An Immunochemical Study of the Combining Sites of the Second Lectin Isolated from *Bandeiraea simplicifolia* (BS II)

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An Immunochemical Study of the Combining Sites of the Second Lectin Isolated from *Bandeiraea simplicifolia* (BS II)

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**INTRODUCTION**

Two lectins with different binding specificities have been isolated from extracts of seeds of *Bandeiraea simplicifolia*. The first, *Bandeiraea* lectin I [11] was specific for terminal non-reducing αDGalactosyl residues. It reacted with B substances from human ovarian cysts and with several galactomannans to form precipitin lines in agar gels. Polysaccharides with terminal αDGalactosyl residues, such as larch galactan, did not react. The lectin agglutinated B erythrocytes strongly but also reacted to a lower titre with A₁ and very weakly with A₂ erythrocytes [15, 28] indicating that terminal non-reducing αDGalNAc [24] can be accommodated in the-site to some extent. Recently, it was shown that *B. simplicifolia* lectin I (BS I) consists of five isolectins each of which is a tetrameric glycoprotein composed of A and B subunits; the A subunits are specific for αDGalNAc, the B subunits for αDGal [30].

The second lectin, *Bandeiraea* lectin II (BS II), isolated by affinity chromatography on chitin [13], is a glycoprotein (molecular weight 113,000) of four subunits of molecular weight 30,000. It does not agglutinate A, 13 or () erythrocytes. Quantitative precipitin assays showed it to react better with BSA conjugated to p-azophenyl αDGalNAc than with the β compound. In inhibition studies, the unusual observation was made that N,N'-diacetylchitobiose (DGlcNAcβ1 → 4DGlcNAc) and pNO₂ phenyl αDGalNAc were highly active; methyl αDGalNAc was only one half as active but was eight times more active than methyl βDGlcNAc.

The variety of blood group substances and oligosaccharides available made it possible to obtain more information about the combining site of BS II by quantitative immunochemical methods [16, 17, 20]. The lectin precipitated with various blood group substances, polysaccharide and glycoproteins with terminal non-reducing α and β-linked DGlcNAc but to different extents. Assays by inhibition of the precipitin reaction using various oligosaccharides and glycosides showed that
only the terminal DglcNAc and part of the second sugar contribute significantly to the binding specificity. The lectin is most specific for terminal reducing α-linked DglcNAc. It is unusual that certain disaccharides with β linkages react as well or better than others with α linkages; \( p\text{NO}_2 \) phenyl αDglcNAc, phenyl αDglcNAc and DglcNAc\( \alpha l \rightarrow 5\text{Dglc} \) were the best inhibitors. Molecular models account for these findings; overall shape and contour of the molecule and hydrophobic bonds are the decisive factors in binding.

EXPERIMENTAL

Materials.

BS II was purified from \( B.\) simplicifolia seeds [13]. The following blood group substances were used: hog gastric mucin A + H [4] with blood group A and H activity; B substances, Beach \( \text{ØOH} \) insoluble [2] and horse 4 25% [5]; \( A_1 \) MSS 10% 2X, \( A_2 \) WG \( \text{ØOH} \) insoluble [19]; H, Tighe \( \text{ØOH} \) insoluble [6] and JS \( \text{ØOH} \) insoluble [35]. JS 1st and \( 2^{nd} \text{IO}_4^-/\text{BH}_4^- \) were obtained after two sequential stages of periodate oxidation and Smith degradation [23]. Le\( ^a \) active cyst material (N-l \( \text{ØOH} \) insoluble) was described earlier [24]. The Tij fractions with B, I-MA and I-Step activity were those studied by Maisonrouge-McAuliffe and Kabat [26]. Fractions of precursor blood group substance OG were described by Vicari and Kabat [37]. Carcinoembryonic antigen (CEA) was provided by Dr. P. Gold [9], agalactoosomucoid by Dr. G. Ashwell [31] and a synthetic antigen (antigen A) DglcNAc\( \beta 1 \rightarrow 4 \text{DglcNAc}\beta 1 \rightarrow \text{N-polyAsn} \) by Dr. T. W. Shier [36]. The blood group oligosaccharides used were isolated and characterized previously [8, 22, 27], N,N'-diacetylcitobiose and N,N',N''-triacetylcitotriose were from Dr. N. Sharon [1], N,N',N''-tetraacetylcitotetraose was described previously [10]; DglcNAc\( \beta 1 \rightarrow 3\text{Dgal} \), DglcNAc\( \beta 1 \rightarrow 6\text{Dgal} \) and DglcNAc\( \beta 1 \rightarrow 3\text{DglcNAc}\beta 1 \rightarrow 6\text{Dgal} \) were from Dr. Z. Yosizawa [40]; DglcNAc\( \alpha l \rightarrow 5\text{Dglc} \) was from Dr. van Heeswijk. Monosaccharides were obtained commercially (Nutritional Biochemicals [12] Corp. and Schwartz/Mann Research Laboratories).

Immunchemical methods.

Quantitative precipitin and inhibition assays were by the quantitative microprecipitin technique [20] in a final volume of 200 μl; 5.6 μg of lectin nitrogen was used in each assay unless otherwise stated. The tubes were incubated at 37°C for 1 h then kept at 4°C for one week with mixing twice daily.

\[
\begin{align*}
\text{Dglc} &= \text{DGlucopyranose.} \\
\text{Dglc/} &= \text{DGlucofuranose.} \\
\text{Dgal} &= \text{DGalactopyranose.} \\
\text{DglcNH}_2 &= 2\text{-amino-2-deoxy-}\text{-D-glucopyranose.} \\
\text{DglcNAc} &= 2\text{-acetamido-2-deoxy-}\text{-D-glucopyranose.} \\
\text{DgalNAc} &= 2\text{-acetamido-2-deoxy-}\text{-D-galactopyranose.} \\
\text{DmanNAc} &= 2\text{-acetamido-2-deoxy-}\text{-D-mannopyranose.}
\end{align*}
\]
The precipitates were centrifuged, washed, and total nitrogen determined by the ninhydrin method [35].

RESULTS

Quantitative precipitin assays.

The lectin varied substantially in its capacity to precipitate with various blood group substances. Reactions with H substances are shown in figure 1A, hog mucin A + H precipitated 94% of the lectin N added with 4 μg giving 50% precipitation; human ovarian cyst JS ØOH insoluble reacted less well, 13 μg being needed for 50% precipitation. However, the first stage of periodate oxidation and Smith degradation, JS IΟ₄/ΒΗ₄ 1st stage, was more active than JS ØOH insoluble and almost as active as hog mucin A + H precipitating 4.8 μg of lectin N with 4 μg giving 50% precipitation. JS IΟ₄/ΒΗ₄ 2nd stage was inactive, 21 μg precipitating only 0.3 μg of N. Cyst Tighe ØOH insoluble, another H substance was more active than JS ØOH insoluble but not as potent as JS IΟ₄/ΒΗ₄ 1st stage or hog mucin A + H with 9 μg giving 50% precipitation.

The activities of two fractions from ovarian cyst fluid, Tij [26] are also given in figure 1A. Tij 10% 2X with high B3 and I Ma activity requiring 10% ethanol for precipitation from phenol was relatively inactive while Tij 20% 2X, precipitating at 20% ethanol from phenol, with low B but reacting strongly with anti-I Step, anti-i Den and conA, was highly active, 50% precipitation of BS II requiring

![Quantitative precipitation curves of BS II with various blood group substances and glycoproteins.](image-url)
only 5μg. OG 10% 2X, a precursor blood group fraction with I and i activity, did not react while another, OG 20% 2X, showed weak activity. Only one point of OG 20% 2X was used because of the limited amount available. The lectin did not react with an A1 substance from human ovarian cyst MSS 10% 2X nor with an A2 substance WG OOH insoluble from human saliva (Figure 1B). It reacted with various B substances to different extents (Figure 1B); with horse 4 25%, almost all the added lectin was precipitated, 3 μg giving 50% inhibition, while a human ovarian cyst B substance, Beach OOH insoluble, was almost completely inactive. N-I OOH insoluble, a Leα active cyst substance, showed no activity.

Of two preparations of a synthetic antigen A, D\text{GlcNAc}β1 \rightarrow 4\text{DGlcnAc}β1 \rightarrow N\text{-poly (Asn)} (Figure 1B), one reacted well giving 4.3 μg of specific precipitate N while the other preparation was inactive. These two antigen A samples showed the same differences with Aaptos lectin II [7]. Agalactoorosomucoid [31], prepared from the asialoglycoprotein by removing the terminal DGal enzymatically to expose multiple terminal non-reducing \text{DGlcnAc}β1 \rightarrow 4 residues, was highly potent, precipitating all 5 μg N of the added lectin and was about half as active as horse 4 25%. CEA which lacks terminal non-reducing \text{DGlcnAc} did not react with the lectin.

Quantitative precipitin inhibition assays.

Various sugars and blood group oligosaccharides were tested for their ability to inhibit the precipitin reaction between BS II and B substance horse 4 25% (Figure 2). Of the monosaccharides tested 220 nmoles of \text{DGlcNAc} gave 50% inhibition while 450 nmoles of \text{DGal}, 1,000 nmoles of \text{DManNAc}, 640 nmoles of \text{DGal}, 770 nmoles of \text{DGlc} and 300 nmoles of \text{DGlcNH}_2 gave 0, 18, 16, 11 and 10 percent inhibition respectively (Figure 2B).

Methyl α\text{DGlcNAc} was more active than \text{DGlcNAc}; 110 nmoles gave 50% inhibition while 880 nmoles of methyl β\text{DGlcNAc} only inhibited to 35%; p\text{NO}_2 phenyl α\text{DGlcNAc} and phenyl α\text{DGlcNAc} were the most potent inhibitors, both being 3, 6 and 20 times better than methyl α\text{DGlcNAc}, \text{DGlcNAc} and p\text{NO}_2 phenyl β\text{DGlcNAc} respectively; p\text{NO}_2 phenyl α\text{DGlc} showed no inhibition with 310 nmoles; N,N’-diacetylchitobiose, N,N’N’-triacetylchitotriose and N,N’,N”-tetraacetylchitotetraose were of equal potency and as active as methyl α\text{DGlcNAc}.

Of the di- and trisaccharides tested, those with terminal non-reducing β-linked \text{DGlcNAc} were relatively inactive (Figure 2B); \text{DGlcNAc}β1 \rightarrow 3\text{DGal}, \text{DGlcNAc}β1 \rightarrow 6\text{DGal}, \text{DGlcNAc}β1 \rightarrow 3[\text{DGlcNAc}β1 \rightarrow 6]\text{DGal} [40] and the blood group oligosaccharides, \text{DGlcNAc}β1 \rightarrow 6\text{DGal} (R_L 0.95) [8] and \text{DGlcNAc}β1 \rightarrow 3\text{DGalactitol} [7] all were of similar activities with 600 nmoles inhibiting not more than 25%; higher concentrations of these inhibitors were not used because of the limited amounts available. A disaccharide and a trisaccharide with terminal non-reducing α-linked \text{DGlcNAc} were also tested (Figure 2A); 140 nmoles of \text{DGlcNAca}1 \rightarrow 4\text{DGal} (R_L 1.53) [8] gave 50% inhibition, being 1.8 time as active as \text{DGlcNAc} but
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only about 80% as active as methyl α-DGlcnAc or the chitin oligosaccharides. DGlcnAcα1 → 4DGalβ1 → 3DGalNAc (RL 0.97a) [8] was like pNO2 phenyl βDGlcnAc with 190 nmoles inhibiting only 30%. The reduced oligosaccharide DGlcnACac1 → 4DGalβ1 → 3DGalNAc (RL 0.42) [25] was only as active as oligosaccharides with terminal β-linked DGlcnAc, 430 nmoles inhibiting only 24%.

DISCUSSION

BS II, while specific for terminal non-reducing DGlcnAc [13], was unusual in that disaccharides with both α and β linkages were good inhibitors. Indeed, the most active compounds in inhibiting precipitation of BS II by βDGlcnAc-BSA
[13] were $\text{DGlcNAc}\beta_1 \rightarrow 4\text{DGlcNAc}$, $p\text{NO}_2$ phenyl $\alpha\text{DGlcNAc}$ and methyl $\alpha\text{DGlcNAc}$.

This study generally confirms these observations and provides a structural basis which accounts for the activity of both $\alpha$ and $\beta$ compounds. Some quantitative differences in inhibiting power were found using BS II and horse 4 25% as the precipitating system; $p\text{NO}_2$ phenyl $\alpha\text{DGlcNAc}$ and phenyl $\alpha\text{DGlcNAc}$ (Figure 2A) were the best inhibitors, being equal and about 6 and 20 times better than $\text{DGlcNAc}$ and their $\beta$ anomers. Earlier studies with conA [33], Sophora japonica [34] and peanut agglutinin [35] have shown the $p\text{NO}_2$ phenyl glycosides to be better inhibitors than the methyl glycosides of the same anomeric conformation, indicating hydrophobic interactions between the phenyl ring and the combining sites [18]; phenyl glycosides were not tested. Since with BS II, $p\text{NO}_2$ phenyl $\alpha\text{DGlcNAc}$ and phenyl $\alpha\text{DGlcNAc}$ were of equal potency, the $\text{NO}_2$ group does not contribute. Molecular models were constructed in an attempt to account for differences in reactivity of the phenyl $\alpha$ and $\beta$ $\text{DGlcNAc}$ (Figure 3A); when the models were placed in similar conformations the only apparent difference is the angle of the phenyl ring relative to the $\text{DGlcNAc}$. For phenyl $\beta\text{DGlcNAc}$ the ring is at an angle to the plane of the sugar while in the $\alpha$ anomer it is in the same plane and the molecule is relatively flat. This could be of importance for interaction, and the angle of the $\beta$ compound might sterically hinder reaction in the binding site. Methyl $\alpha\text{DGlcNAc}$ was much more active than methyl $\beta\text{DGlcNAc}$ but only about 1/3 as active as phenyl $\alpha\text{DGlcNAc}$, and models (Figure 3D) show the methyl group in the $\beta$ anomer to be at a slight angle to the $\text{DGlcNAc}$ while in the $\alpha$ compound the molecule is flatter.

Of the free sugars tested only $\text{DGlcNAc}$ showed considerable activity. $\text{DGlc}$, $\text{DGlcNH}_2$, $\text{DGalNAc}$ and $\text{DManNAc}$ were almost completely inactive. Thus an equatorial N-acetamido group at C2 and the $\text{DGlc}$ conformation are required as noted earlier [13]. Disaccharides of $\text{DGlcNAc}$ linked $\beta_1 \rightarrow 3$ or $\beta_1 \rightarrow 6$ to $\text{DGal}$ and $\text{DGlcNAc}\beta_1 \rightarrow 3[\text{DGlcNAc}\beta_1 \rightarrow 6]\text{DGal}$ were inactive. The disaccharide $\text{DGlcNAc}\alpha_1 \rightarrow 4\text{DGal}$ was highly active but only 80% as active as methyl $\alpha\text{DGlcNAc}$. Reduction of $\text{DGlcNAc}\alpha_1 \rightarrow 4\text{DGal}$ to $\text{DGlcNAc}\alpha_1 \rightarrow 4\text{DGalactitol}$ reduced its activity to about that of $\beta\text{DGlcNAc}$; thus opening the ring to give galactitol interferes with binding of the lectin to the terminal non-reducing $\alpha\text{DGlcNAc}$.

$\text{N,N',N''-diacetylchitobiose}$, $\text{N,N',N''-triacetylchitotriose}$ and $\text{N,N',N''-tetraacetylchitotetraose}$ were all equal in potency to methyl $\alpha\text{DGlcNAc}$ and 1.25 time better than $\text{DGlcNAc}\alpha_1 \rightarrow 4\text{DGal}$. Molecular models of $\text{N,N'-diacetylchitobiose}$ ($\text{DGlcNAc}\beta_1 \rightarrow 4\text{DGlcNAc}$) and $\text{DGlcNAc}\alpha_1 \rightarrow 4\text{DGal}$ were constructed (Figure 3C) and the similar regions outlined by a length of polyethylene tubing. For these two structures, portions below and to the left of the polyethylene tubing are quite similar (Figure 3C). These include the non-reducing $\text{DGlcNAc}$ and the $\text{CH}_2\text{OH}$ of the subterminal residue. The side view shows the most striking difference; the molecule of $\text{N,N'-diacetylchitobiose}$ is flat like phenyl $\alpha\text{DGlcNAc}$ while in $\text{DGlcNAc}\alpha_1 \rightarrow 4\text{DGal}$ the two are at an angle as in phenyl $\beta\text{DGlcNAc}$ (Figure 3A).
Addition of a third sugar to $\text{DGlcNac}_\alpha \rightarrow 4 \text{DGal}$ to give $\text{DGlcNac}_\alpha \rightarrow 4 \text{DGal}_\beta \rightarrow 4 \text{DGlcNac}$ increased its activity to that of $\text{DGlcNac}_\beta \rightarrow 4 \text{DGlcNac}$ and methyl $\alpha \text{DGlcNac}$. Molecular models of $\text{DGlcNac}_\alpha \rightarrow 4 \text{DGal}_\beta \rightarrow 4 \text{DGlcNac}$ (Figure 3E) showed striking similarities to $\text{DGlcNac}_\beta \rightarrow 4 \text{DGlcNac}$; the model of the trisaccharides can assume conformation, if the $\text{DGal}$ residue is rotated so that it is perpendicular to the two $\text{DGlcNac}$ residues, such that the molecule is shortened with $\text{CH}_2\text{OH}$ group of the reducing $\text{DGlcNac}$ coming into close contact with N-acetamido group of the terminal non-reducing $\text{DGlcNac}$ and aligning the two $\text{DGlcNac}$ residues in the same plane as in $\text{DGlcNac}_\beta \rightarrow 4 \text{DGlcNac}$ (Figure 3C). The polyethylene tubing outlines the regions of between these molecules.

$\text{DGlcNac}_\alpha \rightarrow 5 \text{DGlc}$ was as good as $p\text{NO}_2 \text{phenyl } \alpha \text{DGlcNac}$ and phenyl $\alpha \text{DGlcNac}$ and better than the other oligosaccharides tested. Its activity may be accounted for by the reduced ring size of the second residue. Molecular models (Figure 3B) showed a general flatness, and the residue with its furanose ring is of comparable size to the phenyl of phenyl $\alpha \text{DGlcNac}$ and permits increased adaptability in the site. Interactions may also be involved; there is an H at C5 beneath furanose ring of $\text{DGlcNac}_\alpha \rightarrow 5 \text{DGlc}/$ (not seen in models) which could contribute to the interaction; such an H can be found in similar positions in all active compounds, H at C4 in the reducing $\text{DGlcNac}$ of $\text{DGlcNac}_\beta \rightarrow 4 \text{DGlcNac}$ and $\text{DGlcNac}_\alpha \rightarrow 4 \text{DGal}_\beta \rightarrow 4 \text{DGlcNac}$ in the conformations shown in Figure 3C and E. For $\text{DGlcNac}_\alpha \rightarrow 4 \text{DGal}$, the H underneath the pyranose ring of the $\text{DGal}$ is not in the same position because of the angle of the $\text{DGal}$ to the $\text{DGlcNac}$ and this might prevent it from contacting in the binding site (Figure 3C). Similarly, $\text{DGlcNac}_\alpha \rightarrow 4 \text{DGal}_\beta \rightarrow 3 \text{DGalNac}$ which showed greatly reduced activity (Figure 2B) as compared with $\text{DGlcNac}_\alpha \rightarrow 4 \text{DGal}_\beta \rightarrow 4 \text{DGlcNac}$ may assume a conformation similar to it (Figure 3F) but the $\text{CH}_2\text{OH}$ of the reducing $\text{DGalNac}$ at a slightly different angle to the terminal non-reducing $\text{DGlcNac}$ and is not in as close contact with N-acetamido group of $\text{DGlcNac}$. An axial OH group is present at C4 of the reducing $\text{DGalNac}$ instead of the H in $\text{DGlcNac}$, and this inhibits sterically and prevents the molecule from assuming a flat conformation. This OH is indicated by an arrow in the bottom view of the compound in figure 3G. The absence of the H at this position may further reduce hydrophobic interaction in the site and account for the greatly reduced activity of the $\text{DGalNac}$ containing trisaccharide. The axial H in the comparable position of $\text{DGlcNac}_\alpha \rightarrow 4 \text{DGal}_\beta \rightarrow 4 \text{DGlcNac}$ is shown by the arrow in figure 3H. In methyl $\alpha \text{DGlcNac}$ an H of the $\text{CH}_2$ is at the same position as the H at C5 of $\text{DGlcNac}_\alpha \rightarrow 5 \text{DGlc}$ and the axial H on C4 of the reducing $\text{DGlcNac}$ in N,N’-diacetylchitobiose. This hydrophobic interaction could explain methyl $\alpha \text{DGlcNac}$ is as active as $\text{DGlcNac}_\beta \rightarrow 4 \text{DGlcNac}$ but inhibited less strongly than phenyl $\alpha \text{DGlcNac}$ with which additional or stronger hydrophobic interactions could be involved.
The findings by inhibition assays that the lectin can react with terminal $\alpha$- and $\beta$-linked $\text{D}\text{GlcNAc}$ account for the precipitin data in Figure 1. Thus the lectin is not blood group specific since A, B, H, Le$^a$, Le$^b$ and I activities do not involve terminal non-reducing $\text{D}\text{GlcNAc}$, and it does not react with A$_1$, A$_2$ and Le$^a$ substances. The reactions with B and H substances are due to the extensively documented [24, 37, 39] heterogeneity of these materials. The presence of terminal non-reducing $\alpha$-linked $\text{D}\text{GlcNAc}$ in hog mucin A + H was established by isolation of $\text{D}\text{GlcNAc}\alpha \rightarrow 4\text{DGalactitol}$ [25] and subsequently of $\text{D}\text{GlcNAc}\alpha \rightarrow 4\text{DGal}$ [8]; individuals immunized with hog mucin A + H produced antibody specific for terminal non-reducing $\alpha$-linked $\text{D}\text{GlcNAc}$ [29]; terminal non-reducing $\alpha\text{D}\text{GlcNAc}$ is also responsible for precipitation with conA [25] and in this study with BS II. Isolation of penta- and hexasaccharides with terminal non-reducing $\text{D}\text{GlcNAc}$ linked $\alpha$ and $\beta$1 $\rightarrow$ 4 from intact hog H substance linings by alkaline borohydride degradation [21] could also explain the reaction of BS II with various H substances. The high activity of Tij 20% 2X is also accounted for by the isolation of oligosaccharides with terminal non-reducing $\text{D}\text{GlcNAc}$ linked $\alpha$1 $\rightarrow$ 4 [27]. Similarly, the activity of JS $\text{OOH}$ insoluble and Tighe $\text{OOH}$ insoluble may be due to the heterogeneity of these substances with exposed terminal non-reducing $\beta\text{D}\text{GlcNAc}$. A mouse IgA myeloma immunoglobulin with specificity for terminal $\beta$-linked $\text{D}\text{GlcNAc}$ [38] reacted with JS $\text{IO}_4/\text{BH}_4$ 1st stage which has terminal $\beta$-linked $\text{D}\text{GlcNAc}$ while JS $\text{IO}_4/\text{BH}_4$ 2nd stage, obtained by removing the terminal $\text{D}\text{GlcNAc}$, did not react, findings identical to those with BS II (Figure 1B). Since BS II has high inhibiting activity for oligosaccharides of $\text{D}\text{GlcNAc}$ linked $\beta$ $\rightarrow$ 4 and not for those linked $\beta$1 $\rightarrow$ 4 or $\beta$1 $\rightarrow$ 6, this might suggest that some $\beta$1 $\rightarrow$ 4 linked $\text{D}\text{GlcNAc}$ residues could be present in JS $\text{IO}_4/\text{BH}_4$ 1st stage. Aston et al. [3] had isolated $\text{DGal}\beta$1 $\rightarrow$ 3 $\text{DGlcNAc}\beta$1 $\rightarrow$ 4$\text{DGal}$ from ovarian cyst H substance which would, on $\text{IO}_4/\text{BH}_4$ degradation, yield a $\text{DGlcNAc}\beta$1 $\rightarrow$ 4 terminal residue. However, despite the weak reactions of BS II with $\beta$1 $\rightarrow$ 3 or $\beta$1 $\rightarrow$ 6 linked oligosaccharides of $\text{DGlcNAc}$, the multivalence of the $\text{IO}_4/\text{BH}_4$ 1st stage with respect to terminal non-reducing $\text{DGlcNAc}$ might also lead to precipitation without the linkage being $\beta$1 $\rightarrow$ 4. Of the B substances tested, horse 4 25% is highly active as observed for $\text{Aapto}\text{sc lectin I}$ [7] which is very specific for terminal $\beta$ $\text{D}\text{GlcNAc}$. However, another B substance, Beach $\text{OOH}$ insoluble which reacted with $\text{Aapto}\text{sc lectin I}$, did not react with BS II, again demonstrating heterogeneity of individual blood group substances. Agalactoorosomucoid having multiple terminal non-reducing $\beta$-linked $\text{D}\text{GlcNAc}$ [14] subsequently shown (G. Ashwell, personal communication) to be $\beta$1 $\rightarrow$ 4, and one sample of antigen A ($\text{DGlcNAc}\beta$1 $\rightarrow$ 4$\text{DGlcNAc}\beta$1 $\rightarrow$ N-poly Asn) reacted very strongly with BS II.

The findings with molecular models appear to account for the unusual behavior of BS II in reacting with certain compounds containing $\text{DGlcNAc}$ linked $\alpha$1 $\rightarrow$ 4 and $\beta$1 $\rightarrow$ 4. While it is essentially specific for terminal non-reducing $\alpha$-linked $\text{D}\text{GlcNAc}$, it can also react with an oligosaccharide with terminal non-reducing
DGlcNAc linked β1 → 4 if the oligosaccharide can assume a conformation similar to that of the α anomer (Figure 3D). The binding site requires a terminal DGlcNAc plus a second sugar ring; hydrophobic forces are probably involved in the subsite at which the second sugar reacts, and an axial H adjacent to the glycosidic bond as in methyl αDGlcNAc, on C4 of DGlcNAcβ1 → 4DGlcNAc or C5 of DGlcNAcα1 → 5DGlcNAc and the second sugar or aglycones giving the best inhibition fit (Figure 3).

The finding that a particular conformation of the trisaccharide DGlcNAcα1 → 4DGalβ1 → 4DGlcNAc could mimic that of the disaccharide DGlcNAcβ1 → 4DGlcNAc and could account for their similar activities provides an entirely new perspective to the problem of elucidating the structures of specific receptor sites. It has usually been accepted that carbohydrate determinants are sequential [17, 20] and that even though they could assume a variety of conformations the receptor sites would have amino acid side chains in contact with a portion of any sugar, the addition of which in a given linkage resulted in increased binding. This is no longer necessarily the case. These findings, while made with a lectin site, must be considered as potentially applicable to antibody combining sites. It is conceivable that, in certain instances, an antibody combining site might not have amino acid side chains contacting each successive sugar residue but may be smaller and involve only a conformation in which non-sequential sugars constitute the contacting elements with the connecting sugars playing a structural role.

SUMMARY

The binding specificity of a second lectin purified from seeds of Bandeiraea simplicifolia (BS II) was studied by quantitative precipitin and inhibition assays. The lectin is not blood group specific and did not precipitate with A1, A2, Lea and a precursor blood group substance with I and i activity. Individual human B and H substances reacted to different extents due to their heterogeneity, those with terminal non-reducing αDGlcNAc reacting well; those lacking such residues did not precipitate. Glycoproteins with terminal βDGlcNAc such as agalactoosomucoid also precipitated the lectin. Inhibition of precipitation showed phenyl αDGlcNAc and pNO2 phenyl αDGlcNAc to be the best inhibitors, while their β anomers were relatively inactive. Of the free sugars tested only DGlcNAc showed considerable activity; methyl αDGlcNAc was twice as good as DGlcNAc but only 1/3 as active as phenyl αDGlcNAc, while methyl βDGlcNAc was relatively inactive. DGlcNAcβ1 → 3 or β1 → 6 linked to DGal and DGlcNAcβ1 → 3(DGlcNAcβ1 → 6)DGal were not active. DGlcNAcα1 → 4DGal was 80% as active as methyl αDGlcNAc; reduction of the DGal to galactitol reduced its activity greatly. The presence of a third sugar giving DGlcNAcα1 → 4DGalβ1 → 4DGlcNAc made it as active as methyl
αDGlcNAc. N,N',N'-triacteylchitotriose and N,N',N',N'-tetraacetylchitotetraose which were all equal and as active as methyl αDGlcNAc and DGlcNAca1 → 4DGlcNAc. However, DGlcNAca1 → 4DGalβ1 → 3DGalNAc had much lower activity. DGlcNAca1 → 5DGlc/ had the same activity as phenyl αDGlcNAc.

To explain the unusual finding that αx and β linked oligosaccharides of DGlcNAc were of comparable activity, molecular models were constructed. The best inhibitors showed a basic similarity in three dimensional-structure, the overall planarity of the molecule and hydrophobic interactions are of importance.

REFERENCES


