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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 α**D**Galactosyl 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**D**Galactosyl residues, such as larch galactan, did not react. The lectin agglutinated B erythrocytes strongly but also reacted to a lower titre with A_1 and very weakly with A_2 erythrocytes [15, 28] indicating that terminal non-reducing α**D**GalNAc [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**D**GalNAc, the B subunits for α**D**Gal [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 α**D**GalNAc than with the β compound. In inhibition studies, the unusual observation was made that N,N′-diacetylchitobiose (**D**GlcNAcβ1 → 4**D**GlcNAc) and *p*NO_2 phenyl α**D**GalNAc were highly active; methyl α**D**GalNAc was only one half as active but was eight times more active than methyl β**D**GlcNAc.

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 **D**GlcNAc but to different extents. Assays by inhibition of the precipitin reaction using various oligosaccharides and glycosides showed that

only the terminal **D**GlcNAc and part of the second sugar contribute significantly to the binding specificity. The lectin is most specific for terminal reducing α-linked **D**GlcNAc. It is unusual that certain disaccharides with β linkages react as well or better than others with α linkages ; *p*NO_2 phenyl α**D**GlcNAc, phenyl α**D**GlcNAc and **D**GlcNAcαl → 5**D**Glcf 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 ØOH insoluble [2] and horse 4 25% [5]; A_1 MSS 10% 2X, A_2 WG ØOH insoluble [19]; H, Tighe ØOH insoluble [6] and JS ØOH insoluble [35]. JS 1st and 2nd IO_4/BH_4 were obtained after two sequential stages of periodate oxidation and Smith degradation [23]. Le^a active cyst material (N-l 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], agalactoorosomucoid by Dr. G. Ashwell [31] and a synthetic antigen (antigen A) **D**GlcNAcβ1 → 4 **D**GlcNAcβ1 → 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‴-tetraacelylchitotetraose was described previously [10]; **D**GlcNAβ1 → 3**D**Gal, **D**GlcNAcβ1 → 6**D**Gal and **D**GlcNAcβ1 → 3**D**GlcNAcβ1 → 6**D**Gal were from Dr. Z. Yosizawa [40]; **D**GlcNAcαl → 5**D**Glc*f* 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 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° for one week with mixing twice daily.

DGlc = **D**Glucopyranose.
DGlc/ = **D**Glucofuranose.
DGal = **D**Galactopyranose.
DGlcNH$_2$ = 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 lA, 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 heing needed for 50% precipitation. However, the first stage of periodate oxidation and Smith degradation, JS IO_4/BH_4 lst 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 IO_4/BH_4 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 IO_4/BH_4 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

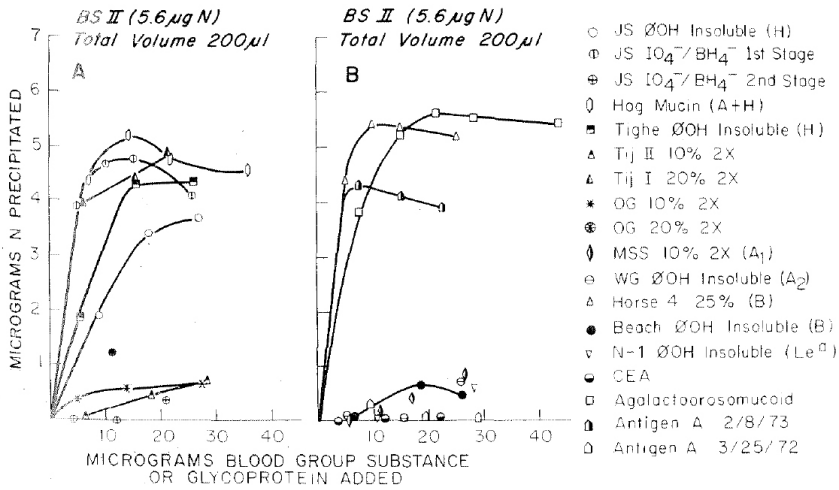

FIG. 1. — *Quantitative precipitation curves of BS II with various blood group substances and glycoproteins.*

only 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 A_1 substance from human ovarian cyst MSS 10% 2X nor with an A_2 substance WG ØOH 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 ØOH insoluble, was almost completely inactive. N-l ØOH insoluble, a Le^a active cyst substance, showed no activity.

Of two preparations of a synthetic antigen A, **D**GlcNAcβl → 4**D**GlcNAcβl → N-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 **D**Gal enzymatically to expose multiple terminal non-reducing **D**GlcNAcβ1 → 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 **D**GlcNAc did not react with the lectin.

Quanitative 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 **D**GlcNAc gave 50% inhibition while 450 nmoles of **D**GalNAc, 1,000 nmoles of **D**Gal, 640 nmoles of **D**ManNAc, 770 nmoles of **D**Glc and 300 nmoles of **D**GlcNH$_2$ gave 0, l8, 16, 11 and 10 percent inhibition respectively (Figure 2B).

Methyl α**D**GlcNAc was more active than **D**GlcNAc; 110 nmoles gave 50% inhibition while 880 nmoles of methyl β**D**GlcNAc only inhibited to 35%; *p*NO$_2$ phenyl α**D**GlcNAc and phenyl α**D**GlcNAc were the most potent inhibitors, both being 3, 6 and 20 times better than methyl α**D**GlcNAc, **D**GlcNAc and *p*NO$_2$ phenyl β**D**GlcNAc respectively; *p*NO$_2$ phenyl α**D**Glc 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**D**GlcNAc.

Of the di- and trisaccharides tested, those with terminal non-reducing β-linked **D**GlcNAc were relatively inactive (Figure 2B); **D**GlcNAcβ1 → 3**D**Gal, **D**GlcNAcβ1 → 6**D**Gal, **D**GlcNAcβ1 → 3[**D**GlcNAcβ1 → 6]**D**Gal [40] and the blood group oligosaccharides, **D**GlcNAcβ1 → 6**D**Gal (R_L 0.95) [8] and **D**GlcNAcβ1 → 3**D**Galactitol [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 **D**GlcNAc were also tested (Figure 2A); 140 nmoles of **D**GlcNAcα1 → 4**D**Gal (R_L 1.53) [8] gave 50% inhibition, being 1.8 time as active as **D**GlcNAc but

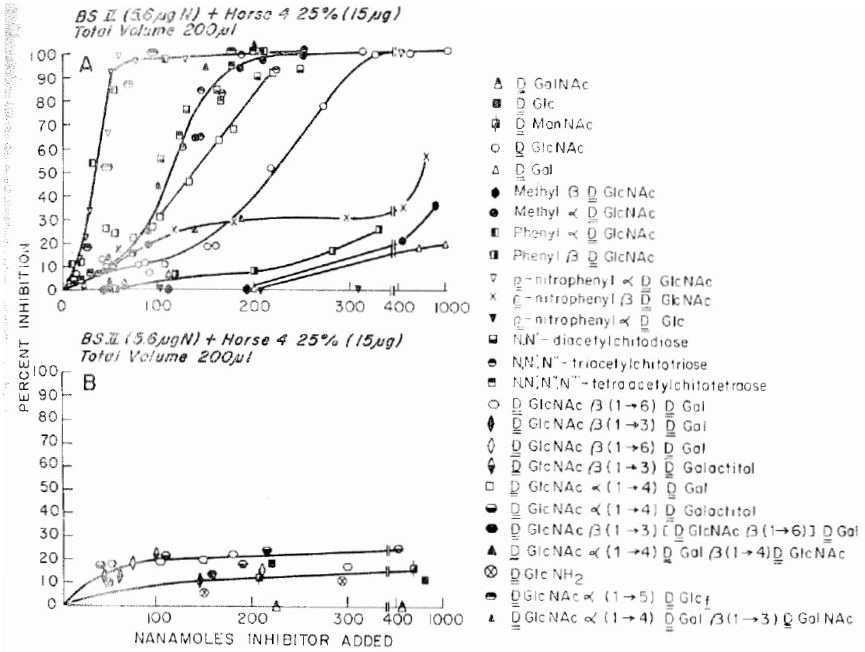

FIG. 2. — *Inhibition of precipitation of BS II with B substance, horse 4 25 %, by monosaccharides and various oligosaccharides.*

only about 80% as active as methyl α-**D**GlcNAc or the chitin oligosaccharides. **D**GlcNAcαl → 5**D**Glc/ [12] was as active as *p*NO_2 phenyl α**D**GlcNAc and phenyl α**D**GlcNAc, 47 nmoles giving 50% inhibition. **D**GlcNAcα1 → 4**D**Galβ1 → 4**D**GlcNAc (R_L 0.97b) [8] was as active as the methyl α**D**GlcNAc and the β1 → 4 linked oligosaccharides of **D**GlcNAc. However, another trisaccharide, **D**GlcNAcαl → 4**D**Galβ1 → 3**D**GalNAc (R_L 0.97a) [8] was like *p*NO_2 phenyl β**D**GlcNAc with 190 nmoles inhibiting only 30%. The reduced oligosaccharide **D**GlcNAcα1 → 4**D**Galactitol (R_G 0.42) [25] Was only as active as oligosaccharides with terminal β-linked **D**GlcNAc, 430 nmoles inhibiting only 24%.

DISCUSSION

BS II, while specific for terminal non-reducing **D**GlcNAc [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 β**D**GlcNAc-BSA

[13] were **D**GlcNAcβ1 → 4**D**GlcNAc, *p*NO_2 phenyl α**D**GlcNAc and methyl α**D**GlcNAc.

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*NO_2 phenyl α**D**GlcNAc and phenyl α**D**GlcNAc (Figure 2A) were the best inhibitors, being equal and about 6 and 20 times better than **D**GlcNAc and their β anomers. Earlier studies with conA [33], *Sophora japonica* [34] and peanut agglutinin [35] have shown the *p*NO_2 phenyl glycosidcs 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*NO_2 phenyl α**D**GlcNAc and phenyl α**D**GlcNAc 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 **D**GlcNAc (Figure 3A); when the models were placed in similar conformations the only apparent difference is the angle of the phenyl ring relative to the **D**GlcNAc. For phenyl β**D**GlcNAc 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 α**D**GlcNAc was much more active than methyl β**D**GlcNAc but only about 1/3 as active as phenyl α**D**GlcNAc, and models (Figure 3D) show the methyl group in the β anomer to be at a slight angle to the **D**GlcNAc while in the α. compound the molecule is flatter.

Of the free sugars tested only **D**GlcNAc showed considerable activity. **D**Glc, **D**GlcNH_2, **D**GalNAc and **D**ManNAc were almost completely inactive. Thus an equatorial N-acetamido group at C2 and the **D**Glc conformation are required as noted earlier [13]. Disaccharides of **D**GlcNAc linked β1 → 3 or β1 → 6 to **D**Gal and **D**GlcNAβ1 → 3[**D**GlcNAcβ1 → 6]**D**Gal were inactive. The disaccharide **D**GlcNAcαl → 4**D**Gal was highly active but only 80% as active as methyl α**D**GlcNAc. Reduction of **D**GlcNAcα1 → 4**D**Gal to **D**GlcNAcα1 → 4**D**Galactitol reduced its activity to about that of β**D**GlcNAc; thus opening the ring to give galactitol interferes with binding of the lectin to the terminal non-reducing α**D**GlcNAc.

N,N′-diacetylchitobiose, N,N′,N″-triacetylchitotriose and N,N′,N″N‴-tetraacetylchitotetraose were all equal in potency to methyl α**D**GlcNAc and 1.25 time better than **D**GlcNAcαl → 4**D**Gal. Moleeular models of N,N′-diacetylchitobiose (**D**GlcNAcβ1 → 4**D**GlcNAc) and **D**GlcNAcα1 → 4**D**Gal 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 **D**GlcNAc and the CH_2OH of the subterminal residue. The side view shows the most striking difference; the molecule of N,N′-diacetylchitobiose is flat like phenyl α**D**GlcNAc while in **D**GlcNAcα1 → 4**D**Gal the two are at an angle as in phenyl β**D**GlcNAc (Figure 3A).

Addition of a third sugar to **D**GlcNAcα1 → 4**D**Gal to give **D**GlcNAcαl → 4**D**Galβ1 → 4**D**GlcNAc increased its activity to that of **D**GlcNAcβ1 → 4**D**GlcNAc and methyl α**D**GlcNAc. Molecular models of **D**GlcNAcαl → 4**D**Galβ1 → 4**D**GlcNAc (Figure 3E) showed striking similarities to **D**GlcNAcβ1 → 4**D**GlcNAc; the model of the trisaccharides can assume conformation, if the **D**Gal residue is rotated so that it is perpendicular to the two **D**GlcNAc residues, such that the molecule is shortened with CH_2OH group of the reducing **D**GlcNAc coming into close contact with N-acetamido group of the terminal non-reducing **D**GlcNAc and aligning the two **D**GlcNAc residues in the same plane as in **D**GlcNAcβl → 4**D**GlcNAc (Figure 3C). The polyethylene tubing outlines the regions of between these molecules.

DGlcNAcα1 → 5**D**Glc was as good as *p*NO_2 phenyl α**D**GlcNAc and phenyl α**D**GlcNAc 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 α**D**GlcNAc and permits increased adaptability in the site. Interactions may also be involved; there is an H at C5 beneath furanose ring of **D**GlcNAcαl → 5**D**Glc/ (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 **D**GlcNAc of **D**GlcNAcβl → 4**D**GlcNAc and **D**GlcNAcαl → 4**D**Galβ1 → 4**D**GlcNAc in the conformations shown in Figure 3C and E. For **D**GlcNAcαl → 4**D**Gal, the H underneath the pyranose ring of the **D**Gal is not in the same position because of the angle of the **D**Gal to the **D**GlcNAc and this might prevent it from contacting in the binding site (Figure 3C). Similarly, **D**GlcNAcαl → 4**D**Galβ1 → 3**D**GalNAc which showed greatly reduced activity (Figure 2B) as compared with **D**GlcNAcαl → 4**D**Galβl → 4**D**-GlcNAc may assume a conformation similar to it (Figure 3F) but the CH_2OH of the reducing **D**GalNAc at a slightly different angle to the terminal non-reducing **D**GlcNAc and is not in as close contact with N-acetamido group of **D**GlcNAc. An axial OH group is present at C4 of the reducing **D**GalNAc instead of the H in **D**GlcNAc, 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 **D**GalNAc containing trisaccharide. The axial H in the comparable position of **D**GlcNAcαl → 4**D**Galβ1 → 4**D**GlcNAc is shown by the arrow in figure 3H. In methyl α**D**GlcNAc an H of the CH_2 is at the same position as the H at C5 of **D**Glc*f* in **D**GlcNAcα1 → 5**D**Glc*f* and the axial H on C4 of the reducing **D**GlcNAc in N,N′-diacetylchitobiose. This hydrophobic interaction could explain methyl α**D**GlcNAc is as active as **D**GlcNAcβ1 → 4**D**GlcNAc but inhibited less strongly than phenyl α**D**GlcNAc 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 **D**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 **D**GlcNAc, and it does not react with A_1, A_2 and Le^a substances. The reactions with B and H substancesare due to the extensively documented [24, 37, 39] heterogeneity of these materials. The presence of terminal non-reducing α-linked **D**GlcNAc in hog mucin A + H was established by isolation of **D**GlcNAcαl → 4**D**Galactitol [25] and subsequently of **D**GlcNAcαl → 4**D**Gal [8]; individuals immunized with hog mucin A + H produced antibody specific for terminal non-reducing α-linked **D**GlcNAc [29]; terminal non-reducing α**D**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 **D**GlcNAc linked α and β1 → 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 **D**GlcNAc linked α1 → 4 [27]. Similarly, the activity of JS ØOH insoluble and Tighe ØOH insoluble may be due to the heterogeneity of these substances with exposed terminal non-reducing β**D**GlcNAc. A mouse IgA myeloma immunoglobulin with specificity for terminal β-linked **D**GlcNAc [38] reacted with JS IO_4/BH_4 1st stage which has terminal β-linked **D**GlcNAc while JS IO_4/BH_4 2nd stage, obtained by removing the terminal **D**GlcNAc, did not react, findings identical to those with BS II (Figure 1B). Since BS II has high inhibiting activity for oligosaccharides of **D**GlcNAc linked βl → 4 and not for those linked β1 → 4 or β1 → 6, this might suggest that some β1 → 4 linked **D**GlcNAc residues could be present in JS IO_4/BH_4 1st stage. Aston *et al.* [3] had isolated **D**Galβl → 3 **D**GlcNAcβ1 → 4**D**Gal from ovarian cyst H substance which would, on IO_4/BH_4 degradation, yield a **D**GlcNAcβ1 → 4 terminal residue. However, despite the weak reactions of BS II with β1 → 3 or β1 → 6 linked oligosaccharides of **D**GlcNAc, the multivalence of the IO_4/BH_4 1st stage with respect to terminal non-reducing **D**GlcNAc might also lead to precipitation without the linkage being β1 → 4. Of the B substances tested, horse 4 25% is highly active as observed for *Aaptos* lectin I [7] which is very specific for terminal β**D**GlcNAc. However, another B substance, Beach ØOH insoluble which reacted with *Aaptos* lectin I, did not react with BS II, again demonstrating heterogeneity of individual blood group substances. Agalactoorosomucoid having multiple terminal non-reducing β-linked **D**GlcNAc [14] subsequently shown (G. Ashwell, personal communication) to be β1 → 4, and one sample of antigen A (**D**GlcNAcβ1 → 4**D**GlcNAcβ1 → 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 **D**GlcNAc linked α1 → 4 and β1 → 4. While it is essentially specific for terminal non-reducing α-linked **D**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 **D**GlcNAc 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**D**GlcNAc, on C4 of **D**GlcNAcβl → 4**D**GlcNAc or C5 of **D**GlcNAcα1 → 5**D**Glc*f*, is essential. The site is perhaps best pictured as a planar shallow groove into which the lower half of the terminal non-reducing **D**GlcNAc and the second sugar or aglycones giving the best inhibition fit (Figure 3).

The finding that a particular conformation of the trisaccharide **D**GlcNAcα1 → 4**D**Galβl →4**D**GlcNAc could mimic that of the disaccharide **D**GlcNAcβl → 4**D**GlcNAc 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**D**GlcNAc reacting well; those lacking such residues did not precipitate. Glycoproteins with terminal β**D**GlcNAc such as agalactoorosomucoid also precipitated the lectin. Inhibition of precipitation showed phenyl α**D**GlcNAc and *p*NO_2 phenyl α**D**GlcNAc to be the best inhibitors, while their β anomers were relatively inactive. Of the free sugars tested only **D**GclNAc showed considerable activity; methyl α**D**GlcNAc was twice as good as **D**GlcNAc but only 1/3 as active as phenyl α**D**GlcNAc, while methyl β**D**GlcNAc was relatively inactive. **D**GlcNAcβl → 3 or β1 → 6 linked to **D**Gal and **D**GlcNAcβl → 3[**D**GlcNAcβ1 → 6]**D**Gal were not active. **D**GlcNAcα1 → 4**D**Gal was 80% as active as methyl α**D**GlcNAc; reduction of the **D**Gal to galactitol reduced its activity greatly. The presence of a third sugar giving **D**GlcNAcα1 → 4**D**Galβ1 → 4**D**GlcNAc made it as active as methyl

α**D**GlcNAc. N,N′,N″-triacetylchitotriose and N,N′,N″,N‴-tetraacetylchitotetraose which were all equal and as active as methyl α**D**GlcNAc and **D**GlcNAcα1 → 4**D**GlcNAc. However, **D**GlcNAcα1 → 4**D**Galβ1 → 3**D**GalNAc had much lower activity. **D**GlcNAcα1 → 5**D**Glc/ had the same activity as phenyl α**D**GlcNAc.

To explain the unusual finding that αx and β linked oligosaccharides of **D**GlcNAc 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

[1] ALLEN, A. K., NEUBERGER, A. & SHARON, N., The purification, composition and specificity of wheat-germ agglutinin. *Biochem. J.*, 1973, 131: 155-162.

[2] ALLEN, P. Z. & KABAT, E. A., Immunochemical studies on blood groups. – XXII. Immunochemical studies on the non-dialyzable residue from partially hydrolyzed blood group A, B and O(H) substances (PI fractions) *J. Immunol.*, 1959, 82: 340-357.

[3] ASTON, W. P., DONALD, A. S. R. & MORGAN, W. T. J., Oligosaccharides containing a (1 → 6) glycosidic linkage obtained from human blood-group specific glycoproteins. *Biochem. Biophys. Res. Commun.*, 1968, 30: 1-6.

[4] BENDICH, A., KABAT, E. A. & BEZER, A. E., Immunochemical studies on blood groups. – V. Further characterization of blood group A and O substances from individual hog stomachs. *J. Amer. Chem. Soc.*, 1947, 69: 2,163-2,167.

[5] BAER, H., KABAT, E. A. & KNAUB, V., Immunochemical studies on blood groups. – X. The preparation of blood group A and B substances and an inactive substance from individual horse stomachs group B substance from human saliva. *J. Exp. Med.*, 1950, 91: 105-114.

[6] BEYCHOK, S. & KABAT, E. A., Optical activity and conformation of carbohydrates. – 1. Optical rotatory dispersion studies on immunochemically reactive amino sugars and their glycosides, milk oligosaccharides, oligosaccharides of glucose, and blood group substances. *Biochemistry,* 1965, 4: 2,565-2,574.

[7] BRETTING, H., KABAT, E. A., LIAO, J. & PEREIRA, M. E. A., Purification and characterization of the agglutinins from the sponge *Aaptos papillata* and a study of their combining sites. *Biochemistry,* 1976, 15: 5,029-5,038.

[8] ETZLER, M. E., ANDERSON, B., BEYCHOK, S., GRUEZO, F., LLOYD, K. O., RICHARDSON, N. G. & KABAT, E. A., Immunochemical studies on blood groups. – XLVI. Oligosaccharides isolated after hydrolysis of hog gastric mucin blood group A + H substance previously treated with the blood group de-N-acetylating enzyme. *Arch. Biochem. Biophys.*, 1970, 141: 588-601.

[9] FUKS, A., BANJO, C., SHUSTER, J., FREEDMAN, S. O. & GOLD, P., Carcino-embryonic antigen (CEA): molecular biology and clinical significance. *Biochem. Biophys. Acta* (Amst.), 1975, 417: 123-152.

[10] GOLDSTEIN, I. J., HAMMERSTRÖM, S. & SUNDBLAD, G. Precipitation and carbohydrate-binding specificity studies on wheat germ agglutinin. *Biochem. Biophys. Acta* (Amst.) 1975, 405: 53-61.

[11] HAYES, C. E., GOLDSTEIN, I. J., An α-**D**-galactosyl-binding lectin from *Bandeiraea simplicifolia* seeds. Isolation by affinity chromatography and characterization. *J. Biol. Chem.*, 1974, 249: 1,904-1,914.

[12] VAN HEESWIJK, W. A. R., DE HAAN, P. & VLIEGENTHART, J. F. G., The synthesis of 5-O-(α-acetamido-α-deoxy-α-**D**-glucopyranosyl)-β-**D**-gluco-furanose. *Carbohydr. Res.*, 1976, 48: 187-196.

[13] IYER, P. N., SHANKAR, WILKINSON, K. D. & GOLDSTEIN, I. J., An N-acetyl-**D**-glucosamine binding lectin from *Bandeiraea simplicifolia* seeds. *Arch. Biochem. Biophys.*, 1976, 117: 330-333.

[14] JEANLOZ, R. W. α1-acid glycoprotein *in: Glycoproteins,* 2nd ed., A. Gottschalk, p. 565-607. Amsterdam : Elsevier, 1972.

[15] JUDD, W. J., STEINER, E. A., FRIEDMAN, B. A., HAYES, C. E. & GOLDSTEIN, I. J. Serological studies in an α-**D**-galactosyl binding lectin isolated from *Bandeiraea simplicifolia* seeds. *Vox Sanq.* (Basel), 1976, 30: 261-267.

[16] KABAT, E. A., *Blood Group Substances: Their Chemistry and Immunochemistry.* New York : Academic Press, 1956.

[17] KABAT, E. A., *Structural Concept in Immunology and Immunochemistry,* 2nd ed. New York : Holt, Reinhart and Winston, 1976.

[18] KABAT, E. A. Dimensions and specificities of recognition sites on lectins and antibodies. *J. Supramol. Structure,* 1977.

[19] KABAT, E. A., BENDICH, A., BEZER, A. E. & BEISER, S. M. Immunochemical studies on blood groups. – IV. Preparations of blood group A substances from human sources and a comparison of their chemical and immunological properties with those of the blood group A substance from hog stomach. *J. Exp. Med.*, 1947, 85: 685-699.

[20] KABAT, E. A., *Kabat and Mayer's Experimental Immunochemistry,* 2nd ed. Springfield, Ill. : Charles C. Thomas, 1961.

[21] KOCHETROV, N. K., DEREVITSKAYA, V. A. & ARBATSKY, N. P., The structure of pentasaccharides and hexasaccharides from blood group substance H. *Europ. J. Biochem.*, 1976, 67: 129-136.

[22] LLOYD, K. O., KABAT, E. A., LAYUG, E. J. & GRUEZO, F., Immunochemical studies on blood groups. XXXIV. Structures of some oligosaccharides produced by alkaline degradation of blood group A, B and H substances. *Biochemistry,* 1966, 5: 1,489-1,501.

[23] LLOYD, K. O. & KABAT, E. A., Immunochemical studies on blood groups. – XLI. Proposed structures for carbohydrate portions of blood group A, B, H, Lewis^a, Lewis^b substances. *Proc. Nat. Acad. Sci.*, 1968, 61: 1,470-1,477.

[24] LLOYD, K. O., KABAT, E. A. & LICERIO, E., Immunochemical studies on blood groups. XXXVIII. Structures and activities of oligosaccharides produced by alkaline degradation of blood group Lewis^a substance. Proposed structure of the carbohydrate chains of human blood-group A, B, H, Le^a and Le^b substances. *Biochemistry,* 1968, 7: 2,976-2,990.

[25] LLOYD, K. O., KABAT, E. A. & BEYCHOK, S., Immunochemical studies on blood groups. – XLIII. The interaction of blood group substances from various sources with a plant lectin, concanavalin A. *J. Immunol.*, 1969, 102: 1,354-1,362.

[26] MAISONROUGE-MCAULIFFE, F. & KABAT, E. A., Immunochemical studies on blood groups. Fractionation, heterogeneity and chemical and immunochemical properties of a blood group substance with B, I and i activities purified from human ovarian cyst fluid. *Arch. Biochem. Biophys.*, 1976, 175: 71-80.

[27] MAISONROUGE-MCAULIFFE, F. & KABAT, E. A., Immunochemical studies on blood groups. Structures and immunochemical properties of oligosaccharides from two fractions of blood group substance from human ovarian cyst fluid differing in B, I and i activities and reactivity toward concanavalin A. *Arch. Biochem. Biophys.*, 1976, 175: 90-113.

[28] MAKELA, O. & MAKELA, P., Some new blood group specific phytoagglutinins. *Ann. Med. Exp. Biol. Fenn.*, 1956, 34: 402-404.

[29] MORENO, C. & KABAT, E. A., Immunochemical studies on blood groups. – XLIV. Human antibodies against a new determinant present in blood group substances from hog gastric mucosa. *J. Immunol.*, 1969, 102: 1,363-1,367.

[30] MURPHY, L. A. & GOLDSTEIN, I. J., Five α-**D**-galactopyranosyl-binding isolectins from *Bandeiraea simplicifolia* seeds. *J. Biol. Chem.*, 1977.

[31] PAULSON, J. C., REARICK, J. L., SADLER, J. E., HILL, R. L., TANABE, T. & ASHWELL, G., Bovine colostrum sialyltransferase: a useful probe of oligosaccharide structure and function. *Fed. Proc.*, 1977, 36: 653 (abstract).

[32] PEREIRA, M. E. A., KABAT, E. A. & SHARON, N., Immunochemical studies on the specificity of soybean agglutinin. *Carbohydr. Res.*, 1974, 37: 89-102.

[33] PORETZ, R. D. & GOLDSTEIN, I. J., An examination of the topography the saccharide binding sites of concanavalin A and of the forces involved in complexation. *Biochemistry,* 1970, 9: 2,890-2,896.

[34] PORETZ, R. D., RISS, H., TIMBERLAKE, J. W. & CHIEN, S. M., Purification and properties of the hemagglutinin from *Sophora joponica* seeds. *Biochemistry,* 1974, 13: 250-256.

[35] SCHIFFMANN, G., KABAT, E. A. & THOMPSON, W., Immunochemical studies on blood groups. – XXX. Cleavage of A, B and H blood-group substances by alkali. *Biochemistry*, 1964, 3: 113-120.

[36] SHIER, W. T., Preparation of a "chemical vaccine" against tumor progression (substituted glucosaminyl poly(L-aspartate). *Proc. Nat. Acad. Sci.*, 1971, 68: 2,078-2,082.

[37] VICARI, G. & KABAT, E. A., Immunochemical studies on blood groups. – XLII. Isolation and characterization from ovarian cyst fluid of a blood group substance lacking B, H, Le^a and Le^b specificity. *J. Immunol.*, 1969, 102: 821-825.

[38] VICARI, G., SHER, A., COHN, M. & KABAT, E. A., Immunochemical studies on a mouse myeloma protein with specificity for certain β-linked terminal residues of N-acetyl-**D**-glucosamine. *Immunochem.*, 1970, 7: 829-838.

[39] ROVIS, L., ANDERSON, B., KABAT, E. A., GRUEZO, F. & LIAO, J., Structures of oligosaccharides produced by base-borohydride degradation of human ovarian cyst blood group H, Le^a and Le^b active glycoprotein. *Biochemistry,* 1973, 12: 5,340-5, 360.

[40] YOSIZAWA, Z., α-Amino sugar-containing oligosaccharides isolated from hydrazinolyzate of blood group A mucopolysaccharide of hog gastric mucus. *J. Biohem.* (Tokyo), 1962, 51: 145-154.