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Charles Wood

University of Nebraska-Lincoln, cwood1@unl.edu

E. A. Kabat

Columbia University

S. Ebisu

Columbia University

Irwin J. Goldstein

University of Michigan - Ann Arbor, irwin.j.goldstein@med.umich.edu

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Wood, Charles; Kabat, E. A.; Ebisu, S.; and Goldstein, Irwin J., "An Immunochemical Study of the Combining Sites of the Second Lectin Isolated from *Bandeiraea simplicifolia* (BS II)" (1978). *Virology Papers*. 176.

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

by C. Wood, E. A. Kabat, S. Ebisu and I. J. Goldstein

Departments of Microbiology, Human Genetics and Development and Neurology,
College of Physicians and Surgeons, Columbia University, New York, NY,
and the Department of Biological Chemistry, University of Michigan, Ann Arbor, Michigan

KEY WORDS: Lectin, Binding specificity, Glycoprotein;
Combining sites; Blood group substances; Molecular models.

Aided by grants from the National Science Foundation NSF BMS-72-02219
AO4 and PCM76-81029 to EAK and the National Institutes of Health,
United States Public Health Service, AM10171 to IJG.

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 α DGalactosyl residues. It reacted with B substances from human ovarian cysts and with several galactomannans to form precipitin lines in agar gels. Polysaccharides with terminal α DGalactosyl 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 α DGalNAc [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 α DGalNAc, the B subunits for α DGal [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, B or O erythrocytes. Quantitative precipitin assays showed it to react better with BSA conjugated to *p*-azophenyl α DGalNAc than with the β compound. In inhibition studies, the unusual observation was made that N,N'-diacetylchitobiose (DGlcNAc β 1 \rightarrow 4DGlcNAc) and *p*NO₂ phenyl α DGalNAc were highly active; methyl α DGalNAc was only one half as active but was eight times more active than methyl β DGlcNAc.

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*NO₂ phenyl α DGlcNAc, phenyl α DGlcNAc and DGlcNAc α 1 \rightarrow 5DGlc α 1 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 \emptyset OH insoluble [2] and horse 4 25% [5]; A₁ MSS 10% 2X, A₂ WG \emptyset OH insoluble [19]; H, Tighe \emptyset OH insoluble [6] and JS \emptyset OH insoluble [35]. JS 1st and 2nd IO₄/BH₄ were obtained after two sequential stages of periodate oxidation and Smith degradation [23]. Le^a active cyst material (N-1 \emptyset OH 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], agalactoorosomucoid by Dr. G. Ashwell [31] and a synthetic antigen (antigen A) DGlcNAc β 1 \rightarrow 4 DGlcNAc β 1 \rightarrow N-polyAsn by Dr. T. W. Shier [36]. The blood group oligosaccharides used were isolated and characterized previously [8, 22, 27], N,N'-diacetylchitobiose and N,N',N''-triacetylchitotriose were from Dr. N. Sharon [1], N,N',N'',N'''-tetraacetylchitotetraose was described previously [10]; DGlcNAc β 1 \rightarrow 3DGal, DGlcNAc β 1 \rightarrow 6DGal and DGlcNAc β 1 \rightarrow 3DGlcNAc β 1 \rightarrow 6DGal were from Dr. Z. Yosizawa [40]; DGlcNAc α 1 \rightarrow 5DGlc α 1 was from Dr. van Heeswijk. Monosaccharides were obtained commercially (Nutritional Biochemicals [12] Corp. and Schwartz/Mann Research Laboratories).

Immunochemical methods.

Quantitative precipitin and inhibition assays were by the quantitative microprecipitin technique [20] in a final volume of 200 μ l; 5.6 μ g 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° for one week with mixing twice daily.

DGlc = DGlucopyranose.

DGlc/ = DGlucofuranose.

DGal = DGalactopyranose.

DGlcNH₂ = 2-amino-2-deoxy-D-glucopyranose.

DGlcNAc = 2-aceamido-2-deoxy-D-glucopyranose.

DGalNAc = 2-acetamido-2-deoxy-D-galactopyranose.

DManNAc = 2-acetamido-2-deoxy-D-mannopyranose.

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 IO₄⁻/BH₄⁻ 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 IO₄⁻/BH₄⁻ 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 IO₄⁻/BH₄⁻ 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

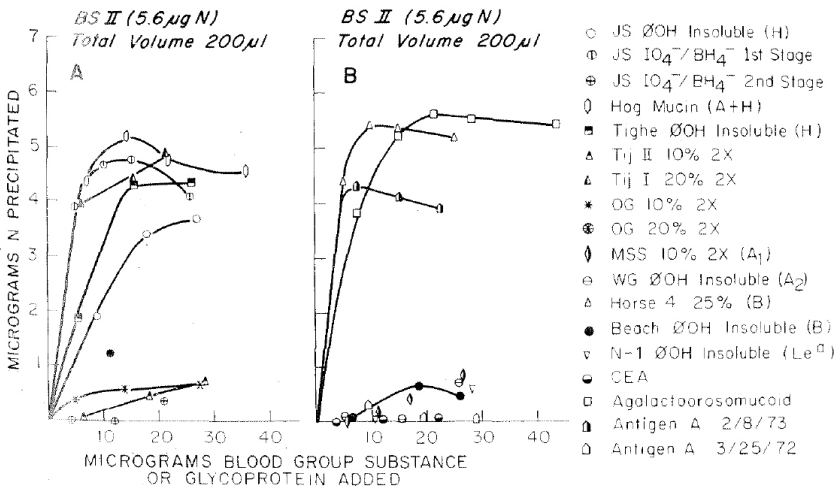


FIG. 1. — Quantitative precipitation curves of BS II with various blood group substances and glycoproteins.

only 5 μ g. 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 \emptyset OH 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 μ g giving 50% inhibition, while a human ovarian cyst B substance, Beach \emptyset OH insoluble, was almost completely inactive. N-I \emptyset OH insoluble, a Le^a active cyst substance, showed no activity.

Of two preparations of a synthetic antigen A, DGlcNAc β 1 \rightarrow 4DGlcNAc β 1 \rightarrow N-poly (Asn) (Figure 1B), one reacted well giving 4.3 μ g 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 DGlcNAc β 1 \rightarrow 4 residues, was highly potent, precipitating all 5 μ g N of the added lectin and was about half as active as horse 4 25%. CEA which lacks terminal non-reducing 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 DGlcNAc gave 50% inhibition while 450 nmoles of DGalNAc, 1,000 nmoles of DGal, 640 nmoles of DManNAc, 770 nmoles of DGlc and 300 nmoles of DGlcNH₂ gave 0, 18, 16, 11 and 10 percent inhibition respectively (Figure 2B).

Methyl α DGlcNAc was more active than DGlcNAc; 110 nmoles gave 50% inhibition while 880 nmoles of methyl β DGlcNAc only inhibited to 35%; *p*NO₂ phenyl α DGlcNAc and phenyl α DGlcNAc were the most potent inhibitors, both being 3, 6 and 20 times better than methyl α DGlcNAc, DGlcNAc and *p*NO₂ phenyl β DGlcNAc respectively; *p*NO₂ phenyl α DGlc showed no inhibition with 310 nmoles; N,N'-diacetylchitobiose, N,N',N''-triacetylchitotriose and N,N',N'',N'''-tetraacetylchitotetraose were of equal potency and as active as methyl α DGlcNAc.

Of the di- and trisaccharides tested, those with terminal non-reducing β -linked DGlcNAc were relatively inactive (Figure 2B); DGlcNAc β 1 \rightarrow 3DGal, DGlcNAc β 1 \rightarrow 6DGal, DGlcNAc β 1 \rightarrow 3[DGlcNAc β 1 \rightarrow 6]DGal [40] and the blood group oligosaccharides, DGlcNAc β 1 \rightarrow 6DGal (R_L 0.95) [8] and DGlcNAc β 1 \rightarrow 3DGalactitol [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 DGlcNAc were also tested (Figure 2A); 140 nmoles of DGlcNAc α 1 \rightarrow 4DGal (R_L 1.53) [8] gave 50% inhibition, being 1.8 times as active as DGlcNAc but

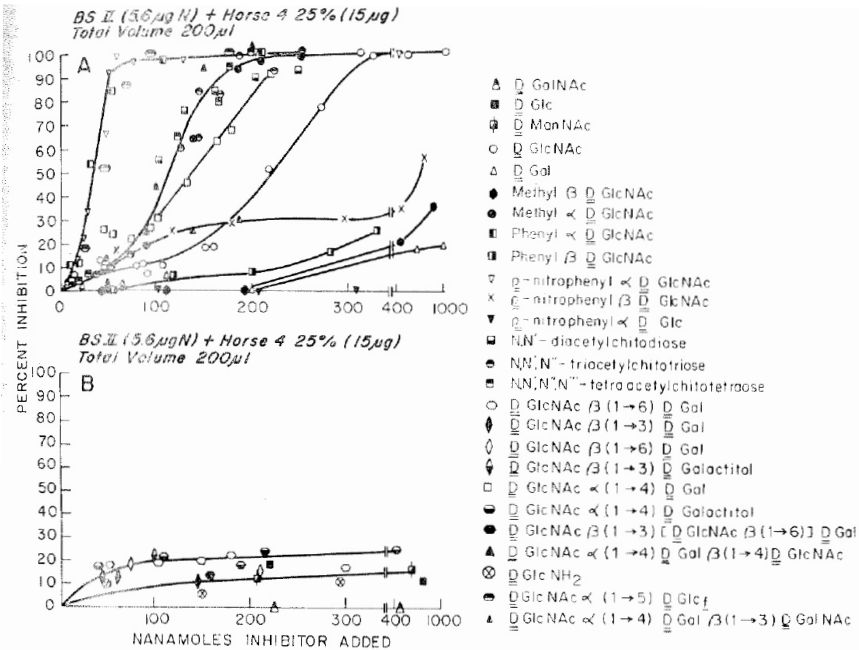


FIG. 2. — Inhibition of precipitation of BS II with B substance, horse 4 25%, by monosaccharides and various oligosaccharides.

only about 80% as active as methyl α-DGlcNAc or the chitin oligosaccharides. DGlcNAcα1 → 5DGlc [12] was as active as pNO₂ phenyl αDGlcNAc and phenyl αDGlcNAc, 47 nmoles giving 50% inhibition. DGlcNAcα1 → 4DGalβ1 → 4DGl-cNAc (R_L 0.97b) [8] was as active as the methyl αDGlcNAc and the β1 → 4 linked oligosaccharides of DGlcNAc. However, another trisaccharide, DGlcNAcα1 → 4DGalβ1 → 3DGalNAc (R_L 0.97a) [8] was like pNO₂ phenyl βDGlcNAc with 190 nmoles inhibiting only 30%. The reduced oligosaccharide DGlcNAcα1 → 4DGa-lactitol (R_G 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 α and β 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 DGlcNAc and their β 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 NO_2 group does not contribute. Molecular models were constructed in an attempt to account for differences in reactivity of the phenyl α and β 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 DGlcNAc . For phenyl $\beta\text{DGlcNAc}$ the ring is at an angle to the plane of the sugar while in the α 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 β 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 β anomer to be at a slight angle to the DGlcNAc while in the α compound the molecule is flatter.

Of the free sugars tested only DGlcNAc showed considerable activity. DGlc , DGlcNH_2 , DGalNAc and DManNAc were almost completely inactive. Thus an equatorial N-acetamido group at C2 and the DGlc conformation are required as noted earlier [13]. Disaccharides of DGlcNAc linked $\beta 1 \rightarrow 3$ or $\beta 1 \rightarrow 6$ to DGal and $\text{DGlcNA}\beta 1 \rightarrow 3[\text{DGlcNAc}\beta 1 \rightarrow 6]\text{DGal}$ 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 $\alpha\text{DGlcNAc}$.

$\text{N,N}'$ -diacetylchitobiose, $\text{N,N}',\text{N}''$ -triacetylchitotriose and $\text{N,N}',\text{N}''\text{N}'''$ -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 DGlcNAc and the CH_2OH 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 $\text{DGlcNAc}\alpha 1 \rightarrow 4\text{DGal}$ to give $\text{DGlcNAc}\alpha 1 \rightarrow 4\text{DGal}\beta 1 \rightarrow 4\text{DGlcNAc}$ increased its activity to that of $\text{DGlcNAc}\beta 1 \rightarrow 4\text{DGlcNAc}$ and methyl $\alpha\text{DGlcNAc}$. Molecular models of $\text{DGlcNAc}\alpha 1 \rightarrow 4\text{DGal}\beta 1 \rightarrow 4\text{DGlcNAc}$ (Figure 3E) showed striking similarities to $\text{DGlcNAc}\beta 1 \rightarrow 4\text{DGlcNAc}$; the model of the trisaccharides can assume conformation, if the DGal residue is rotated so that it is perpendicular to the two DGlcNAc residues, such that the molecule is shortened with CH_2OH group of the reducing DGlcNAc coming into close contact with N-acetamido group of the terminal non-reducing DGlcNAc and aligning the two DGlcNAc residues in the same plane as in $\text{DGlcNAc}\beta 1 \rightarrow 4\text{DGlcNAc}$ (Figure 3C). The polyethylene tubing outlines the regions of between these molecules.

$\text{DGlcNAc}\alpha 1 \rightarrow 5\text{DGlc}$ was as good as *p* NO_2 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 $\text{DGlcNAc}\alpha 1 \rightarrow 5\text{DGlc}$ (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 DGlcNAc of $\text{DGlcNAc}\beta 1 \rightarrow 4\text{DGlcNAc}$ and $\text{DGlcNAc}\alpha 1 \rightarrow 4\text{DGal}\beta 1 \rightarrow 4\text{DGlcNAc}$ in the conformations shown in Figure 3C and E. For $\text{DGlcNAc}\alpha 1 \rightarrow 4\text{DGal}$, the H underneath the pyranose ring of the DGal is not in the same position because of the angle of the DGal to the DGlcNAc and this might prevent it from contacting in the binding site (Figure 3C). Similarly, $\text{DGlcNAc}\alpha 1 \rightarrow 4\text{DGal}\beta 1 \rightarrow 3\text{DGalNAc}$ which showed greatly reduced activity (Figure 2B) as compared with $\text{DGlcNAc}\alpha 1 \rightarrow 4\text{DGal}\beta 1 \rightarrow 4\text{DGlcNAc}$ may assume a conformation similar to it (Figure 3F) but the CH_2OH of the reducing DGalNAc at a slightly different angle to the terminal non-reducing DGlcNAc and is not in as close contact with N-acetamido group of DGlcNAc . An axial OH group is present at C4 of the reducing DGalNAc instead of the H in 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 DGalNAc containing trisaccharide. The axial H in the comparable position of $\text{DGlcNAc}\alpha 1 \rightarrow 4\text{DGal}\beta 1 \rightarrow 4\text{DGlcNAc}$ is shown by the arrow in figure 3H. In methyl $\alpha\text{DGlcNAc}$ an H of the CH_2 is at the same position as the H at C5 of DGlc in $\text{DGlcNAc}\alpha 1 \rightarrow 5\text{DGlc}$ and the axial H on C4 of the reducing DGlcNAc in *N,N'*-diacetylchitobiose. This hydrophobic interaction could explain methyl $\alpha\text{DGlcNAc}$ is as active as $\text{DGlcNAc}\beta 1 \rightarrow 4\text{DGlcNAc}$ 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 α - and β -linked **DGlcNAc** 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 **DGlcNAc**, and it does not react with A₁, A₂ 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 α -linked **DGlcNAc** in hog mucin A + H was established by isolation of **DGlcNAc** α 1 \rightarrow 4**DGalactitol** [25] and subsequently of **DGlcNAc** α 1 \rightarrow 4**DGal** [8]; individuals immunized with hog mucin A + H produced antibody specific for terminal non-reducing α -linked **DGlcNAc** [29]; terminal non-reducing α **DGlcNAc** is also responsible for precipitation with conA [25] and in this study with BS II. Isolation of penta- and hexasaccharides with terminal non-reducing **DGlcNAc** linked α and β 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 **DGlcNAc** linked α 1 \rightarrow 4 [27]. Similarly, the activity of JS \emptyset OH insoluble and Tighe \emptyset OH insoluble may be due to the heterogeneity of these substances with exposed terminal non-reducing β **DGlcNAc**. A mouse IgA myeloma immunoglobulin with specificity for terminal β -linked **DGlcNAc** [38] reacted with JS IO₄/BH₄ 1st stage which has terminal β -linked **DGlcNAc** while JS IO₄/BH₄ 2nd stage, obtained by removing the terminal **DGlcNAc**, did not react, findings identical to those with BS II (Figure 1B). Since BS II has high inhibiting activity for oligosaccharides of **DGlcNAc** linked β 1 \rightarrow 4 and not for those linked β 1 \rightarrow 4 or β 1 \rightarrow 6, this might suggest that some β 1 \rightarrow 4 linked **DGlcNAc** residues could be present in JS IO₄/BH₄ 1st stage. Aston *et al.* [3] had isolated **DGal** β 1 \rightarrow 3 **DGlcNAc** β 1 \rightarrow 4**DGal** from ovarian cyst H substance which would, on IO₄/BH₄ degradation, yield a **DGlcNAc** β 1 \rightarrow 4 terminal residue. However, despite the weak reactions of BS II with β 1 \rightarrow 3 or β 1 \rightarrow 6 linked oligosaccharides of **DGlcNAc**, the multivalence of the IO₄/BH₄ 1st stage with respect to terminal non-reducing **DGlcNAc** might also lead to precipitation without the linkage being β 1 \rightarrow 4. Of the B substances tested, horse 4 25% is highly active as observed for *Aptos* lectin I [7] which is very specific for terminal β **DGlcNAc**. However, another B substance, Beach \emptyset OH insoluble which reacted with *Aptos* lectin I, did not react with BS II, again demonstrating heterogeneity of individual blood group substances. Agalactoorosomucoid having multiple terminal non-reducing β -linked **DGlcNAc** [14] subsequently shown (G. Ashwell, personal communication) to be β 1 \rightarrow 4, and one sample of antigen A (**DGlcNAc** β 1 \rightarrow 4**DGlcNAc** β 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 **DGlcNAc** linked α 1 \rightarrow 4 and β 1 \rightarrow 4. While it is essentially specific for terminal non-reducing α -linked **DGlcNAc**, it can also react with an oligosaccharide with terminal non-reducing

DGlcNAc linked $\beta 1 \rightarrow 4$ 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 α DGlcNAc, on C4 of DGlcNAc $\beta 1 \rightarrow 4$ DGlcNAc or C5 of DGlcNAc $\alpha 1 \rightarrow 5$ DGlcNAc, is essential. The site is perhaps best pictured as a planar shallow groove into which the lower half of the terminal non-reducing DGlcNAc and the second sugar or aglycones giving the best inhibition fit (Figure 3).

The finding that a particular conformation of the trisaccharide DGlcNAc $\alpha 1 \rightarrow 4$ DGal $\beta 1 \rightarrow 4$ DGlcNAc could mimic that of the disaccharide DGlcNAc $\beta 1 \rightarrow 4$ DGlcNAc 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 A_1 , A_2 , Le^a 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 α DGlcNAc reacting well; those lacking such residues did not precipitate. Glycoproteins with terminal β DGlcNAc such as agalactoorosomucoid also precipitated the lectin. Inhibition of precipitation showed phenyl α DGlcNAc and pNO_2 phenyl α DGlcNAc to be the best inhibitors, while their β anomers were relatively inactive. Of the free sugars tested only DGlcNAc showed considerable activity; methyl α DGlcNAc was twice as good as DGlcNAc but only 1/3 as active as phenyl α DGlcNAc, while methyl β DGlcNAc was relatively inactive. DGlcNAc $\beta 1 \rightarrow 3$ or $\beta 1 \rightarrow 6$ linked to DGal and DGlcNAc $\beta 1 \rightarrow 3$ [DGlcNAc $\beta 1 \rightarrow 6$]DGal were not active. DGlcNAc $\alpha 1 \rightarrow 4$ DGal was 80% as active as methyl α DGlcNAc; reduction of the DGal to galactitol reduced its activity greatly. The presence of a third sugar giving DGlcNAc $\alpha 1 \rightarrow 4$ DGal $\beta 1 \rightarrow 4$ DGlcNAc made it as active as methyl

α DGlcNAc. N,N',N''-triacylchitotriose and N,N',N'',N'''-tetraacylchitotetraose which were all equal and as active as methyl α DGlcNAc and DGlcNAc α 1 \rightarrow 4DGlcNAc. However, DGlcNAc α 1 \rightarrow 4DGal β 1 \rightarrow 3DGalNAc had much lower activity. DGlcNAc α 1 \rightarrow 5DGlc/ had the same activity as phenyl α DGlcNAc.

To explain the unusual finding that α 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.

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