

Observation of Unique Cross-Linked Lattices between Multiantennary Carbohydrates and Soybean Lectin. Presence of Pseudo-2-fold Axes of Symmetry in Complex Type Carbohydrates[†]

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ABSTRACT: The tetrameric lectin from *Glycine max* (soybean) (SBA) has been shown to cross-link and precipitate with N-linked multiantennary complex type oligosaccharides containing nonreducing terminal Gal residues (Bhattacharyya, L., Haraldsson, M., & Brewer, C. F. (1988) *Biochemistry* 27, 1034–1041). In the present study, negative stain electron micrographs of the precipitates of SBA with a series of naturally occurring and synthetic multiantennary carbohydrates with terminal Gal or GalNAc residues show the presence of highly ordered cross-linked lattices for many of the complexes. The precipitates of SBA with a "bisected" and "nonbisected" N-linked biantennary complex type oligosaccharide containing Gal residues at the nonreducing termini show similar two-dimensional patterns. However, the pattern observed for the precipitates of a tetraantennary complex type oligosaccharide with SBA is distinct from those of the two biantennary carbohydrates. Furthermore, the precipitates formed between the lectin and a synthetic O-linked biantennary ("cluster") glycoside with terminal GalNAc residues show a pattern that is different from those above. Four biantennary pentasaccharide analogs of the blood group I antigen containing β -LacNAc moieties at the 2,3-, 2,4-, 2,6-, and 3,6-positions of the core Gal also showed ordered patterns in their precipitates with SBA. X-ray crystallographic data and mixed quantitative precipitation profiles of binary mixtures of the four analogs demonstrate that each analog possesses a unique cross-linked lattice with the protein. A common structural feature of the naturally occurring and synthetic carbohydrates that show highly organized cross-linked lattices with SBA is the presence of a pseudo-2-fold axis of symmetry in each oligosaccharide relating the terminal binding epitopes on each arm. This suggests that the symmetry features of certain naturally occurring branch chain oligosaccharides facilitate formation of highly ordered, homogeneous cross-linked complexes with specific lectins.

Lectin-carbohydrate interactions play major roles in a variety of cellular events including cellular recognition, adhesion, and signal transduction (Monsigny, 1984; Brandley & Schnaar, 1986; Liener et al., 1986; Lis & Sharon, 1991). As a means of obtaining insight into the specificity of lectin-carbohydrate interactions that may mediate cellular recognition events, we have been investigating the interaction of asparagine-linked (N-linked)¹ oligosaccharides with lectins. Our studies have shown that many N-linked oligosaccharides are multivalent and can cross-link and precipitate with multivalent lectins (Bhattacharyya & Brewer, 1989; Bhattacharyya et al., 1987a,b, 1988a, 1989a, 1990). Furthermore,

the specificity of carbohydrate-lectin interactions appears to be greater in the cross-linked complexes (precipitates) than in the corresponding soluble complexes (Bhattacharyya & Brewer, 1992; Bhattacharyya et al., 1988b, 1989a, 1990). For example, quantitative precipitation studies of binary mixtures of a series of oligomannose glycopeptides and a bisected hybrid type glycopeptide with the Glc/Man-specific lectin concanavalin A (ConA) indicate that each glycopeptide forms unique, homopolymeric cross-linked complexes with the lectin (Bhattacharyya et al., 1988b). Complexes containing two different glycopeptides bound to the lectin fail to precipitate. Similar studies have also been carried out with mixtures of complex type oligosaccharides and several Gal-specific lectins (Bhattacharyya & Brewer, 1992). In addition, recent investigations also indicate that individual glycoproteins form unique homopolymeric cross-linked complexes with a lectin, even in the presence of mixtures of glycoproteins (Mandal & Brewer, 1992).

The ability of lectins to form distinct cross-linked complexes with specific multivalent carbohydrates and glycoconjugates appears to be related to the formation of long-range order in the lattices. For example, the precipitates formed between the Fuc-specific isolectin A from *Lotus tetragonolobus* (LTL-A) and three structurally related biantennary fucosyl oligosaccharides show distinct, highly organized cross-linked lattices for each carbohydrate when viewed by negative stain or freeze fracture electron microscopy (Bhattacharyya et al., 1989b, 1990). Furthermore, recent studies show that the precipitates of LTL-A and one of the oligosaccharides are

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¹ Abbreviations: SBA, lectin from *Glycine max* (soybean); NMSBA, N-methylated derivative of SBA; ConA, Jack bean lectin concanavalin A; LTL-A, isolectin A from *Lotus tetragonolobus*; LTL-C, isolectin C from *Lotus tetragonolobus*; N-linked, asparagine-linked; O-linked, threonine/serine-linked; LacNAc, Gal β (1,4)GlcNAc; NMR, nuclear magnetic resonance. All sugars are in the D-configuration except Fuc, which is in the L-configuration.

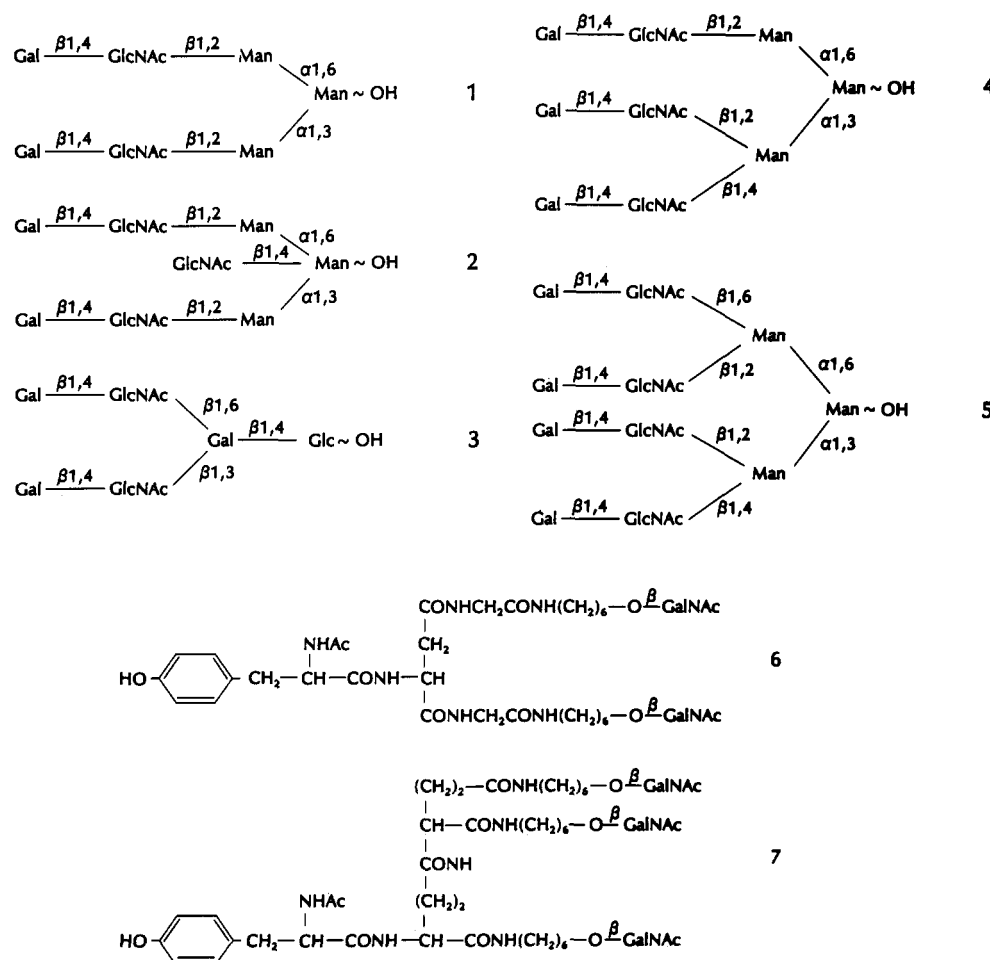


FIGURE 1: Structures of biantennary (1 and 2), triantennary (4), and tetraantennary (5) complex type oligosaccharides, biantennary O-linked oligosaccharide (3, I antigen), and bi- and triantennary synthetic glycosides (6 and 7).

crystalline and suitable for structural analysis by combined X-ray diffraction and electron microscopy (C. F. Brewer and L. Makowski, unpublished results).

In the present paper, we present the results of X-ray crystallographic, quantitative precipitation and negative stain electron microscopy studies of the precipitates formed between the Gal/GalNAc specific tetrameric lectin from *Glycine max* (soybean) (SBA) and a variety of naturally occurring and synthetic multiantennary oligosaccharides with terminal Gal or GalNAc residues. The results provide direct evidence for the formation of highly organized, unique cross-linked lattices for the precipitates of SBA with certain branch chain carbohydrates. The presence of a pseudo-2-fold axis of symmetry in both the naturally occurring and synthetic oligosaccharides is shown to correlate with their ability to form unique cross-linked lattice patterns with the lectin.

MATERIALS AND METHODS

The lectin from soybean (*Glycine max*) was purified from soyfluff (Central Soya, Chicago) as reported previously (Bhattacharyya et al., 1988a). [^{14}C]-*N*-Methyl SBA (^{14}C -NMSBA) was prepared with [^{14}C]formaldehyde as described (Jentoft & Dearborn, 1983). The protein was desalted on Sephadex G-25 in 10 mM sodium phosphate buffer, pH 7.2, containing 0.15 M NaCl, 0.1 mM MnCl_2 , and 0.1 mM CaCl_2 , and the denatured protein was removed by affinity chromatography (Bhattacharyya et al., 1988a). The free amino groups of the native and *N*-methyl lectins were estimated by the TNBS method as described (Habeeb, 1967). Oligosac-

charides 1, 2, 4, and 5 were generous gifts from Dr. Martin Haraldsson, and their synthesis have been previously described (Arnarp et al., 1982; Arnarp & Lonngren, 1981; Lonn & Lonngren, 1983). Glycosides 6 and 7 were gifts from Dr. Y. C. Lee (Lee & Lee, 1987). Oligosaccharide 3 was obtained from BioCarb, Sweden. The syntheses of glycosides 8–12 have been reported elsewhere (Sabesan et al., 1992). The concentrations of oligosaccharides 1–5 and 8–12 were determined by the phenol–sulfuric acid method (Dubois et al., 1956), whereas those of 6 and 7 were determined from their dry weights. The monosaccharides were obtained from Sigma Chemical Co., St. Louis, MO. The structures and purities of the carbohydrates were established by ^1H NMR spectroscopy at 500 MHz.

Hemagglutination–Inhibition Assays. Hemagglutination–inhibition assays were performed at 22 °C by 2-fold serial dilution in 10 mM Tris-HCl buffer, pH 7.2, containing 0.15 M NaCl, 1 mM MnCl_2 , and 1 mM CaCl_2 , using 3% suspensions of rabbit erythrocytes (Osawa & Matsumoto, 1972).

Quantitative Precipitation Analyses. Quantitative precipitation reactions were carried out in 10 mM sodium phosphate buffer, pH 7.2, containing 0.15 M NaCl, 1 mM MnCl_2 , and 1 mM CaCl_2 . The precipitation of SBA with oligosaccharide 3 was analyzed as previously described (Bhattacharyya et al., 1987a). The precipitation of SBA with 6 and 7 was done using ^{14}C -NMSBA. After the ^{14}C -labeled lectin was mixed with the glycosides and the mixture incubated for 24 h at 4 °C, the precipitates were collected by

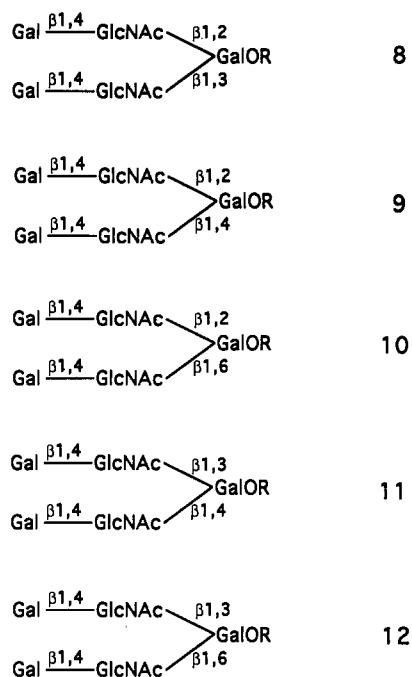


FIGURE 2: Structures of synthetic biantennary pentasaccharides 8–12. The aglycon moiety R is $(\text{CH}_2)_5\text{COOCH}_3$, and R' is $(\text{CH}_2)_5\text{-CONHNH}_2$. The Gal residue is in the β -anomeric configuration.

centrifugation, washed twice with 50 μL of buffer, and dissolved in 2 mL of buffer containing 0.1 M Gal, and the CPMs were then determined. Quantitative precipitation experiments of SBA with binary mixture of pentasaccharides 8, 9, 10, and 12 were carried out in 0.1 M Hepes buffer, pH 7.2, containing 0.15 M NaCl, 1 mM MnCl_2 , and 1 mM CaCl_2 , at 4 $^\circ\text{C}$ for 24–96 h. The amount of protein in the precipitates was determined by dissolving them in 0.1 M lactose and measuring the absorption at 280 nm.

Electron Microscopy. Negative stain electron microscopy of the precipitates was performed by placing the samples on 300-mesh carbon-coated Parlodion grids which had been freshly glow discharged for 2 min, touched to filter paper and floated on a drop of 1% phosphotungstic acid, pH 7.0, and blotted immediately. Samples were observed at 80 kV in a JEOL 1200EX electron microscope.

RESULTS

Relative Affinities of the Carbohydrates for SBA. The relative affinities of oligosaccharides 1–3 in Figure 1 (Bhattacharyya et al., 1988a) and synthetic pentasaccharides 9–12 in Figure 2 (Gupta et al., 1993) for SBA are similar to that of LacNAc while 8 binds with 7-fold-higher affinity. The affinities of 4 and 5 have not been determined because of the scarcity of these compounds. Synthetic cluster glycosides 6 and 7 possess affinities similar to that of GalNAc (Bhattacharyya & Brewer, 1992).

Quantitative Precipitation Analysis. Since glycosides 6 and 7 (Figure 1) contain tyrosine residues, quantitative precipitation studies of the precipitated lectin could not be monitored by absorption measurements at 280 nm as done previously (Bhattacharyya et al., 1988a). Instead, studies were performed with ^{14}C -labeled NMSBA. Comparison of the properties of SBA with NMSBA shows that about 85% of the free amino groups are modified in NMSBA, that the latter is a tetramer like SBA (Goldstein & Poretz, 1986), and that both have similar carbohydrate-binding affinities. However, in the presence of oligosaccharides 4 and 5, the amount

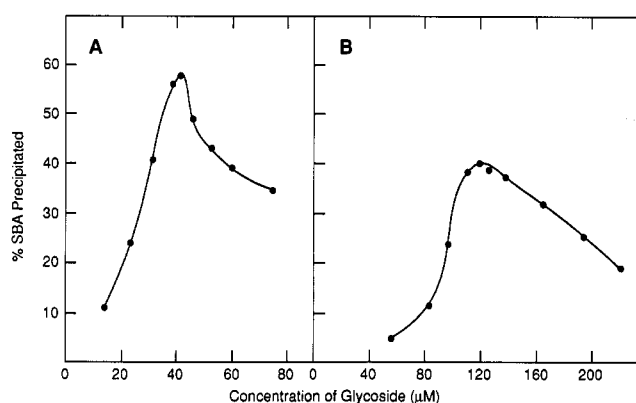


FIGURE 3: Quantitative precipitation profiles of (A) ^{14}C -NMSBA (112 μM) with glycoside 7 at 22 $^\circ\text{C}$ and (B) ^{14}C -NMSBA (216 μM) with glycoside 6 at 4 $^\circ\text{C}$.

of lectin precipitated at the equivalence points of the respective precipitation profiles is somewhat lower with NMSBA than with SBA (Bhattacharyya & Brewer, 1992).

The quantitative precipitation profiles of SBA in the presence of oligosaccharide 3 (not shown) and those of ^{14}C -NMSBA in the presence of glycosides 6 and 7 (Figure 3) are bell shaped with slight broadness in the carbohydrate excess region of the respective precipitation profiles. The profiles are similar to those obtained previously with the lectin and oligosaccharides 1, 2, 4, and 5 (Bhattacharyya et al., 1988a). Oligosaccharide 3 precipitates with the lectin at 4 $^\circ\text{C}$, but not at 22 $^\circ\text{C}$. At 570 μM SBA, approximately 26% of the lectin precipitates at the equivalence point (point of maximum precipitation) (Kabat, 1976) with an oligosaccharide to lectin monomer ratio of 1:1.6.

In the presence of 216 μM ^{14}C -NMSBA, glycoside 6 precipitates at 4 $^\circ\text{C}$ but not 22 $^\circ\text{C}$. About 40% of the lectin precipitates at the equivalence point with a glycoside to lectin monomer ratio of 1:1.8 (Figure 3B). Glycoside 7, on the other hand, precipitates with NMSBA at 22 $^\circ\text{C}$, and approximately 57% of the protein (112 μM) precipitates at the equivalence point with a glycoside to lectin monomer ratio of 1:2.8 (Figure 3A). Since SBA (and therefore NMSBA) possesses one carbohydrate-binding site per monomer (Goldstein & Poretz, 1986), the data indicate that 3 and 6 are divalent for lectin binding, and 7 is trivalent.

Synthetic biantennary pentasaccharides 8–12 (Figure 2) did not precipitate with SBA at 22 $^\circ\text{C}$, but did at 4 $^\circ\text{C}$. Analogs 9 and 12 precipitated with 275 μM SBA, whereas 8 and 11 required 343 μM SBA and 10 precipitated with 440 μM SBA. Individual quantitative precipitation profiles of SBA with all five pentasaccharides were bell shaped (not shown), which is similar to that observed with other complex type carbohydrates (Bhattacharyya et al., 1988a). Due to their precipitation activities with SBA, all five pentasaccharides were considered bivalent. In all of the above cases, the precipitation reactions could be prevented with 0.1 M lactose, but not with 0.1 M Fuc or Man. This demonstrates that the precipitates are stabilized by specific lectin–carbohydrate interactions.

Mixed Quantitative Precipitation Analysis. Figure 4 shows the quantitative precipitation profiles of SBA in the presence of binary mixtures of 8, 9, 10, and 12 at 4 $^\circ\text{C}$. The 8/9 and 9/12 mixtures both show widely separated peaks (Figure 4A,D, respectively), the 8/10 mixture shows intermediate separation of the peaks (Figure 4B), and the 8/12, 9/10, and 10/12 mixtures show small separations in the two protein peaks (Figure 4C,E,F, respectively). However, in each case, the protein precipitation profile shows two peaks.

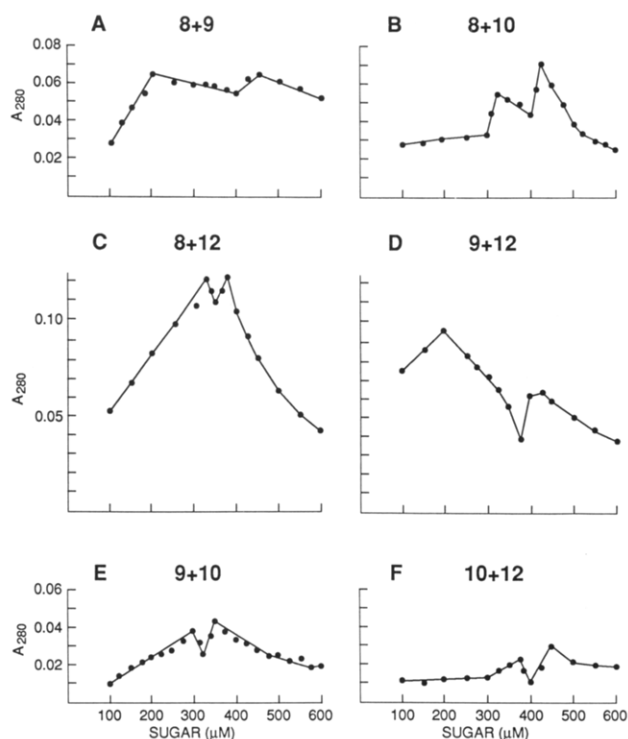


FIGURE 4: Quantitative precipitation profiles at 4 °C of (A) SBA (400 μ M) with a 1:1 ratio of glycosides 8 and 9, (B) SBA (400 μ M) with a 1:1 ratio of glycosides 8 and 10, (C) SBA (350 μ M) with a 7:3 ratio of glycosides 8 and 12, (D) SBA (400 μ M) with a 1:1 ratio of glycosides 9 and 12, (E) SBA (821 μ M) with a 1:1 ratio of glycosides 9 and 10, and (F) SBA (400 μ M) with a 1:1 ratio of glycosides 10 and 12.

Electron Microscopy of SBA Precipitates with the Oligosaccharides. Figure 5 shows the results of negative stain electron microscopy of the precipitates of SBA with oligosac-

charides 1, 2, 5, and 6. The pattern with 2 has been reported previously (Bhattacharyya et al., 1989b) and is included here for comparison. The precipitates were prepared in each case using equivalent concentrations of SBA and the carbohydrates, in terms of their binding valencies. The results show characteristic lattice patterns for the SBA precipitates with each carbohydrate. Neither SBA nor the oligosaccharides alone show any lattice pattern under similar conditions. When the precipitates were examined at different points across their respective precipitation profiles, similar electron micrographs were obtained. No patterns were observed for the precipitates of 3, 4, and 7 with the lectin.

Figure 6 shows the electron micrographs of the precipitates of SBA with pentasaccharides 8, 9, 10, and 12. No pattern was observed for SBA precipitates with 11. The cross-linked complexes of SBA with 8, 9, 10, and 12 were formed by using equivalent concentrations (in terms of binding valencies) of SBA and the carbohydrates. Different ratios of SBA to a given pentasaccharide showed the same lattice image. As can be seen in Figure 6, the patterns observed for the precipitates of SBA with 8, 9, 10, and 12 appear different for each cross-linked complex and also different from those seen in Figure 5.

Electron Microscopy of SBA Precipitates with Mixtures of Oligosaccharides. Figure 7 shows the electron micrograph of the precipitates of SBA with an equimolar mixture of oligosaccharides 1 and 6. The results show the characteristic patterns of both carbohydrate cross-linked complexes (Figure 5A,D, respectively) in the mixture, and the absence of any hybrid pattern. These findings demonstrate the presence of separate cross-linked complexes for each carbohydrate in the mixture.

Table 1 contains the results of the electron micrographs of the precipitates of SBA with 1/5 and 2/5 mixtures in different

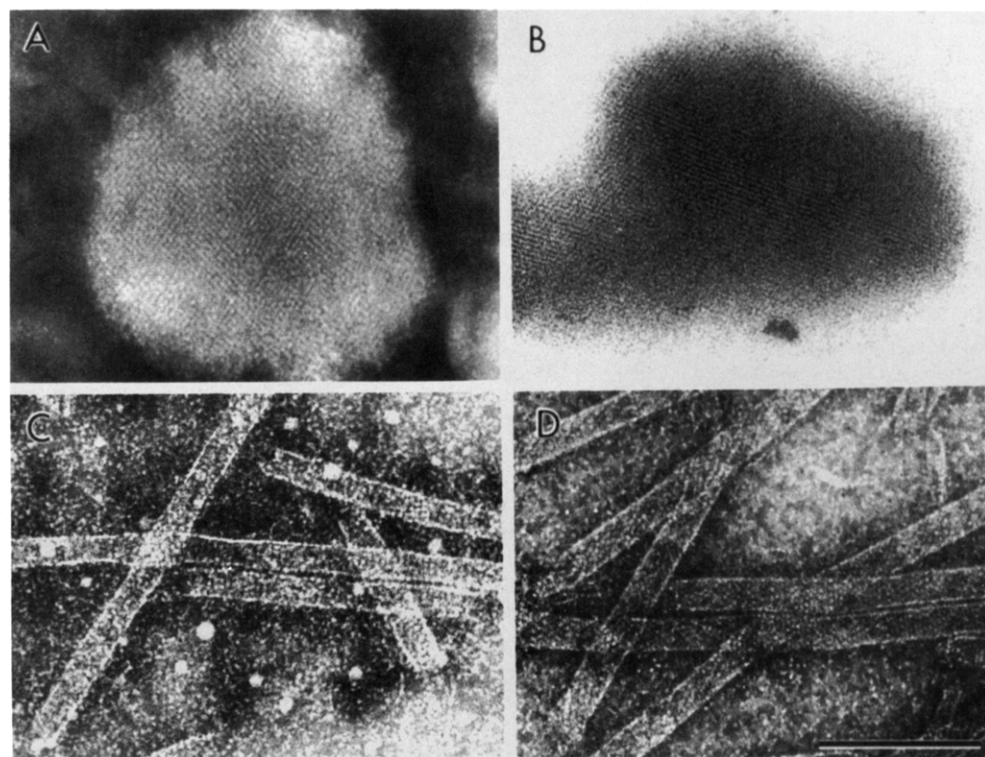


FIGURE 5: Negative stain electron micrographs of the precipitates of SBA with oligosaccharides 1 (A), 2 (B), 5 (C), and 6 (D). The concentrations of SBA and the oligosaccharides are (A) 168 μ M SBA, 93 μ M 1; (B) 175 μ M SBA, 100 μ M 2; (C) 280 μ M SBA, 73 μ M 5; and (D) 113 μ M SBA, 62 μ M 6, respectively. All precipitates were generated at 4 °C, and electron microscopy was carried out with grids precooled at 4 °C. The bar in D represents 0.1 μ m. Magnification in all panels is the same.

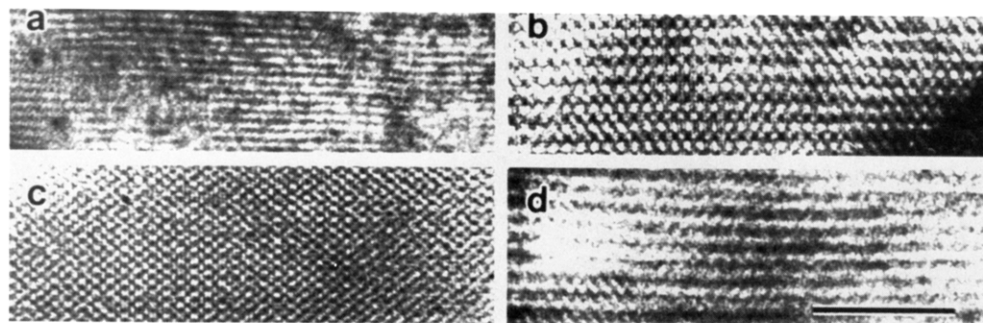


FIGURE 6: Negative stain electron micrographs of the precipitates of SBA with pentaasaccharide **8** (a), **9** (b), **10** (c), and **12** (d). The concentrations of SBA and the oligosaccharides are (a) 343 μ M SBA, 215 μ M **1**; (b) 275 μ M SBA, 155 μ M **2**; (c) 440 μ M SBA, 361 μ M **3**; (d) 275 μ M SBA, 153 μ M **5**, respectively. All precipitates were generated at 4 °C, and electron microscopy was carried out with grids precooled at 4 °C. The bar in panel d represents 0.4 μ m. Magnification in all panels is the same.

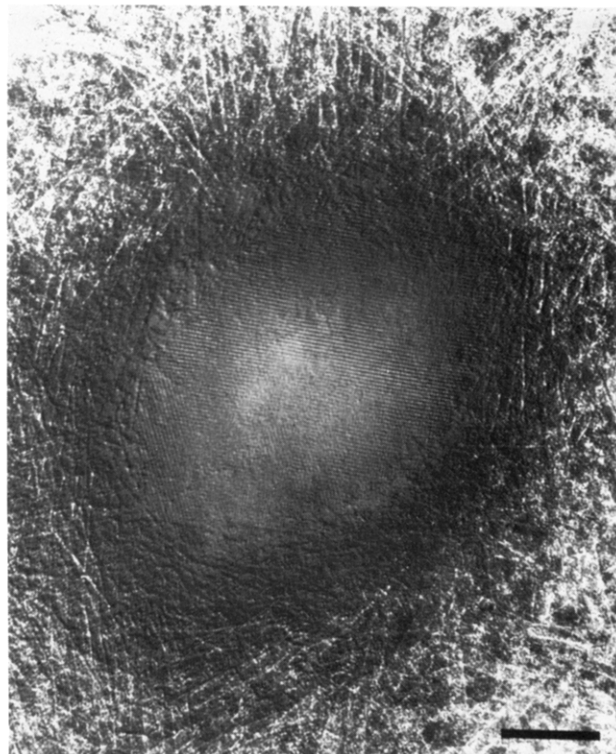


FIGURE 7: Negative stain electron micrographs of the precipitates of SBA (165 μ M) in the presence of an equimolar mixture of **1** (42 μ M) and **6** (44 μ M). The precipitation reaction was carried out at 4 °C, and electron microscopy was carried out with grids precooled at 4 °C. The bar represents 0.1 μ m.

Table 1: Electron Micrograph Patterns of the Precipitates of SBA in the Presence of Mixtures of Oligosaccharides

mix- ture	SBA (μ M)	1 (μ M)	2 (μ M)	5 (μ M)	approx ratio of SBA binding sites to oligosaccharides ^a	pattern ^b
1/5	254	72		36	1:1	A
	265	53		54	1:2	A
	259	7		66	1:19	A
	273	4		78	1:39	C (diffused)
	305	2		77	1:77	C
2/5	214		61	30	1:1	B
	264		50	49	1:2	B
	262		4.5	75	1:33	B

^a The number of SBA binding sites on each molecule of **1** and **2** is two, and that on each molecule of **5** is four (Bhattacharyya et al., 1988a).

^b The letters A, B, and C represent patterns A, B, and C in Figure 5, respectively.

ratios. In the presence of a mixture of **1** and **5**, electron micrographs show the characteristic lattice pattern of SBA

with **1** up to a 20-fold excess of **5**. However, above a 40-fold excess of **5** to **1**, the SBA lattice pattern with **5** is seen.

Similarly, in the presence of a mixture of **2** and **5**, electron micrographs of the precipitates clearly show the characteristic lattice pattern of only **2** up to a 33-fold excess of **5**. The paucity of **5** excluded electron microscopy at a higher ratio of the oligosaccharides.

DISCUSSION

Quantitative Precipitation Analyses. Oligosaccharides **1** and **2** are biantennary complex type oligosaccharides, whereas **4** and **5** are tri- and tetraantennary complex type oligosaccharides, respectively (Figure 1). Oligosaccharide **3** is the nonreducing moiety of the I antigen found in glycolipids (Marcus, 1984). Glycosides **6** and **7** are synthetic bi- and triantennary carbohydrates containing nonreducing terminal GalNAc residues and resemble, in part, the structures of serine/threonine-linked (O-linked) oligosaccharides. Quantitative precipitation profiles of the oligosaccharides with SBA indicate that **1** and **2** are bivalent, **4** is trivalent, and **5** is tetravalent in binding to the lectin (Bhattacharyya et al., 1988a). Quantitative precipitation analysis indicates that the lectin can cross-link and precipitate with **3**, **6**, and **7**, that **3** and **6** are bivalent, and that **7** is trivalent.

Biantennary analogs **8–12** in Figure 2 have the common pentasaccharide moiety (β -LacNAc)₂GalR, where R is O(CH₂)₅COOCH₃ (R is O(CH₂)₅CONHNH₂ for **12**). **12** is similar to **3**; however, it differs in its aglycon moiety. All five pentasaccharides precipitate as bivalent ligands with SBA. Quantitative precipitation profiles of SBA in the presence of binary mixtures of **8**, **9**, **10**, and **12** all show the presence of two protein peaks. Oligosaccharide **11** also showed two peaks in similar experiments with the other carbohydrates (not shown). The presence of two protein peaks in mixed quantitative precipitation profiles has been previously used to demonstrate the formation of unique homopolymeric cross-linked complexes between ConA and a series of oligomannose glycopeptides and a bisected hybrid type glycopeptide (Bhattacharyya et al., 1988b). Similar experiments have also demonstrated the formation of homogeneous cross-linked complexes between tri- and tetraantennary complex type oligosaccharides with several Gal-specific lectins including SBA (Bhattacharyya & Brewer, 1992). Thus, the results in Figure 4 indicate that **8**, **9**, **10**, and **12** all form unique cross-linked complexes with SBA.

Many lectins including SBA have previously been shown to precipitate with multiantennary N-linked oligosaccharides. Precipitation of SBA with **3**, **6**, **7**, and **12** indicates that O-linked branch chain oligosaccharides including glycolipids can also cross-link Gal/GalNAc-specific lectins.

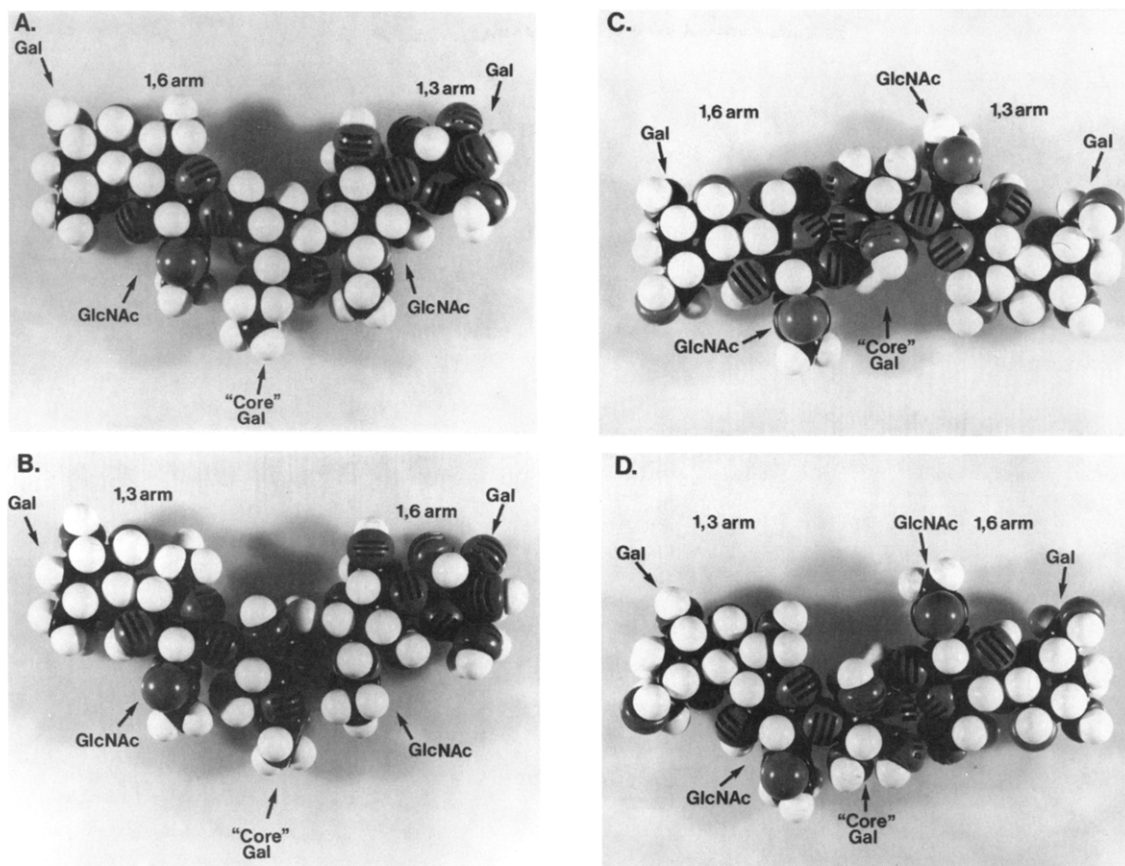


FIGURE 8: Corey–Pauling–Koltun (CPK) space-filling model of complex type oligosaccharide **1** with dihedral angle ω (O-5, C-5, C-6, and O-6 of the α (1-6) arm of the core β -Man residue) = 60° (gt) in A and B and $\omega = -60^\circ$ (gg) in C and D. The symmetry relationships of the molecules in A and B and in C and D are described in the text. GlcNAc, Gal, and Man represent *N*-acetylglucosamine, galactose, and mannose, respectively.

Electron Micrographs of the Precipitates. The electron micrographs of the negative stain precipitates of SBA with many of the carbohydrates directly show the presence of highly organized cross-linked lattices for certain complexes (Figures 5 and 6). The results establish the existence of long-range order in these SBA–carbohydrate cross-linked complexes. Similar results were obtained using electron microscopy for LTL-A with three biantennary fucosyl oligosaccharides (Bhattacharyya et al., 1990).

The negative stain electron micrographs of the precipitates of biantennary complex type oligosaccharides **1** and **2** with SBA show similar two-dimensional type patterns (Figure 5A,B, respectively). The cross-linked complexes of tetraantennary oligosaccharide **5** and synthetic cluster oligosaccharide **6** with SBA, on the other hand, show electron micrographs consisting of highly organized filaments which differ from each other in their internal patterns (Figure 5C,D, respectively). The geometries of these structures contrast with the two-dimensional type patterns observed for **1** and **2** (Figure 5A,B, respectively). Thus, the lattice pattern for tetravalent oligosaccharide **5** is unique and distinct from those of the two bivalent oligosaccharides, **1** and **2**. The fact that **6** also gives rise to a unique pattern demonstrates that a synthetic “cluster” glycoside can also form a highly organized cross-linked complex with a lectin.

Although all five synthetic pentasaccharides in Figure 2 precipitate with SBA, only the precipitates from **8**, **9**, **10**, and **12** show observable patterns by electron microscopy (Figure 6). A combination of X-ray crystallographic data and mixed precipitation experiments establish that the cross-linked lattices for **8**, **9**, **10**, and **12** are different from each other. The cross-

linked complexes of **9** and **10** with SBA, in fact, form crystals suitable for analysis by X-ray diffraction techniques (C. F. Brewer and J. Sacchettini, unpublished results). Both complexes diffract to approximately 2.5–3.0-Å resolution (detailed results will be presented elsewhere) and have the same hexagonal space group, $P6_422$. However, their unit cell dimensions are similar but distinct: $a = 143$ Å, $b = 143$ Å, $c = 106$ Å, $\alpha = 90^\circ$, $\beta = 90^\circ$, and $\gamma = 120^\circ$ for **9**; and $a = 143$ Å, $b = 143$ Å, $c = 109$ Å, $\alpha = 90^\circ$, $\beta = 90^\circ$, and $\gamma = 120^\circ$ for **10**. Thus, the difference between the two cross-linked lattices is the length of their c axes, 106 Å versus 109 Å (± 0.1 Å). This small difference in the lattice structures of **9** and **10** is detected in the mixed quantitative precipitation profile of the two oligosaccharides with SBA (Figure 4E), which shows the presence of two distinct protein precipitation peaks. (The two carbohydrates were not labeled, and thus individual peak assignments for the oligosaccharides could not be made.) Thus, the X-ray crystallographic data confirm the sensitivity of mixed quantitative precipitation analysis for determining differences in the lattice structures of **9** and **10** and, by analogy, **8**, **11**, and **12**. (Insufficient amounts of **1** and **2** precluded doing mixed precipitation studies with these two oligosaccharides.) The presence of different lattice structures of SBA with oligosaccharides **8**–**12** demonstrates the sensitivity of lectin–carbohydrate cross-linking interactions to the pattern of branching of biantennary carbohydrates.

Although lattice patterns were observed for many of the precipitates of SBA with the carbohydrates in Figures 1 and 2, the precipitates formed by oligosaccharides **3**, **4**, **7**, and **11** did not show any lattice patterns. Since these precipitates result from specific carbohydrate–protein interactions and

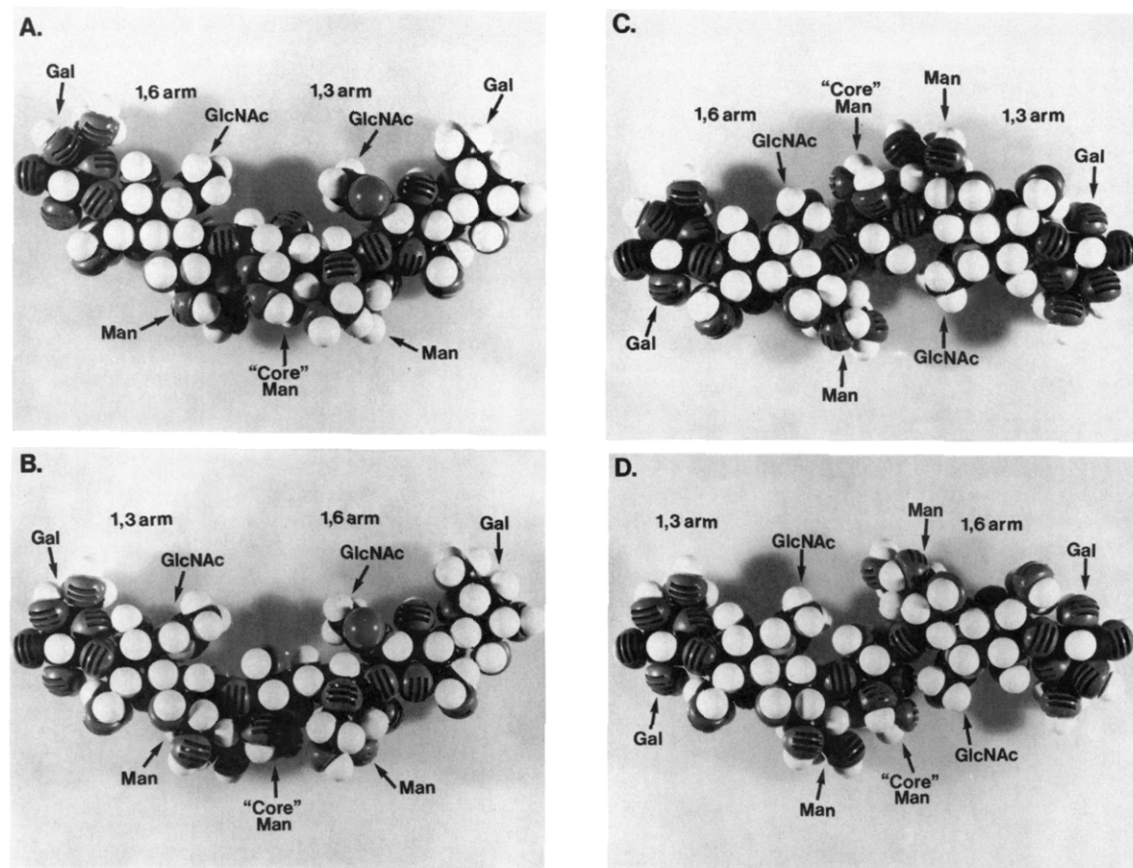


FIGURE 9: Corey–Pauling–Koltun (CPK) space-filling model of oligosaccharide **12** with dihedral angle $\omega = 60^\circ$ (*gt*) in A and B and $\omega = -60^\circ$ (*gg*) in C and D. The “core” Gal residue has a β -ethoxy aglycon moiety attached to the C-1 carbon in A and B to help visualize this region of the molecule. The symmetry relationships of the molecule in A and B and in C and D are described in the text.

defined stoichiometries, they must form specific complexes. However, it appears that these complexes may not be stable enough or possess a high enough degree of order (or symmetry) to be visualized by negative stain electron microscopy. Indeed, SBA precipitates weakly with **3** and **4**, and it is possible that sample treatment causes dissolution of the precipitates. However, the lack of observable patterns for **7** and **11** suggests that the degree of long-range order in the respective complexes may not be great enough, since both precipitate strongly with the lectin. This latter possibility is supported by a study of the cross-linked lattice structure of LTL-A with a biantennary fucosyl oligosaccharide (C. F. Brewer and L. Makowski, unpublished results).

Electron Micrographs of Binary Mixtures of Carbohydrates. Electron micrographs of the precipitates of SBA with a mixture of **1** and **6** show the coexistence of the individual patterns of both carbohydrate cross-linked complexes with the lectin (Figure 7). Formation of a heterogeneous lattice by cross-linking of two different carbohydrates with the lectin is expected to result in a pattern distinct from those observed for each carbohydrate. These results provide further evidence for the formation of homopolymeric cross-linked lattices for each carbohydrate.

The precipitates of SBA with **1/5** and **2/5** mixtures also show the characteristic patterns of the individual carbohydrate lattices in the electron micrographs. However, only one pattern is observed at a time, which depends on the relative concentrations of the two oligosaccharides in the mixture (Table 1). These observations are similar to those for LTL-A in the presence of mixtures of biantennary fucosyl oligosaccharides (Bhattacharyya et al., 1990).

The observations that the lattice pattern of **1** and **2** in the presence of **5** dominates the mixed precipitation experiments, even at relatively high concentrations of **5**, appear to be due to the differences in affinities of the oligosaccharides for SBA. Although the affinity of **5** for SBA is not known, the relatively weak precipitation activity of the lectin with **5**, compared to **1** and **2**, suggests that **5** binds more weakly than the latter two carbohydrates (Bhattacharyya et al., 1988a). Thus, in an equivalent mixture of **1** and **5** (a 2:1 ratio since the former is bivalent and the latter tetravalent), the proportion of cross-linked complexes of SBA with **1** is much higher than with **5**, since the ratio of their affinities is even greater in their respective cross-linked complexes due to the multiple binding interactions that occur in these lattices. Under these conditions, the characteristic lattice pattern of **1** is observed (Table 1). At approximately a 1:40 or higher equivalent ratio of **1** and **5**, the pattern of **5** appears in the electron micrographs. Similar results were obtained for mixtures of **2** and **5**.

Presence of Pseudo-2-fold Axes of Symmetry in Naturally Occurring and Synthetic Multivalent Oligosaccharides. The presence of long-range order in the lattice patterns observed in the electron micrographs of many of the SBA–carbohydrate precipitates requires symmetrical repeating units in the respective cross-linked lattices. The symmetry properties of SBA can be assessed since the crystal structure of the lectin in the cross-linked complexes with **9** and **10** has been determined (C. F. Brewer and J. Sacchettini, unpublished results). The crystal structure of SBA is similar to that of ConA, which is known to 1.75-Å resolution (Hardman et al., 1982), in that it is a highly symmetric tetrameric protein with several 2-fold axes of symmetry relating the carbohydrate-binding sites on each monomer.

Inspection of the structures of the carbohydrates that give observable cross-linked lattice patterns with SBA reveals that each one possesses at least one 2-fold axis of symmetry relating the Gal or GalNAc residues in the molecule. The overall symmetry of these carbohydrates, however, is pseudo-2-fold since the “core” region of these molecules contains either a trimannoside moiety or Gal residue, which are not symmetric moieties as are the outer binding epitopes. For example, CPK models of **1** in the $\omega = 60^\circ$ (*gt*) and -60° (*gg*) conformations (ω is the dihedral angle formed by O-5, C-5, C-6, and O-6 of the core β -Man residue), which are preferred conformations of the molecule (Brisson & Carver, 1983; Homans et al., 1987), show that the two terminal Gal residues are related by a pseudo-2-fold axis of symmetry in each conformation. With **1** in the $\omega = 60^\circ$ conformation, the terminal Gal residues on the α -(1–6) and α -(1–3) arms are related by a pseudo-2-fold axis of symmetry, as shown in Figure 8A,B, respectively. In this case, the axis of symmetry is parallel to the arrow designating the “core” Man (in the plane of the figure), with Figure 8A,B related by a 180° rotation of the molecule around this axis. With **1** in the $\omega = -60^\circ$ conformation, the terminal Gal residues on the two arms are also related by a pseudo-2-fold axis of symmetry, as shown in Figure 8C,D. In this case, the axis of symmetry is perpendicular to the arrow designating the “core” Man (perpendicular to the plane of the figure), with Figure 8C,D related by 180° rotation around this axis. Thus, both conformations of the oligosaccharide possess a pseudo-2-fold axis of symmetry, although their rotation axes are different. The presence of these symmetry axes in **1** permits the oligosaccharide to be part of a long-range symmetric lattice with SBA. It is not possible at this time, however, to determine which conformation of **1** occupies the lattice or if the lattice is composed of both conformers.

The same two pseudo-2-fold axes of symmetry in **1** also exist in **2**, which differs only in the presence of a bisecting GlcNAc residue. Thus, **2** must be able to assume conformation(s) similar to **1** since the cross-linked lattice patterns of the two carbohydrates with SBA are similar. Likewise, synthetic biantennary glycoside **6** also readily assumes conformations with a pseudo-2-fold axis of symmetry relating its two GalNAc residues. Biantennary oligosaccharides **8**, **9**, **10**, and **12** also possess one or more pseudo-2-fold axes of symmetry relating the terminal Gal residues in each molecule. Oligosaccharides **10** and **12**, like **1** and **2**, can exist in two different conformations which possess different pseudo-2-fold axes of symmetry. This is shown for **12** in the $\omega = 60^\circ$ (*gt*) conformation in Figure 9A,B, which is the preferred conformation of the oligosaccharide in solution as determined by ^1H NMR (Sabesan et al., 1992). The axis of symmetry is essentially parallel to the arrow designating the “core” Gal (in the plane of the figure), with Figure 9A,B related by 180° rotation around this axis. With **12** in the $\omega = -60^\circ$ (*gg*) conformation, the terminal Gal residues on the two arms are related by a pseudo-2-fold axis of symmetry, as shown in Figure 9C,D. In this case, the axis of symmetry is perpendicular to the arrow designating the “core” Gal, with Figure 9C,D related by a 180° rotation around this axis. Thus, both possible conformations of **12** also possess pseudo-2-fold axes of symmetry.

Although **8** and **9** exist in only one major preferred conformation (Sabesan et al., 1992), they both possess pseudo-2-fold axes of symmetry. This is shown for **9** in Figure 10A,B. The axis of symmetry in **9** nearly parallels the arrow designating the “core” Gal (in the plane of the figure), with Figure 10A,B related by a 180° rotation about this axis.

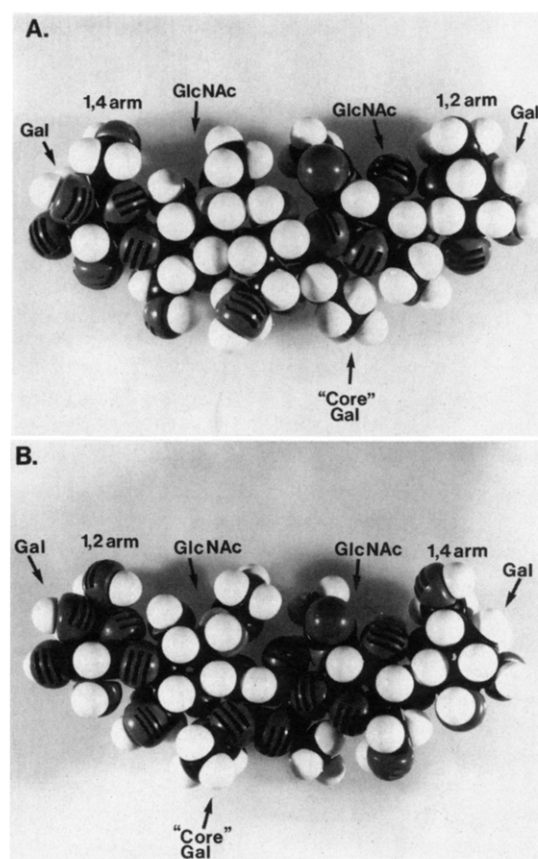


FIGURE 10: Corey–Pauling–Koltun (CPK) space-filling model of oligosaccharide **9** in A and B. The “core” Gal residue has a β -ethoxy aglycon moiety attached to the C-1 carbon in A and B to help visualize this region of the molecule. The symmetry relationship of the molecule in A and B is described in the text.

Interestingly, **11** appears not to possess a pseudo-2-fold axis of symmetry relating its two Gal residues due to unfavorable steric interactions of the two *N*-acetyl groups in the molecule, which may explain its lack of an observable electron micrograph pattern with SBA.

Tetraantennary oligosaccharide **5**, which shows an observable electron micrograph pattern with SBA, also possesses a pseudo-2-fold axis of symmetry as shown in Figure 11A,B. The axis of symmetry of the molecule is through the center “core” Man residue, perpendicular to the plane of the molecule. Interestingly, neither triantennary oligosaccharide **4** nor synthetic cluser glycoside **7** possesses a pseudo-2-fold axis of symmetry, nor does each carbohydrate show observable electron micrograph patterns with SBA even though both form homogeneous cross-linked complexes with the lectin as indicated by mixed quantitative precipitation studies (Bhattacharyya & Brewer, 1992). However, if two molecules of **4** or **7** are positioned head to head with each other (as in two Y-shaped molecules with their tops facing each other), separated by cross-linked protein, this pairing gives rise to a potential pseudo-2-fold axis of symmetry relating the two carbohydrates, which may explain their ability to form ordered homogeneous cross-linked lattices with lectins but not observable electron micrograph patterns for their precipitates.

Conclusions. The present findings using quantitative precipitation analysis and electron microscopy demonstrate the presence of long-range order in the precipitates of SBA with a variety of naturally occurring and synthetic biantennary oligosaccharides as well as a tetraantennary complex type carbohydrate. Furthermore, the existence of unique lattice structures for individual oligosaccharides and the presence of

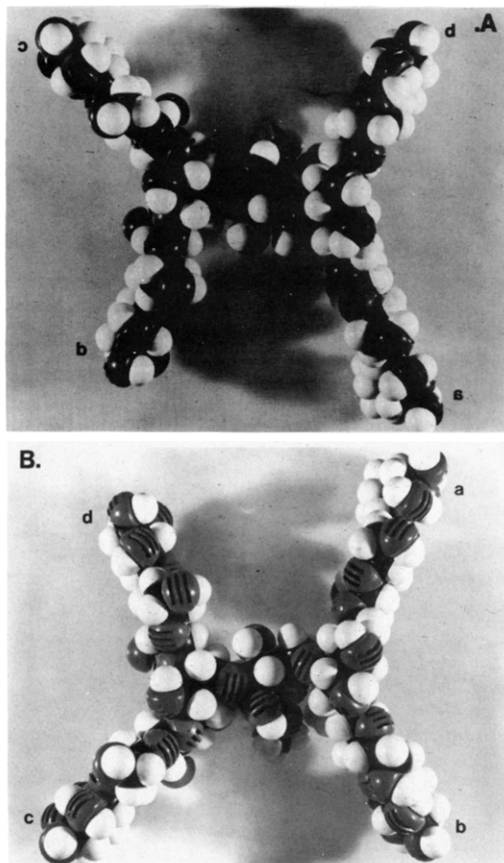


FIGURE 11: Corey-Pauling-Koltun (CPK) space-filling model of tetraantennary oligosaccharide **5** in A and B. The dihedral angle ω of the core $\alpha(1-6)$ and outer $\beta(1-6)$ linkages were set to -60° (gg). The arms a and b in both figures are the $\text{Gal}\beta(1-4)\text{GlcNAc}\beta(1-6)$ and $\text{Gal}\beta(1-4)\text{GlcNAc}\beta(1-2)$ residues, respectively, connected to the $\alpha(1-6)\text{Man}$ residue. The arms c and d are the $\text{Gal}\beta(1-4)\text{GlcNAc}\beta(1-4)$ and $\text{Gal}\beta(1-4)\text{Gal}\beta(1-2)$ residues, respectively, connected to the $\alpha(1-3)\text{Man}$ residue. The "core" Man has an α -ethoxy aglycon moiety attached to the C-1 carbon. The symmetry relationship of the molecule in A and B is described in the text.

the lattice patterns of the individual carbohydrates in binary mixed precipitates with SBA provide evidence for the formation of unique molecular packing interactions as the basis for the formation of homogeneous carbohydrate-SBA cross-linked complexes. Studies of the molecular interactions that give rise to the differences in the structures of the cross-linked complexes of **9** and **10** with SBA are currently underway (C. F. Brewer and J. Sacchettini, unpublished results).

The present results also demonstrate the existence of pseudo-2-fold axes of symmetry in naturally occurring and synthetic branch chain carbohydrates that correlate with observable electron micrograph patterns in their precipitates with SBA. The presence of such symmetry elements in both the carbohydrates and the lectin suggests that both types of molecules are designed to form unique homogeneous cross-linked complexes. These results may relate to the structure-function properties of lectins and branch chain carbohydrates and, in turn, to their possible roles in biological systems.

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