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Comparison of the binding properties of the mushroom *Marasmius oreades* lectin and *Griffonia simplicifolia* I-B_4 isolectin to αgalactosyl carbohydrate antigens in the surface phase

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Abstract: The binding of two α-galactophilic lectins, *Marasmius oreades* agglutinin (MOA), and *Griffonia simplicifolia* I isolectin B_4 (GS I-B_4) to neoglycoproteins and natural glycoproteins were compared in a surface phase assay. Neoglycoproteins carrying various α-galactosylated glycans and laminin from basement membrane of mouse sarcoma that contains the xenogenic Galα1–3Gal1–4GlcNAc epitope were immobilized in microtiter plate wells and lectin binding determined with an enzyme-linked assay. After 24 h of incubation, MOA had higher affinity for the xenogenic pentasaccharide (Galα1–3Gal1–4GlcNAcβ1–3Galβ1–4Glc) than for the Galα-monosaccharide. The binding properties of MOA and GS I-B_4 to the xenogenic disaccharide (Galα1–3Galβ1) were comparable while the binding of MOA to the xenogenic pentasaccharide was much stronger than the binding of GS I-B_4 to the same epitope. Non-xenogenic disaccharide-coupled neoglycoproteins having galactose end groups linked α1–2 or α1–4 to Gal or linked α1–3 to GalNAc bound very weakly to MOA, whereas GS I-B_4 recognized all of these disaccharides with similarly high affinity. MOA also showed high affinity for laminin. The results indicate that the *Marasmius oreades* lectin has nearly the same affinities as does GS I-B_4 for the simple xenogenic carbohydrate antigens, but MOA has greater affinity for the pentasaccharide and is far more specific in its binding preferences than the *Griffonia* lectin.

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Key words: Galα – *Griffonia simplicifolia* – lectins – *Marasmius oreades* – xenotransplantation

Abbreviations: BSA: bovine serum albumin; ELLA: enzyme-linked lectin assay; GS I-B_4, *Griffonia simplicifolia* I isolectin B_4; HRP: horseradish peroxidase; HSA: human serum albumin; MOA: *Marasmius oreades* agglutinin; PBS: phosphate-buffered saline (10 mM $NaHPO_4$, pH 7.2, 0.15 M NaCl, 0.04% NaN_3).

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Introduction

Pig to human xenotransplantation may be an achievable way to overcome the acute shortage of suitable human donor organs. However, at present this procedure is not possible because pig organs, transplanted to primates, are strongly rejected by circulating natural human antibodies [1]. The xenogenic porcine epitopes that are recognized by the natural human antibodies consists of terminal galactose residues in α1–3 linkage to subterminal galactose [2–5]. Antibody binding sites for such carbohydrate antigens can have a binding requirement for as few as two saccharides or for the pentasaccharide Galα1–3Galβ1–4GlcNAcβ1–3Galβ1–4Glc as isolated from glycolipid or glycoprotein fractions of pig aorta and kidney [6,7]. A variety of lectins and antibodies have previously been used to determine and characterize the xenogenic antigens [8–13] but none of them seem to fulfill the demands of sufficiently high specificity and affinity that are required when used as tools for

detection of the extended Galα1–3Gal antigens. Recently, a lectin isolated from the mushroom *Marasmius oreades* [*Marasmius oreades* agglutinin (MOA)] has proved to have high affinity for both the disaccharide Galα1–3Gal and for the trisaccharide Galα1–3Galβ1–4GlcNAcβ1 and could therefore be an important tool in transplantation research [14, 15] by proving useful to identify the galactosylated xenogenic antigens. To decide if this is true, we have compared the binding properties of the α-galactophilic lectins MOA and *Griffonia simplicifolia* B_4 isolectin with glycoproteins having terminal, non-reducing α-galactopyranosyl groups. The present study also involves a comparison of the surface phase assay used here with the solution phase approach used in titration calorimetry [14].

Material and methods

Lectins and glycoproteins

Purification of recombinant MOA was performed as previously described [15]. GS I-B_4 was obtained from EY Laboratories (San Mateo, CA, USA). The investigations of lectin–carbohydrate interactions presented in this study were performed using a lectin stock solution of 1 mg lectin in 1 ml phosphate-buffered saline (PBS). Unless other concentrations are indicated, a dilution of the lectin stock solution of 1 : 400 was used for the binding measurements. The lectin–carbohydrate interactions were studied in solid phase surface systems by means of avidin–biotin techniques as previously described [16]. Lectins were biotinylated as follows: 1 mg lectin was dissolved in 1 ml $NaHCO_3$ (0.1 M, pH 8.2) containing 0.1 M lactose to protect the active site, and mixed with Biotin-NHS dissolved in 0.2 ml dimethylformamide. The mixture was stirred for 2 h at room temperature and dialyzed in 5 l 0.05 M Tris/0.15 M NaCl (pH 7.2) overnight at 4 °C.

Neoglycoproteins with human or bovine serum albumin (HSA or BSA) as carrier proteins were obtained from Glycorex (Lund, Sweden): (Galα1-O-spacer)$_n$-HSA (H-1021), (Galα1–2Gal-O-spacer)$_n$-HSA (H-1022), (Galα1–3Galβ1-O-spacer)$_n$-BSA (B-1008), (Galα1–3GalNAcβ1-O-spacer)$_n$-HSA (H-1030), (Galα1–4Galβ1-O-spacer)$_n$-HSA (H-1026), (Galα1–3Galβ1–4GlcNAcβ-O-spacer)$_n$-BSA (B-1009), and (Galα1–3Galβ1–4GlcNAcβ1–3Galβ1–4Glc-O-spacer)$_n$-HSA (H-1025). The neoglycoproteins contained a neutral spacer molecule of approximately 10Å in length to avoid possible steric hindrance of the lectin–carbohydrate binding. The number of carbohydrate chains attached per BSA/HSA molecule, n, was in the range of 20 to 25. Melibiose (Galα1–6Glc), other monosaccharides, and laminin from basement membrane of Engelbreth-Holm-Swarm mouse sarcoma were purchased from Sigma (St Louis, MO, USA).

Solid surface phase measurements

The binding of the lectins to the glycoconjugates was determined by enzyme-linked lectin assay (ELLA). Microtiter plates (Maxisorb; Nunc, Roskilde, Denmark) were coated with glycoproteins or neoglycoproteins in amount varying from 1 to 500 ng per well in 50 mM carbonate buffer pH 9.6 and thereafter shaken overnight at 4 °C. The following day each well was washed three times with washing buffer (0.05 M PBS pH 7.2 containing 0.1% Tween 20). To block non-specific binding the wells were incubated with washing buffer containing 5% BSA for 30 min at room temperature. Before incubation with lectins the wells were washed four times with washing buffer. A total volume of 50 µl incubation solution of biotinylated lectin (2.5 µg/ml) in PBS was applied to each well. The plates were incubated with shaking for 18 h at 4 °C, or at 37 °C where indicated.

After lectin incubation the wells were washed four times with PBS to remove unbound lectin completely, and 30 µl horseradish peroxidase (HRP)-conjugated avidin (DAKO, Copenhagen, Denmark) diluted 1/4000 with washing buffer was applied to each well. After shaking for 1 h at room temperature the HRP-containing solution was discarded and the wells were thoroughly washed five times with washing buffer. For chromogenic visualization of the reaction product a tablet of *o*-phenylenediamine (DAKO) was dissolved in 0.1 M citrate buffer, pH 5.0, containing 4 µl of 25% H_2O_2 per 10 ml buffer. The enzymatic reaction was stopped with 0.5 M H_2SO_4 and the absorbance in each well was read at 490 nm using a microtiter plate reader. GraphPad Prism software (San Diego, CA, USA) was used for data analysis and graphing. Absorbance readings at 490 nm were corrected for background (non-specific) binding obtained by measuring absorbance in wells coated with non-glycosylated BSA or HSA, so values reflects specific binding of lectin to the glycosylated proteins.

The data presented are mean values of experiments performed in triplicate. The standard deviation did not exceed 10% and in most experiments was $< 5\%$ of the mean value. Error bars are therefore not shown in the graphs. The specificity of the lectins toward free saccharides was investigated as follows: Melibiose, N-acetylgalactosamine,

glucose or mannose at concentrations 0.1 to 2 mM were added to diluted solutions of lectin (0.2 μg/ml MOA or GS I-B_4). Microtiter wells coated with Galα1–3Galβ1–4GlcNAcβ-BSA were incubated with the lectin–monocarbohydrate solutions. As controls, identical wells were incubated with the lectin solutions alone.

Results

The results of the solid phase surface measurements of reaction between MOA and various neoglycoproteins are shown in Fig 1. The absorbance units read at 490 nm, corrected for non-specific binding, are referred to as specific binding. The non-specific absorbance caused by serum albumin amounted $< 5\%$ of the total absorbance. The data obtained fit saturation binding curves using non-linear regression, from which K_D and B_{max} values for the reactions between lectin and carbohydrate were calculated. B_{max} is the plateau value of absorption and thus reflects the maximum number of lectin binding sites. K_D is the variable for the surface concentration of ligand, which represents half of the maximum ligand concentration (termed BC_{50} in [16]), expressed as nanogram of glycoconjugate. This value represents a surface concentration of binding sites because the surface area in the microtiter plate wells is constant. Furthermore, variation in the molecular weight per ligand unit because of the size of the ligand and its density on the protein is no more than 20%, so that the B_{max} and K_D values for the different neoglycoconjugates are comparable. In this experiment the concentration of lectin was 2.5 μg/ml. The calculated saturation binding data were as follows:

$$(\text{Gal}\alpha 1-\text{O}-\text{spacer})_n-\text{BSA}: B_{max}=4.29; K_D=49.4\,\text{ng}$$

$$(\text{Gal}\alpha 1-3\text{Gal}\beta 1-4\text{GlcNAc}-\text{O}-\text{spacer})_n-\text{BSA}: B_{max}=4.38; K_D=69.5\,\text{ng}$$

$$(\text{Gal}\alpha 1-3\text{Gal}\beta 1-4\text{GlcNAc}\beta 1-3\text{Gal}\beta 1-4\text{Glc}-\text{O}-\text{spacer})_n-\text{HSA}: B_{max}=3.66; K_D=18.8\,\text{ng}$$

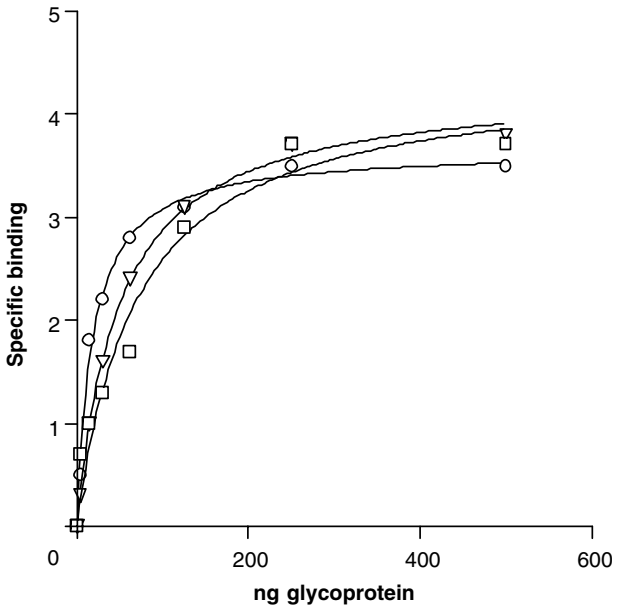

Fig. 1. Binding of MOA to neoglycoproteins. The neoglycoproteins were immobilized for 24 h at 4 °C and incubated with 50 μl of a lectin concentration of 2.5 μg/ml for 24 h at 4 °C. There was no binding of MOA to BSA or HSA alone. ∇, $(\text{Gal}\alpha 1\text{-O-spacer})_n$-BSA; □, $(\text{Gal}\alpha 1–3\text{Gal}\beta 1–4\text{GlcNAc-O-spacer})_n$-BSA; ○, $(\text{Gal}\alpha 1–3\text{Gal}\beta 1–4\text{GlcNAc}\beta 1–3\text{Gal}\beta 1–4\text{Glc-O-spacer})_n$-HSA.

To study further the affinities of MOA and GS I-B_4 toward xenoantigens of different lengths, small amounts of neoglycoproteins were incubated with lectin solutions at 4 and 37 °C as illustrated in Fig. 2. The plots show that the affinity of MOA for Galα-structures is higher with a long chain carbohydrate (the pentasaccharide) than with a short chain (the monosaccharide), whereas the reverse is true for GS I-B_4, especially at 4 °C. All affinities increase at 37 °C, and differences between mono- and pentasaccharide are much less evident.

To compare the specificities of MOA and GS I-B_4 towards disaccharides with terminal Galα, low levels (generally less than the expected K_D) of non-xenogenic disaccharide neoglycoproteins and the xenogenic disaccharide (Galα1–3Gal) were immobilized on microtiter plates and subsequently incubated with lectin for 24 h at 4 °C. The results are shown in Fig. 3A–D. The difference in the binding of GS I-B_4 and MOA to Galα1–2Gal, Galα1–3GalNAc and Galα1–4Gal is apparent; MOA recognizes these possible non-xenogenic disaccharides very poorly compared with GS I-B4 (Fig. 3A–C). When the binding affinities of MOA and GS I-B4 to the xenoreactive disaccharide (Galα1–3Gal) were compared, the two lectins showed almost identical binding curves (Fig 3D). As the amount of ligands was kept low, linear regression analyses could be used to estimate the initial slope of each lectin–carbohydrate interaction. The slope quantifies the strength of specific binding of the carbohydrate ligands attached to the neoglycoproteins. For comparison of the lectin–glycoprotein interactions the reciprocal of the slope values, which is comparable in the same sense as the K_D values, are shown in Table 1.

To determine the binding specificity of free sugars, several were used as inhibitors of lectin–neoglycoconjugate binding. The effects of melibiose

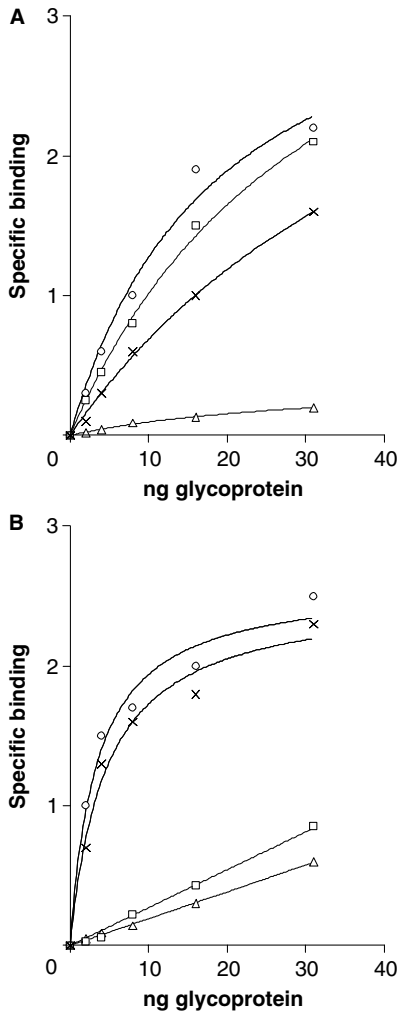

Fig. 2. The influence of incubation temperature on binding of MOA and GS I-B_4 to neoglycoproteins. The time of incubation was 24 h and the incubation temperatures were 4 °C (A) and 37 °C (B). ×, MOA and (Galα1–3Galβ1-O-spacer)$_n$-BSA; ○, MOA and (Galα1–3Galβ1–4GlcNAcβ1–3Galβ1–4Glc-O-spacer)$_n$-HSA; □, GS I-B_4 and (Galα1–3 Galβ1-O-spacer)$_n$-BSA; Δ, (Galα1–3Galβ1–4GlcNAcβ1–3Galβ1–4Glc-O-spacer)$_n$-HSA.

(Galα1–6Glc) on MOA and GS I-B_4 interaction with mono- or tri-α-galactosylated neoglycoproteins are shown in Fig. 4A–B. In each experiment, 200 ng neoglycoprotein was immobilized in all wells. Before lectin–neoglycoprotein incubation, various concentrations of melibiose were added to solutions of MOA and GS I-B_4 (2.5 μg/ml). After 30 min the lectin–melibiose mixtures were transferred to the wells and incubated for 24 h at 4 °C. It is apparent that MOA binding is very poorly inhibited by the Galα1–6Glc disaccharide, whereas GS I-B_4 is potently inhibited by this sugar. In comparable experiments, N-acetylgalactosamine, glucose or mannose had no effect on either MOA or GS I-B_4 binding to immobilized glycoproteins (data not shown).

Beside neoglycoproteins, a number of native glycoproteins display Galα1–3Gal extended glycans. One of the best characterized of these native glycoproteins is murine laminin from basement membrane of mouse sarcoma. The influence of temperature on the affinity and rate of binding of MOA and GS I-B_4 to laminin is shown in Fig. 5A,B. Figure 5A shows that after 24 h at 4 °C and at 37 °C both lectins show higher affinity for the glycoprotein at 37 °C than at 4 °C. Further, specific binding values were considerably higher when laminin was incubated with MOA than with GS I-B_4. The lectin binding curves obtained after short periods of incubation (Fig 5B) show no binding between GS I-B_4 and laminin could be detected when the incubation was performed at 4 °C.

To estimate the initial rate of lectin binding to the neoglycoprotein, we immobilized 100 ng of neoglycoprotein in each well and incubated the plates with MOA for 2 to 15 min at 4 °C and 37 °C (Fig. 6). The results indicate that at an incubation temperature of 4 °C, the rate of binding to the Galα-pentasaccharide was slow compared with the binding to the tri- and monosaccharide (solid symbols). Elevating the temperature of the incubation to 37 °C enhanced the rate of MOA binding to the pentasaccharide while binding to the monosaccharide was moderately slowed (open symbols). Interestingly, the intermediate rate of binding to the trisaccharide was unaffected by temperature.

Discussion

This work supports the contention that the *Marasmius oreades* agglutinin is an appropriate tool for detection of carbohydrate xenoantigens terminating in Galα1–3Gal. Because the xenogenic epitopes are located on the surface of vascular endothelial cells, we have used solid phase surface measurements to study the lectin binding to α-galactosylated glycoproteins, in contrast to previous studies with MOA using solution binding and precipitation measurements [14].

At present the reagents of choice to distinguish such antigens are natural human anti-Galα1–3Gal antibodies and the galactophilic lectin GS I-B_4, but neither of these appears to be able to detect all of the Galα1–3Gal extended glycoconjugates on cell surfaces. Additionally, there are reports indicating that Galα1–3Gal residues may be expressed in association with various internal sequences or in various three-dimensional arrays that differ in accessibility and avidity for immunoglobulins and the *Griffonia* lectin [17,18]. Therefore, it could be suggested that the number of sites on a cell

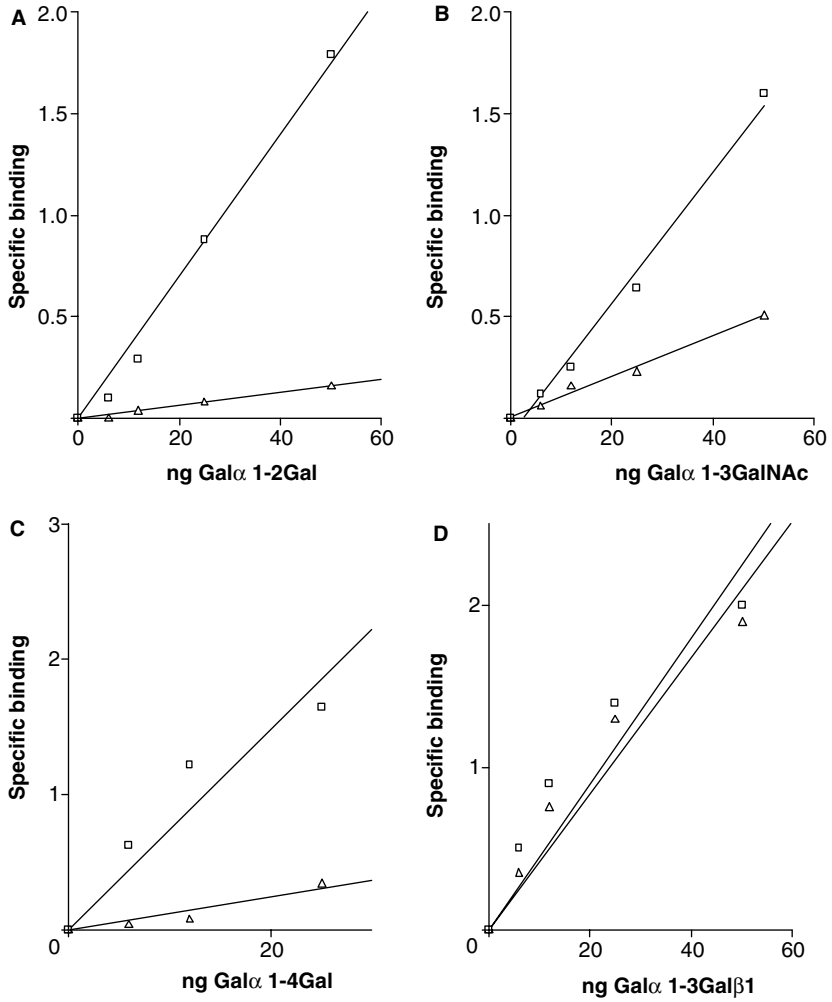

Fig. 3. (A–D) Binding of lectins to small amounts of immobilized neoglycoproteins containing dissacharides: □, GS I-B_4; Δ, MOA; conditions are as in Fig. 1.

Table 1. Lectin–carbohydrate interactions showing the reciprocal of the slope values

	1/Slope	
Carbohydrate	MOA	GS I-B_4
Galα1–2Gal	298	27
Galα1–3GalNAc	100	31
Galα1–4Gal	93	26
Galα1–3Galβ1	24	22

recognized by GS I-B_4 may not correspond to the number of sites recognized by anti-Galα1–3Gal immunoglobulins. In a study on recognition of Galα1–3Gal residues on porcine endothelial cell surfaces, Lin and colleagues [19] noted that a fraction of Galα1–3Gal residues bound by anti-Galα1–3Gal IgM was not recognized by GS I-B_4 and that some residues bound by GS I-B_4 were not bound by anti-Galα1–3Gal IgM.

Another drawback for the use of xenoreactive antibodies and GS I-B_4 lectin in transplantation research could be a lack of specificity, as they may bind to determinants other than Galα1–3Gal-R. Galili and colleagues [20] have shown that human anti-Galα1–3Gal antibodies interact with senescent human erythrocytes. As the human α1,3-galactosyltransferase genes are pseudogenes [21] human cells do not have Galα1–3Gal-R on their surface. The reaction between the antibody and human red blood cells thus indicate that xenoreactive natural antibodies may be polyreactive. However, it could also be that senescent human erythrocytes may express B-like antigen as a result of the exposure of cryptic antigens exposed during the process of normal aging [22].

GS I-B_4 is the only lectin that has been used extensively to detect Galα1-antigens, but like the anti-Gal antibodies the lectin lacks specificity for extended α-galactosylated carbohydrates. It reacts

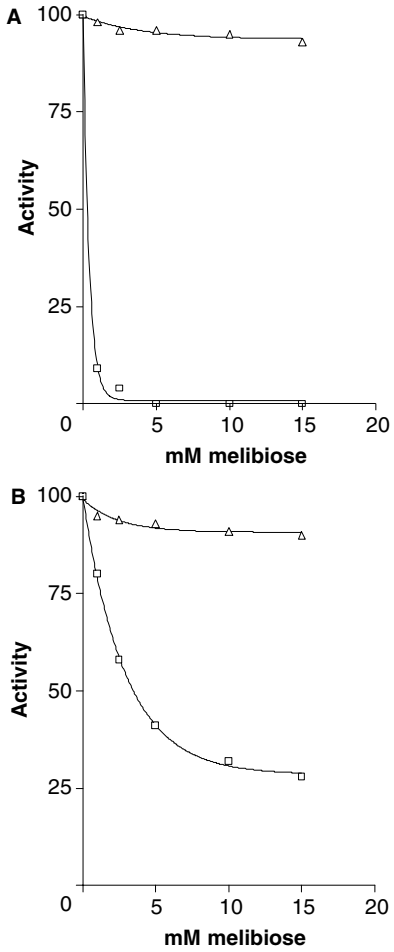

Fig. 4. (A–B) Melibiose inhibition on lectin-neoglycoprotein interaction. The *y*-axes shows remaining activity (%). The *x*-axes shows concentration of melibiose added to the lectin incubation medium. The immobilized neoglycoproteins were (Galα1-O-spacer)$_n$-BSA in (A) and (Galα1–3Gal–4GlcNAc-O-spacer)$_n$-HSA in (B). □, GS I-B$_4$; Δ, MOA.

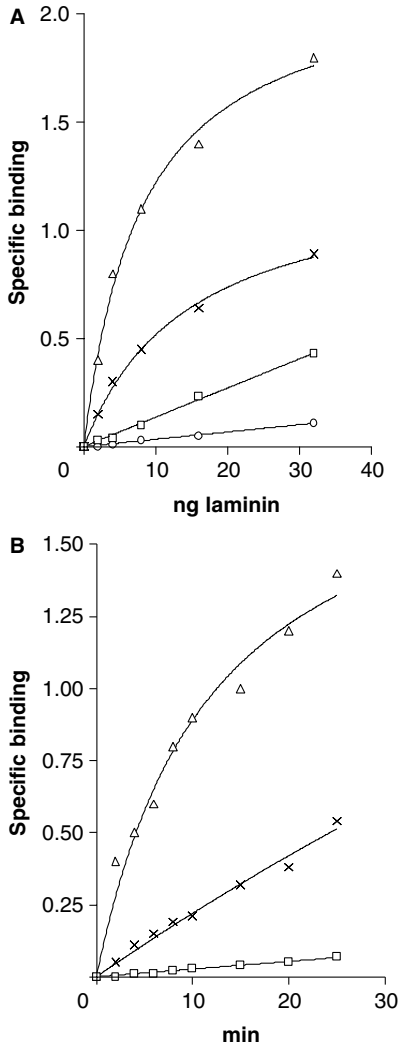

Fig. 5. (A) Measurements of the interaction between lectin and small concentrations of mouse laminin after incubation for 24 h at different temperatures. Δ, MOA 37 °C; ×, MOA 4 °C; □, GS1 B$_4$ 37 °C; ○, GS I-B$_4$ 4 °C. (B) Measurements of the interaction between lectin and a fixed amount of immobilized laminin (100 ng). Incubation time 0 to 25 min. Δ, MOA 37 °C; ×, MOA 4 °C; □, GS-B$_4$ 37 °C.

with terminal Galα1 [23] but seems not to have an extended specificity for the Galα1–3 xenoantigens [24] as it detects α1–2, α1–3, α1–4 galactobioses, and Galα1–6Glc with high affinity [16]. A marker more specific than GS I-B$_4$ for xenoreactive carbohydrate antigens would therefore be desirable. Recently, a blood group B-specific agglutinin from the mushroom *Marasmius oreades* (MOA) was isolated and characterized. Solution phase carbohydrate binding studies indicated that MOA had an extended binding site that accommodated the Galα1–3Gal disaccharide specifically [14].

In future pig-to-man xenotransplantation it will be necessary to be able to decide the presence of possible xenogenic carbohydrate antigens in the tissues from the donor pigs. To choose a suitable lectin for this purpose we have here compared the binding of the two lectins MOA and GS I-B$_4$ to chemically modified proteins that bear well-defined α-galactosylated derivatives. Lectin binding to a native, well-characterized glycoprotein that contains numerous Galα1–3Gal moieties was also investigated. In a study on lectin binding to α-galactosylated neoglycoproteins using ELLA we previously showed that GS I-B$_4$ had higher affinity for the monosaccharide (Galα1) than for the xenogenic pentasaccharide (Galα1–3Galβ1–4GlcNAcβ1–3Galβ1–4Glc) [8] while the results of the present study indicate that MOA has higher affinity for the pentasaccharide than for the Galα monosaccharide.

The interaction between lectin and disaccharide-coupled neoglycoproteins with a αGal end group, linked 1–2 and 1–4 to Gal, or 1–3 to GalNAc

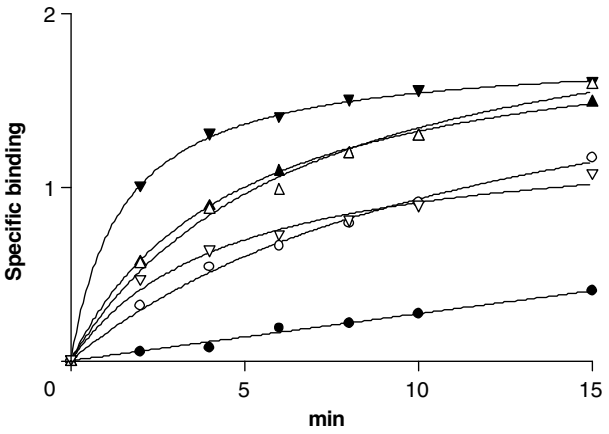

Fig. 6. Binding curves obtained after 2 to 15 min incubation of MOA with neoglycoproteins at 4 °C (solid symbols) and 37 °C (open symbols). ▽, (Gal1-O-spacer)$_n$-BSA; △, (Galα1–3Galβ1–4GlcNAc-O-spacer)$_n$-BSA; ○, (Galα1–3Galβ1–4GlcNAc1–3Galβ1–4Glc-O-spacer)$_n$-HSA.

(Fig. 3) showed that MOA bound very weakly whereas GS I-B$_4$ recognized all the above disaccharides with high avidity. These results agree with precipitation measurements of MOA by glycoconjugates performed by Winter and colleagues [14]. The binding properties of MOA to the xenogenic trisaccharide were comparable with this ligand binding of GS I-B$_4$. Furthermore, MOA showed high affinity to immobilized mouse sarcoma laminin which contains multiple Galα1–3Gal1–4GlcNAcβ1 glycan moieties. Therefore, it may be concluded that MOA has at least as great an affinity for the xenogenic carbohydrate antigens as does GS I-B$_4$, but MOA is far more specific in its binding preferences.

A significant and telling difference between GS I-B$_4$ isolectin and MOA is illustrated in Fig. 4. Panel A shows that melibiose (Galα1–6Gal) inhibits the binding of GS I-B$_4$ to (Galα1-O-spacer)$_n$-BSA at a concentration < 2 mM, whereas melibiose at 15 mM shows barely discernable inhibition of MOA binding to this neoglycoprotein. This observation epitomizes the difference between the two lectins: GS I-B$_4$ with a limited and shallow binding site recognizes any oligosaccharide having a terminal α-Gal residue but MOA has an extended binding site requirement for Galα1–3Gal- end groups so it does not recognize melibiose [14]. It is also interesting to note (Fig. 4B) that binding of GS I-B$_4$ to the trisaccharide (Galα1–3Gal–4GlcNAc-O-spacer)$_n$-HSA requires a higher concentration of melibiose for inhibition. It is possible that the trisaccharide presents additional, although not specific, molecular contacts for binding the lectin, or more likely that a more favorable clustering or presentation of carbohydrate ligands occurs. In a study on hepatic carbohydrate receptors, Lee [25] likewise noted that clustering of target sugars in proper geometric arrangement enhanced the binding of these proteins.

The effect of temperature on the rate and equilibrium binding of MOA to various sizes of α-Gal oligosaccharides provides further insight into MOA binding. The low initial rate of binding of MOA to the pentasaccharide surface is particularly striking, and suggests that binding occurs to a specific conformation of the pentasaccharide, which is in low abundance at 4 °C. Because the equilibrium binding constant, as indicated in Fig. 1, is higher for the pentasaccharide, the dissociation from the pentasaccharide must be very slow relative to that from the monosaccharide. At 37 °C, faster conformational shifts lead to a significantly faster rate of binding, but still with a very slow dissociation rate leading to an increase of equilibrium binding at the higher temperature (Fig. 2). Hydrophobic interactions, which are strengthened at higher temperature, might also possibly play a role in greater affinity at higher temperature. The opposite effect of temperature on the initial rate of MOA binding to the monosaccharide-containing ligand indicates that, as expected, conformational freedom is less of an issue, and that hydrogen bonding may play the larger role. Initial rate of binding of MOA to the trisaccharide appears largely unaffected by temperature, suggesting a balance between these opposing effects in the intermediate structure. In the case of GS I-B$_4$, equilibrium binding of the disaccharide structure is diminished at the higher temperature (Fig. 2), indicating that the interactions involved in its binding to and dissociation from this small, relatively constrained structure, are different than those of MOA, which shows increased equilibrium binding of both mono- and oligosaccharides at the higher temperature. In the case of the naturally-occurring glycoprotein laminin, which contains Galα-trisaccharide structures, equilibrium binding is favored at higher temperatures by both MOA and GS I-B4, suggesting that flexibility of conformation and/or hydrophobic interactions play a significant role in both lectins binding interactions.

Shinohara and colleagues [26] noted that the affinities between lectins and carbohydrates could be different in fluid phase and solid phase surface measurements. However, a comparison between the fluid phase measurements on MOA binding properties as shown by Winter and colleagues [14] and the results of the present study obtained by solid phase surface measurements shows a high degree of concordance. We have previously shown

that fluorescein-labeled MOA-stained endothelial cells lining the capillaries in sections of porcine skeletal muscle [14]. As porcine endothelial cells possesses abundant α-galactosylated antigens it may be concluded that MOA shows a specific binding to Galα-epitopes located on a surface such as cell membranes.

Conclusion

MOA shows a high affinity to the various xenogenic Galα1–3Gal-R epitopes (di-, tri-, and pentasaccharides) both when the glycans are immobilized and in solution. It has low affinity towards other Galα epitopes such as Galα1–2Gal, Ga1α1–4Gal, Galα1–6Glc, and Galα1–3GalNAc. The lectin could therefore serve as a powerful tool for detection of xenogenic carbohydrate antigens for laboratory examinations in the preclinical and further clinical pig-to-man xenotransplantation.

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