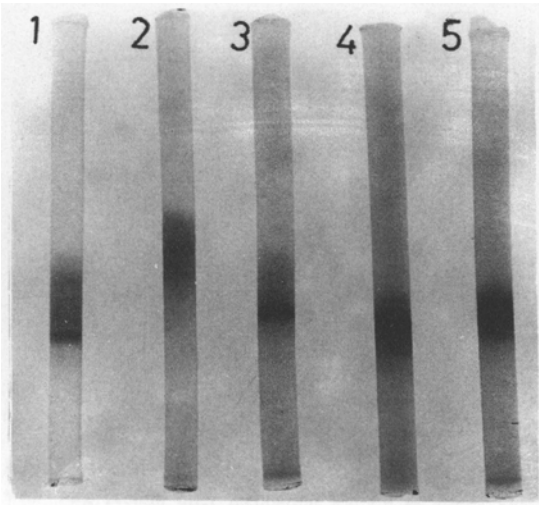

Figure 4. Recognition by BBL of α-galactoside-containing polysaccharides, demonstrated by affinity electrophoresis. BBL (10μg) was run in PAGE at pH 8·2 at 4°C in 10% gel containing no polysaccharide or sugar (1), 50% saturated guar galactomannan (2), 50% saturated guar galactomannan plus 100mM l-0-methyl α-D-galactoside (3), yeast mannan 2 mg per ml (4) or yeast mannan 2 mg per ml plus 100 mM 1-O-methyl-α-D-galactoside (5).

4. Discussion

Oligosaccharide chains containing polylactosamine sequences have been shown to be excellent ligands for L-14 (Zhou and Cummings 1990). Thus laminin containing up to four repeating lactosamine disaccharides in one oligosaccharide side chain has been shown, to be one of the strongest glycoprotein ligands for L-14 (Zhou and Cummings 1990). Also, terminal galactose units have been reported to be unnecessary for L-14 binding to polylactosamine-containing oligosaccharides (Merkle and Cummings 1988). Asialofetuin as well as thyroglobulin used in the present work, however, were devoid of polylactosamine sequences, so that affinity of BBL towards these glycoproteins could be attributed solely to terminal galactose moieties. Though the relative numbers of sialic acid-terminated and α-galactoside-terminated oligosaccharide chains per molecule of thyroglobulin are not known, the observation that non-reducing terminal β-galactoside moieties generated by desialylation could

not facilitate BBL binding to α-galactosidase-treated asialothyroglobulin suggested that a certain minimum density of terminal β-galactoside groups may be required for lectin binding. In support to this contention fetuin glycopeptides, unlike the total fetuin molecule, could not bind to immobilized L-14 (Merkle and Cummings 1988). Thus an unusually dense distribution of terminal β-galactoside groups (Green *et al* 1988), rather than a high affinity for individual β-galactosyl moieties may account for the observed BBL binding to asialofetuin.

Although removal of TAG moiety hardly affected the L-14 recognition of laminin (Zhou and Cummings 1990) due to the presence of adjacent polylactosamine groups, the present results suggest that in intact laminin, the TAG moiety may contribute substantially to L-14 binding. In brain grey matter, especially in adults, however, laminin is too scarce to act as cognate molecules for endogenous L-14 (BBL). Possibly endogenous glycoproteins of the type used here, that bind BBL exclusively through TAG moieties are prominent physiological receptors for this lectin. It was recently reported that endogenous cell surface glycoproteins that bind the 35 kDa galactose-binding lectin of macrophages are characterized by TAG groups (Sato and Hughes 1994). Notably, increased synthesis of TAG moiety is characteristic of brain tissues compared to other organs, in animals in general (Finne and Krusius 1976) and in humans in particular (Jaison *et al* 1993).

A marked affinity for TAG moiety may be significant to the proposed physiological role of L-14 in immune surveillance and in translocation of cancer cells (Raz *et al* 1986). Firstly, its most likely physiological receptor, laminin contains TAG moieties in most non-human animals (Zhou and Cummings 1990) and occasionally in human (Avila *et al* 1988). Secondly TAG moieties, though hardly synthesized in non-brain human tissues (Galili *et al* 1988b) are abundant on the surface of many pathogenic bacteria (Galili *et al* 1988a) and of tumour cells (Castronovo *et al* 1989). A terminal α-galactose-specific lectin from the seeds of the plant *Griffonia simplicifolia* has been shown *in vitro* to mediate sugar-dependent macrophage-tumour cell adhesion (Maddox *et al* 1982).

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