Precipitation of Galactose-Specific Lectins by Complex-Type Oligosaccharides and Glycopeptides: Studies with Lectins from *Ricinus communis* (Agglutinin I), *Erythrina indica*, *Erythrina arborescens*, *Abrus precatorius* (Agglutinin), and *Glycine max* (Soybean)†

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ABSTRACT: We have recently demonstrated that certain oligomannose and bisected hybrid type glycopeptides and bisected complex type oligosaccharides are bivalent for binding to concanavalin A and can precipitate the lectin [Bhattacharyya, L., Ceccarini, C., Lorenzoni, P., & Brewer, C. F. (1987) *J. Biol. Chem. 262*, 1288–1293; Bhattacharyya, L., Haraldsson, M., & Brewer, C. F. (1987) *J. Biol. Chem. 262*, 1294–1299]. The present results show that tri- and tetraantennary complex type oligosaccharides containing nonreducing terminal galactose residues, and a related triantennary glycopeptide, precipitate the D-galactose-specific lectins from *Ricinus communis* (agglutinin I) (RCA-I), *Erythrina indica* (EIL), *Erythrina arborescens* (EAL), and *Glycine max* (soybean) (SBA). Nonbisected and bisected biantennary complex type oligosaccharides can precipitate SBA, which is a tetrameric lectin, but not RCA-I, EIL, or EAL, which are dimeric lectins. The relative affinities of the oligosaccharides and glycopeptide were determined by hemagglutination inhibition measurements and their valencies by quantitative precipitin analyses. The equivalence points of the precipitin curves indicate that the tri- and tetraantennary oligosaccharides are tri- and tetravalent, respectively, for EIL, EAL, and SBA binding. However, the oligosaccharides are all trivalent for RCA-I binding due apparently to the larger size of the monomeric subunit of the lectin. The triantennary glycopeptide was also trivalent for RCA-I and EIL binding. Biantennary oligosaccharides with adequate chain lengths were found to be bivalent for binding to SBA; those with shorter chains did not precipitate the lectin. The results indicate that, in general, each arm of the branched-chain oligosaccharides can bind individual lectin molecules, which leads to cross-linking and precipitation. The extent of precipitation is dependent not only on the valency of the oligosaccharides but, as in the case of the triantennary carbohydrates, on their branching patterns as well. These findings are discussed in terms of the possible structure–function properties of lectins and complex-type oligosaccharides.

Cell surface glycoconjugates have been implicated as receptors in a variety of biological processes including cellular recognition and signal transduction events (Brandley & Schnaar, 1986; Lennarz, 1980; Monsigny, 1984). They are involved in the regulation of cellular functions such as endocytosis, growth, motility, morphology, and differentiation (Brandley & Schnaar, 1986; Lennarz, 1980; Monsigny, 1984). In addition, the glycosylation patterns of many cellular and serum glycoproteins are altered during many of these processes, as well as under pathological conditions including neoplastic transformation (Brandley & Schnaar, 1986; Kobata, 1984; Lennarz, 1980; Monsigny, 1984; Nicolson, 1976).

Asparagine-linked (N-linked) oligosaccharides represent one class of cell surface carbohydrates which are generally classified into three subtypes: oligomannose, complex, and hybrid (Kobata, 1984, Lennarz, 1980). Complex-type oligosaccharides are often the final products of N-glycosylation pathways and are generally present on the surface of cells in greater amounts than the other two subtypes (Kornfeld & Kornfeld, 1985; Snider, 1984). The structures of a wide variety of complex-type glycopeptides and oligosaccharides are known (Kobata, 1984; Vliegenthart et al., 1983); however, little is known about their molecular binding properties other than their interactions with glycosylases (Lennarz, 1980; Monsigny, 1984) and their binding specificities to lectins (Baenziger & Fiete, 1979; Brewer & Bhattacharyya, 1986; Debray et al., 1981; Narasimhan et al., 1986; Ohyama et al., 1985).

Lectins are cell agglutinating proteins of nonimmune origin that bind to specific carbohydrate determinants without chemically modifying them (Goldstein et al., 1980). They are found in plants, animal tissues, and invertebrates. Plant lectins have been the most widely investigated because of their abundance and ease of isolation (Goldstein & Poretz, 1986) and their use in exploring the membrane properties of both normal and transformed cell (Lis & Sharon, 1986). In many cases, the cell surface receptors for lectins are N-linked complex-type glycopeptides (Kobata, 1984; Kornfeld & Kornfeld, 1985; Nicolson, 1976; Snider, 1984). Thus, elucidating their binding interactions provides a means of examining the molecular recognition properties of this group of cell surface carbohydrates.

The molecular binding properties of N-linked glycopeptides have been characterized in the past primarily by their relative

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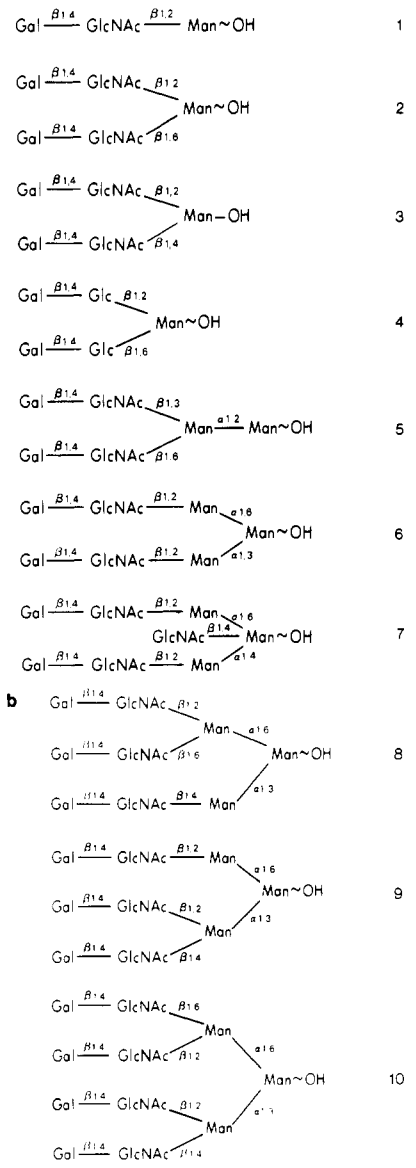

FIGURE 1: (a) Structures of trisaccharide **1**, pentasaccharide **4**, and biantennary complex type oligosaccharides **2**, **3**, **5**, **6**, and **7**. In oligosaccharide **3**, the mannose is a reduced alditol. (b) Structures of triantennary complex type oligosaccharides **8** and **9** and tetraantennary complex type oligosaccharide **10**. Man, Glc, Gal, and GlcNAc represent mannose, glucose, galactose, and *N*-acetylglucosamine residues, respectively.

affinities for lectins (Baenziger & Fiete, 1979; Debray et al., 1981) or abilities to bind to lectin affinity columns (Narasimhan et al., 1986; Ohyama et al., 1985). However, we have recently reported that certain oligomannose and bisected hybrid type glycopeptides (Bhattacharyya & Brewer, 1986a; Bhattacharyya et al., 1987a) and bisected complex type oligosaccharides (Bhattacharyya et al., 1987b) precipitate concanavalin A (Con A)[1] from solution. Quantitative precipitin studies indicate that the carbohydrates are bivalent for Con A binding. These results provide the first evidence of bivalent binding of N-linked glycopeptides to a lectin. More recently, we have reported that triantennary complex type oligosaccharide **8** (Figure 1), which possesses terminal nonreducing galactose residues, is a trivalent ligand for the galactose binding lectin from *Erythrina indica* (EIL) and precipitates the protein (Bhattacharyya & Brewer, 1986b).

In this study, we report the binding and precipitation of bi-, tri-, and tetraantennary complex type oligosaccharides containing terminal nonreducing galactose residues (Figure 1), and of the N-linked triantennary glycopeptide from asialofetuin, with five galactose-specific lectins.

Materials and Methods

The seeds of *Erythrina indica* Lamm. and *Erythrina arborescens* Roxb. were purchased from United Chemicals, Calcutta, India. The lectins were purified by affinity chromatography on partially hydrolyzed ECD–Sepharose as described previously (Bhattacharyya et al., 1981). RCA-I was purchased from Sigma Chemical Co. APA was kindly provided by Dr. N. K. Sinha, Bose Institute, Calcutta, and was purified according to Roy et al. (1976). The purification of SBA is described below. RCA-I and SBA undergo aggregation on storage and were precipitated by ammonium sulfate (80% saturation), dissolved, and dialyzed against buffer before use. Following this treatment, gel filtration on Sephadex G-200 shows a single peak corresponding to a molecular weight of approximately 130 000 for either protein. The protein concentrations were determined spectrophotometrically with the respective extinction coefficients and reported in terms of the monomer. At 280 nm, $A^{1\%,1cm}$ is 13.4, 15.9, 14.6, 11.8, and 12.8 for EIL (Bhattacharyya et al., 1981), EAL (Bhattacharyya et al., 1981), APA (Olsnes et al., 1974), RCA-I (Olsnes et al., 1974), and SBA (Lotan et al., 1974), respectively. The syntheses of trisaccharide **1** and the complex-type oligosaccharides in Figure 1 have been previously reported (Arnarp & Lonngren, 1980, 1981; Arnarp et al., 1981, 1982, 1985; Kaifu & Osawa, 1976; Lonn & Lonngren, 1983). Mono- and disaccharides were obtained from Sigma Chemical Co. The triantennary asialoglycopeptide analogue of oligosaccharide **9** (AFGP) was isolated from fetuin as described (Nilsson et al., 1979). A small amount of oligosaccharide formed due to β-*N*-acetylglucosaminidase activity associated with the commercial Pronase (Lis & Sharon, 1978) was separated from the glycopeptide on a Dowex AG 50-X1 (H^+ form) column. The concentrations of oligosaccharides and glycopeptide were measured by the phenol–sulfuric acid method with D-mannose as the standard (Dubois et al., 1956). The purity of these compounds was checked by 400-MHz ^1H nuclear magnetic resonance spectroscopy.

Purification of Soybean Lectin. Soyfluff (Central Soya, Gibson City, IL) was extracted overnight with 10 mM sodium phosphate buffer, pH 7.2, containing 0.15 M NaCl (PBS) (1:5 w/v) at 4 °C. The supernatant obtained by centrifugation was brought to pH 4.5 by 2 N HCl, and the precipitates were discarded. The supernatant was made 80% saturated with ammonium sulfate, and the precipitates collected by centrifugation were dissolved and dialyzed extensively against PBS. The lectin was purified by affinity chromatography at 4 °C on a partially hydrolyzed ECD–Sepharose (Porath et al., 1971) column preequilibrated in PBS. After the unabsorbed proteins were removed by extensive washing with the equilibrating buffer, the lectin was eluted from the column with 0.2 M galactose in the same buffer. The purity of the preparation was checked by polyacrylamide gel electrophoresis (Davis, 1964), by isoelectric focusing (Wrigley, 1971), and by com-

[1] Abbreviations: Con A, concanavalin A, the lectin from jack bean; EIL, lectin from the seeds of *Erythrina indica*; EAL, lectin from the seeds of *Erythrina arborescens*; RCA-I, aggutinin I from the seeds of *Ricinus communis*; APA, agglutinin from the seeds of *Abrus precatorius*; SBA, lectin from the seeds of *Glycine max*; ECD–Sepharose, ethylenechlorohydrin-cross-linked desulfated Sepharose; AFGP, triantennary N-linked glycopeptide from asialofetuin; PBS, 10 mM sodium phosphate buffered with 0.15 M sodium chloride, pH 7.2; MeβGal, methyl β-D-galactopyranoside; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride. All sugars are in the D configuration.

Table I: Inhibitory Power of Complex-Type Oligosaccharides and AFGP for Hemagglutination by the Galactose-Specific Lectins

oligosaccharide or glycopeptide	relative inhibitory potency[a]				
	EIL	EAL	RCA-I	APA	SBA[b]
MeβGal[c]	1	1	1	1	1
lactose	1	2	1	1	0.5
N-acetyllactosamine	7	10	5	3	2
1	11	11	6	*e*	1
2	34	24	25	7	3
3	36	20	20	nd[d]	2
4	4	6	5	3	1
5	33	24	17	*e*	0.5
6	30	24	25	12	1
7	33	24	25	9	4
8	100	120	26	*e*	nd[d]
9	80	70	36	*e*	nd[d]
10	80	80	40	nd[d]	nd[d]
AFGP	45	45	12	nd[d]	nd[d]

[a] All data normalized to MeβGal. Higher values indicate greater inhibitory potency. [b] Data for SBA were taken at 4 °C. [c] The minimum concentrations of MeβGal required for complete inhibition of agglutination by EIL, EAL RCA-I, APA, and SBA at four hemagglutinating doses (Osawa & Matsumoto, 1972) are 3.1, 12.5, 6.3, 50, and 6.3 mM, respectively. [d] nd = not determined. [e] APA was not inhibited by 9.2 mM **1**, 6.0 mM **5**, 5.3 mM **8**, and 6.2 mM **9**.

parison with a standard sample (Sigma Chemical Co.).

Quantitative Precipitation Assays. The assays were performed either with 0.1 M Tris-HCl buffer, pH 7.2, containing 0.9 M KCl, 1 mM $MnCl_2$ and 1 mM $CaCl_2$ or with PBS as described previously (Bhattacharyya et al., 1987a,b).

Hemagglutination Inhibition Assays. These assays were done at room temperature by the 2-fold serial dilution technique (Osawa & Matsumoto, 1972) in PBS with a 3% (v/v) suspension of rabbit (for EIL, EAL, RCA-I, and SBA) or human blood group O (for APA) erythrocytes.

RESULTS

Hemagglutination Inhibition Assays. Table I shows the results of inhibition of hemagglutination by the five lectins with the oligosaccharides in Figure 1, related simple sugars, and AFGP. The results for RCA-I, EIL, EAL, and APA were similar, although APA binds the mono- and oligosaccharides more weakly than the other three lectins. In general, *N*-acetyllactosamine binds better than lactose (from 3 to 7 times), and **1** binds as good as *N*-acetyllactosamine. Biantennary oligosaccharides containing two nonreducing terminal *N*-acetyllactosamine moieties (**2**, **3**, and **5**–**7**) bind 3–5 times better than *N*-acetyllactosamine. However, **5** binds RCA-I and APA slightly weaker than **2**, **6**, and **7**. Oligosaccharide **4**, which contains two terminal lactose residues, has 3–5 times stronger affinity than lactose but binds 4–8 times weaker than other biantennary oligosaccharides containing terminal *N*-acetyllactosamine residues. Triantennary oligosaccharides **8** and **9** bind EIL and EAL 7–13 times better than *N*-acetyllactosamine and 3–5 times better than the biantennary molecules. Oligosaccharides **8** and **9** are nearly as potent as the biantennary molecules for RCA-I binding. In all cases, tetraantennary oligosaccharide **10** binds as strongly as the triantennary molecules. AFGP binds to EIL, EAL, and RCA-I with somewhat weaker affinities than oligosaccharide **9**. Affinity data for the tri- and tetraantennary oligosaccharides binding to APA were not obtained because of the high concentrations required for inhibition and limited amounts of carbohydrates.

In most cases, the affinities of SBA for the bi-, tri-, and tetraantennary oligosaccharides at approximately 10 mM concentrations were too weak to produce any detectable inhibition of hemagglutination at room temperature; however, inhibition was observed at 4 °C for the biantennary oligosaccharides. [Data for **8**–**10** were not obtained due to the high concentrations of oligosaccharides required and limited amounts of these compounds. However, these oligosaccharides do bind specifically to SBA since their precipitation with the lectin can be inhibited or reversed by MeβGal or GalNAc (see below)]. MeβGal, lactose, and *N*-acetyllactosamine all bind essentially equally well, as do the biantennary oligosaccharides.

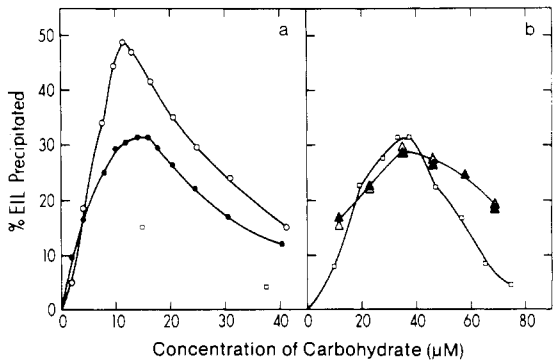

FIGURE 2: Precipitin curves for precipitation of EIL by (a) **8** (●), **9** (□), and **10** (○) and (b) **9** (□) and AFGP (△, ▲). A pH 7.2 Tris-HCl buffer (0.1 M) containing 0.9 M KCl was used for the precipitation by **8**–**10**. The precipitation reactions with AFGP were carried out at pH 7.2 in the same buffer (▲) and in pH 7.2 sodium phosphate buffer (10 mM) containing 0.15 M NaCl (△). The temperature was 22 °C for **8**–**10** and 4 °C for AFGP. See Table II for protein concentrations.

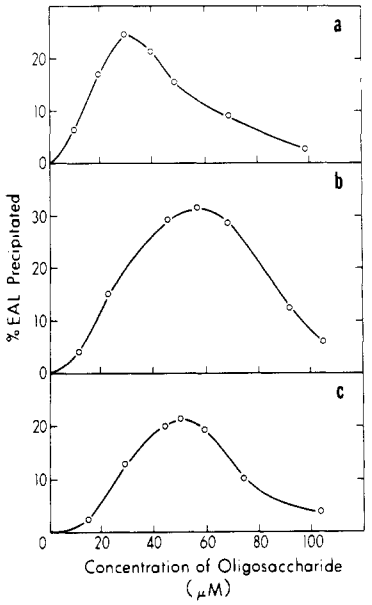

FIGURE 3: Precipitin curves for precipitation of EAL by (a) **10**, (b) **8**, and (c) **9** at 4 °C. The buffer was 0.1 M Tris-HCl containing 0.9 M KCl at pH 7.2. See Table II for protein concentrations.

Quantitative Precipitation Assays. Figures 2–4 show quantitative precipitin curves for EIL, EAL, and RCA-I, respectively, in the presence of **8**, **9**, and **10**. [The profile for **8** with EIL is included from Bhattacharyya and Brewer (1986b) in Figure 2 for the purpose of comparison.] The precipitin profiles of EIL and RCA-I with AFGP are shown in Figures 2 and 4, respectively. Figure 5 shows the profiles for precipitation of SBA by **6**, **7**, **9**, and **10**. In each case, the precipitates were prevented from forming in the presence of 0.1 M MeβGal or GalNAc and dissolved upon addition of either monosaccharide. The concentration of oligosaccharide or glycopeptide at the equivalence point (region of maximum precipitation) of each precipitin curve and the concentration

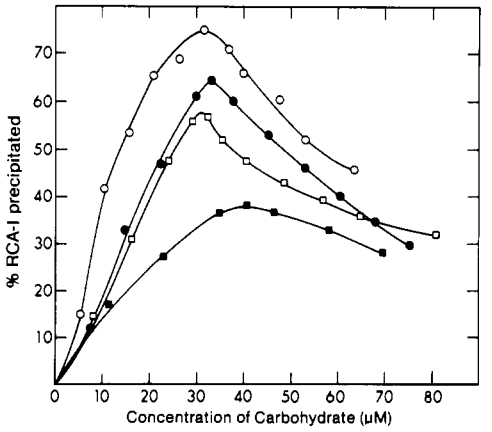

FIGURE 4: Precipitin curves for precipitation of RCA-I by **8** (□), **9** (●), **10** (○), and AFGP (■). The temperature was 22 °C, and the buffer was the same as that in Figure 3. See Table II for protein concentrations.

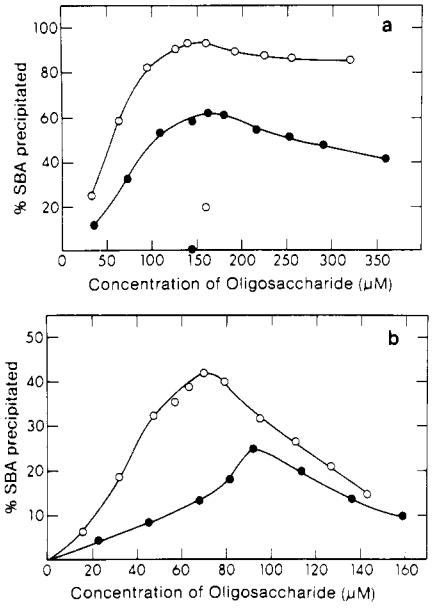

FIGURE 5: Precipitin curves for precipitation of SBA by (a) **6** (●) and **7** (○) and (b) by **9** (●) and **10** (○) at 4 °C. The lower data points in (a) show the results at 22 °C. The buffer was the same as that in Figure 3. See Table II for protein concentrations.

of protein monomer are shown in Table II. The ratio of these concentrations gives the stoichiometry of binding of the oligosaccharide (glycopeptide) to lectin (Kabat, 1976) and is included in Table II. Altering the buffer (Tris-HCl or sodium phosphate) or salt concentration (0.15–0.9 M NaCl or KCl) generally had less than 10% effects on the amounts of precipitates formed and did not change the stoichiometry of binding of carbohydrates to lectins, as shown in Figure 2b for the precipitation of EIL by AFGP.

The results show that the stoichiometries of binding of **8–10** and AFGP to EIL are 1:2.9, 1:2.7, 1:4.0, and 1:3.0, respectively. At a protein concentration of 44 μM, **9** precipitated about 15% of EIL at the equivalence zone, compared to approximately 32 and 49% by **8** and **10**, respectively (Figure 2a). Precipitation of EIL by **9** was thus studied at a higher protein concentration (94 μM) (Figure 2b). The biantennary oligosaccharides failed to precipitate EIL at room temperature or 4 °C. AFGP did not precipitate EIL at room temperature but did so at 4 °C.

In a preliminary experiment, EAL was found to give only weak turbidity with **8** at room temperature. However, at 4 °C the solution gave much stronger precipitation, and therefore

Table II: Stoichiometries of Precipitation Reactions of the Galactose-Specific Lectins with the Bi-, Tri-, and Tetraantennary Oligosaccharides and AFGP[a]

oligosaccharide or glycopeptide	protein concn (μM)	concn of oligosaccharide/ glycopeptide at equivalence point (μM)	ratio of concn of oligosaccharide/ glycopeptide to protein monomer
		EIL	
8	44	15	1:2.9
9	94	35	1:2.7
10	44	11	1:4.0
AFGP[b]	104	37	1:3.0
		EAL	
8	160	57	1:2.8
9	140	50	1:2.7
10	120	31	1:3.9
		RCA-I	
8	92	31	1:3.0
9	92	33	1:2.8
10	92	32	1:2.9
AFGP	118	41	1:2.9
		SBA	
6	260	160	1:1.6
7	260	150	1:1.7
8	280	120	1:2.3
9	260	92	1:2.8
10	260	69	1:3.8

[a] Data for EIL and RCA-I were obtained at 22 °C and for EAL and SBA at 4 °C. [b] Data obtained at 4 °C.

studies were done at this temperature (Figure 4). Table II shows the ratios of concentrations of **8–10** at the equivalence points to those of EAL to be 1:2.8, 1:2.7 and 1:3.9, respectively. The biantennary oligosaccharides also failed to precipitate the lectin at room temperature or 4 °C.

Figure 4 shows the results of similar studies with RCA-I. Table II shows that the ratios of concentrations of **8–10** and AFGP at the equivalence points to RCA-I are 1:3.0, 1:2.8, 1:2.9, and 1:2.9, respectively. As with EIL and EAL, the biantennary oligosaccharides failed to precipitate RCA-I at room temperature or 4 °C.

Figure 5a shows that SBA is precipitated by biantennary oligosaccharides **6** and **7**. At room temperature, **6** does not precipitate the lectin, and **7** precipitates only about 20% of the protein at the equivalence zone. At 4 °C, however, **6** and **7** precipitates approximately 63 and 94% of the lectin, respectively. The shape of the curves indicates weak binding between SBA and the oligosaccharides (Kabat, 1976). Oligosaccharides **2–5** did not precipitate SBA at 4 °C. Tri- and tetraantennary oligosaccharides **8–10** precipitate SBA (Figure 5b), though more weakly than **6** and **7**. Table II shows that the stoichiometries of binding of **6–10** to SBA are 1:1.6, 1:1.7, 1:2.3, 1:2.8, and 1:3.8, respectively.

None of the bi-, tri-, or tetraantennary oligosaccharides precipitated APA at room temperature or 4 °C.

Discussion

Properties of Lectins. EIL and EAL have close physicochemical, structural, and carbohydrate binding properties (Bhattacharyya et al., 1981, 1986). EIL has a molecular weight of 68 000 and consists of two nearly equal subunits (Bhattacharyya et al., 1981). EAL has a molecular weight of 58 000 and consists of two equal subunits (Bhattacharyya et al., 1981, 1986). RCA-I and APA are dimeric lectins with molecular weights of 120 000 (Olsnes et al., 1974) and 126 000 (Olsnes et al., 1974; Roy et al., 1976), respectively. Each monomer of both lectins consists of an A chain and a B chain

connected by a disulfide bridge (Olsnes et al., 1974). SBA is a tetrameric lectin of molecular weight 120 000 with four equal subunits (Lotan et al., 1974; Shaanan et al., 1984).

EIL binds one lactose molecule per monomer with a K_d of 0.5 mM at 25 °C (Bhattacharyya et al., 1981). EAL is reported to possess similar mono- and oligosaccharide binding properties (Bhattacharyya et al., 1981). RCA-I (Podder et al., 1974) and APA (Olsnes et al., 1974) bind one lactose molecule per monomer. SBA is reported to bind one monosaccharide per monomer (De Boeck et al., 1984).

Interaction of Complex-Type Oligosaccharides with the Lectins. The results of inhibition of hemagglutination by the five galactose-specific lectins with complex-type oligosaccharides indicate broad similarity in the carbohydrate binding properties of RCA-I, EIL, EAL, and APA (Table I), with a somewhat different pattern for SBA. Previous studies with simple sugars indicate that RCA-I, EIL, EAL, and APA are specific for nonreducing terminal galactose residues (Goldstein & Poretz, 1986). These findings are supported by our observation that removal of the terminal galactose residues of **7** abolishes binding to the lectins. Similar findings have been reported for RCA-I interactions with complex-type glycopeptides and oligosaccharides (Baenziger & Fiete, 1979; Debray et al., 1981). The results indicate that the nonreducing terminal galactose residue is the primary binding determinant and contributes approximately 80% or more of the free energy of *N*-acetyllactosamine binding, although the lectins have loci for the acetamido group of *N*-acetyllactosamine.

The present findings indicate that the location of the linkages to the branching mannose residues and the length of the individual chains in the oligosaccharides do not significantly influence their affinities for RCA-I, EIL, EAL, and APA. Also, the reduced alditol form of the mannose residue in **3** did not affect binding, nor did the presence of a so-called "bisecting" GlcNAc residue linked $\beta(1-4)$ to the core mannose influence binding of the oligosaccharides (compare **6** with **7**). The latter result contrasts with the finding that the presence of a bisecting GlcNAc strongly influences the interactions of complex-type glycopeptides and oligosaccharides with Con A (Bhattacharyya et al., 1987b; Brewer & Bhattacharyya, 1986; Narasimhan et al., 1986).

The increased inhibitory potencies of **2**, **3**, and **5–9** relative to *N*-acetyllactosamine with EIL and EAL are consistent with their enhanced affinities being due to the presence of multiple terminal *N*-acetyllactosamine residues in individual molecules which statistically increases their probability of binding. Oligosaccharide **4** which has two terminal lactose residues also binds approximately 3–4 times better than lactose in both lectins, which is consistent with this mechanism of binding. The enhanced binding of the bi- and triantennary oligosaccharides is similar to the 5- and 20-fold enhanced affinities of $\alpha(1-2)$-linked di- and trisaccharides of mannose, respectively, for Con A, relative to methyl α-D-mannopyranoside (So & Goldstein, 1968), which have been assigned to a statistical model of increased binding due to multiple binding residues in individual molecules (Brewer & Brown, 1979). Interestingly, the $\alpha(1-2)$ tetrasaccharide of mannose showed no increase in affinity beyond the corresponding trisaccharide (So & Goldstein, 1968), which is similar to that observed for tetraantennary oligosaccharide **10** relative to the triantennary molecules **8** and **9**, in the present study. These results suggest that the "statistical" mechanism enhances the binding affinity of oligosaccharides which possess up to three binding residues.

A similar model appears to operate in the enhancement of affinities of the biantennary oligosaccharides over single-chain oligosaccharides for RCA-I and APA. However, in the case of RCA-I, the triantennary oligosaccharides bind only as strong as the biantennary oligosaccharides, which agrees with previously reported findings (Baenziger & Fiete, 1979).

The binding data of AFGP for EIL and RCA-I indicate that the glycopeptide binds with somewhat weaker affinity to the lectins compared to **9**, the oligosaccharide analogue of AFGP. This may be due to the additional bulkiness of the core region of AFGP. The data for AFGP binding to RCA-I agree with previously reported findings (Baenziger & Fiete, 1979).

SBA, classified as a GalNAc-specific lectin, also binds galactose, though more weakly (Goldstein & Poretz, 1986). The lectin binds the biantennary oligosaccharides in Figure 1, although data had to be acquired at 4 °C. The present results indicate that the affinities of oligosaccharides for SBA are insensitive to differences in the structures of the carbohydrates in Figure 1 and that the lectin binds predominantly to the terminal galactose residue(s) in these oligosaccharides.

Precipitation of Tri- and Tetraantennary Complex Type Oligosaccharides and a Related Triantennary Glycopeptide. The precipitin profiles in the present study are similar to antigen–antibody (Kabat, 1976), lectin–polysaccharide (So & Goldstein, 1969), and glycopeptide– (Bhattacharyya & Brewer, 1986a; Bhattacharyya et al., 1987a) and oligosaccharide–Con A (Bhattacharyya et al., 1987b) precipitin curves and suggest similar multivalent interactions between complex-type oligosaccharides and the galactose-specific lectins.

In general, the precipitation data for tri- and tetraantennary oligosaccharides, **8–10**, with the lectins reflect the relative affinities of the carbohydrates for the proteins. Thus, the oligosaccharides precipitate with RCA-I, EIL, EAL, and SBA but not with APA. Precipitation of RCA-I and EIL occurs at room temperature, whereas EAL and SBA, which bind more weakly, require lower temperature (4 °C). The same conclusion can also be extended to the glycopeptide. AFGP has lower affinity for RCA-I than **9** and precipitates less RCA-I than the oligosaccharide under similar conditions. Also, AFGP has lower affinity for EIL than **9** and precipitates EIL in the cold while the oligosaccharide does so at room temperature.

The stoichiometry of the precipitin reactions are given in Table II. For RCA-I, EIL, EAL, and SBA, and ratios of the concentrations of the triantennary oligosaccharides **8** and **9** to protein monomers are approximately 1:3. Since, the lectins have one sugar binding site per monomer, **8** and **9** are trivalent. The ratios of the concentrations of tetraantennary oligosaccharide **10** to protein monomer are approximately 1:4 for EIL, EAL, and SBA but 1:3 for RCA-I. Thus, **10** is tetravalent in binding the former three lectins but trivalent for RCA-I (discussed below).

As expected (Kabat, 1976), the percentage of precipitated lectin at the equivalence zone for each oligosaccharide is related to the valency of the carbohydrate. Thus, **8–10** have approximately the same affinities for EIL, EAL, and RCA-I, yet tetravalent **10** gives a higher percentage of precipitation with the lectins than trivalent **8** and **9**. (An apparent exception is the precipitation of SBA by the biantennary oligosaccharides, which is discussed below.)

Interestingly, the branching patterns of the two triantennary oligosaccharides influence the extent of their precipitation with the lectins, even though their respective affinities are nearly the same. Thus, **8** gives better precipitation of EIL and EAL compared to **9**. However, for SBA, the reverse is true (data for **8** are not shown), and RCA-I precipitates **8** and **9** almost

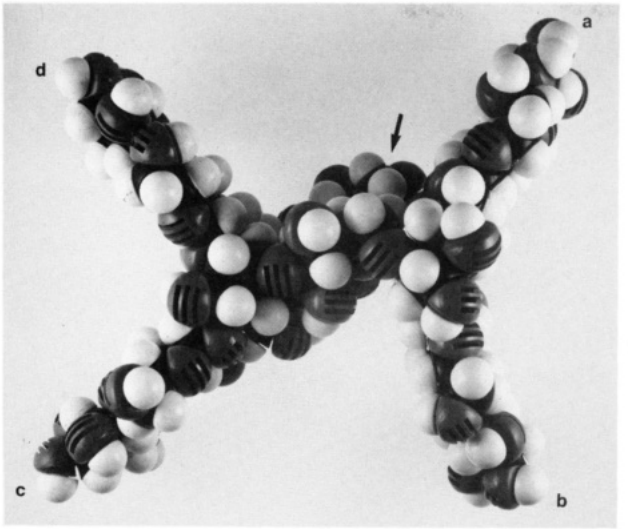

FIGURE 6: Corey–Pauling–Koltun space-filling model of tetraantennary complex type oligosaccharide **10**, together with one β(1–4)GlcNAc residue of the core chitobiose moiety. The rotation angles, ω, of the core α(1–6) and outer β(1–6) arms of mannose are set to 180° and −60°, respectively. The angle, ω, is the dihedral angle formed by the H-5, C-5, C-6, and O-6 atoms of the mannose residues. The arms a and b are the Galβ(1–4)GlcNAcβ(1–6) and Galβ(1–4)GlcNAcβ-(1–2) residues, respectively, connected to the α(1–6)mannose residue. The arms c and d are the Galβ(1–4)GlcNAcβ(1–4) and Galβ(1–4)GlcNAcβ(1–2) residues, respectively, connected to the α(1–3)-mannose residue. The arrow indicates the β(1–4)GlcNAc residue of the core.

to the same extent. Thus, the lectins show different sensitivities to the branching patterns of the two triantennary oligosaccharides.

AFGP also precipitates RCA-I and EIL and is trivalent in both cases as is the corresponding oligosaccharide **9**. These results indicate that the precipitation data for the oligosaccharides in the present study can be extrapolated to the corresponding glycopeptides.

Precipitation of Biantennary Complex Type Oligosaccharides. All of the biantennary oligosaccharides failed to precipitate RCA-I, EIL, and EAL; however, **6** and **7** did precipitate SBA strongly at 4 °C. Since RCA-I, EIL, and EAL are dimers and SBA is a tetramer and since the two oligosaccharides have weaker affinities for SBA than the other lectins, the results suggest that **6** and **7** require a tetravalent lectin to form a precipitate. In addition, **7** results in greater precipitation of SBA than **6**, which may be due to the slightly higher affinity of **7** or the influence of the bisecting GlcNAc of the latter. These results contrast with the findings that only bisected complex type oligosaccharides, but not nonbisected analogues, precipitate Con A (Bhattacharyya et al., 1987b).

Interestingly, biantennary molecules **2**, **4**, and **5**, which have shorter oligosaccharide chains than **6** and **7**, did not precipitate SBA even though the former oligosaccharides have nearly the same affinities as the latter two carbohydrates. Thus, the longer chains of **6** and **7** appear to be required for precipitation of SBA. However, the shorter chains present in **2** and **3** are also found in **8** and **9**, respectively, which do precipitate with the lectin as trivalent ligands. Apparently, the greater valencies of **8** and **9** stabilize the shorter chain cross-links of the oligosaccharides with the protein. It is also interesting to note the weaker precipitation activities of **8**–**10** with SBA, as compared to **6** and **7**. This may be due to weaker binding of the former oligosaccharides (direct measurements of their affinities could not be obtained due to limited amounts of the compounds) or greater stability of the precipitated lattice formed with the latter carbohydrates which contain longer chains.

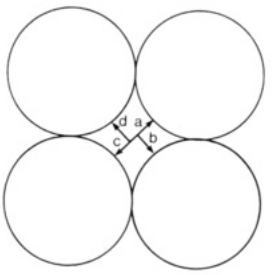

FIGURE 7: Schematic diagram of the binding of four lectin molecules (monomer) to tetraantennary oligosaccharide **10**. The circles represent the four protein monomers. The arms a, b, c, and d represent the respective arms of the tetraantennary oligosaccharide as indicated in Figure 7.

Conformation of the Tetraantennary Oligosaccharide in the Precipitated Complexes. Figure 6 shows a Corey–Pauling–Koltun model of **10**, together with one β(1–4)-linked GlcNAc residue of the core chitobiose moiety, which is in accord with the results of the present study and with previously reported NMR data and hard-sphere exoanomeric calculations (Bock et al., 1982). It is evident from the model that the four chains of the molecule can exist in a planar configuration which allows maximum separation of four protein molecules that bind to the terminal galactose residues. In the case of the corresponding glycopeptide, the core chitobiose and asparagine residue are essentially perpendicular to this plane. Examination of the model suggests that these four chains of the carbohydrate moiety in a glycoprotein would be at a sufficient distance from the surface of the protein to retain tetravalent binding properties. These conclusions are also consistent with the results obtained with AFGP.

Figure 7 shows a schematic diagram of four monomers of a lectin binding to the terminal galactose residues of **10**. Assuming spherical shape for the lectin monomers (which is approximately true for globular proteins), a simple geometric calculation using the distances between the C-4 atoms of the galactose residues of **10** obtained by hard-sphere exoanomeric calculations (Bock et al., 1982) shows that the radius of a monomer should be less than or equal to about 40 Å. However, Table I indicates that in addition to the terminal galactose EIL, EAL, and RCA-I recognize the acetamido group at the C-2 position of the subterminal GlcNAc residues. Thus, the maximum radius of a monomer of these lectins must be less than 40 Å. Allowing for 10 Å between the C-4 atom of the terminal galactose and the C-2 atom of the subterminal GlcNAc and performing a similar calculation show that the maximum radius of a lectin monomer is approximately 24 Å, under these conditions of binding.

Although no crystallographic data on sizes of the lectins are available, a comparison of the molecular weights of the monomers of EIL, EAL, and SBA with that of Con A shows that the radii of the monomers of these lectins are approximately 22–25 Å [considering that each monomer of Con A is approximately a sphere of radius 20 Å (Reeke et al., 1974) and assuming similar shapes of the monomers of these lectins on the basis of similarity in amino acid compositions and sedimentation coefficient values (Agrawal & Goldstein, 1968; Bhattacharyya et al., 1981; Lotan et al., 1974; McKenzie et al., 1972); also, the primary sequence of SBA is highly conserved with that of Con A (Hemperly & Cunningham, 1983)]. Thus, the above calculations suggest that four monomers of EIL, EAL, and SBA can be accomodated around **10**, as observed in the experimental data.

These considerations also suggest an explanation for the trivalent binding of **10** to RCA-I. The molecular weight of the monomer of RCA-I is 60 000, which is nearly twice those of the other lectins. Although X-ray crystallographic data are not available for RCA-I, data on ricin, a closely related galactose-specific monomeric protein from the seeds of *Ricinus communis* of molecular weight 63 000 [cf. Goldstein and Poretz (1986)], show that A and B chains are 55 × 45 and 70 × 30 Å, respectively (Montfort et al., 1987), and the overall length of the molecule is approximately 90 Å [our estimate based on Figure 2 in Montfort et al. (1987)]. Thus, the size of the RCA-I monomer is greater than the calculated upper limit of a 24-Å radius for four lectin monomers to bind to **10**, and therefore, **10** is trivalent for RCA-I binding. In this regard, it is interesting to note that although the overall molecular weights of RCA-I and SBA are identical (120 000), four molecules of SBA can bind to **10**. These results indicate that the size of the monomer and not the overall size of the lectin is important in determining the valency of certain complex-type oligosaccharides.

Additional Comments. Since bi-, tri-, and tetraantennary complex type carbohydrates are putative receptors on the surface of cells (Brandley & Schnaar, 1986; Kobata, 1984; Kornfeld & Kornfeld, 1985; Lennarz, 1980; Monsigny, 1984; Snider, 1984), it would appear that their ability to cross-link binding proteins may be related to their intrinsic biological properties. Indeed, cross-linking of these carbohydrates on the cell surface by what has been termed "patching and capping" by binding proteins is well documented and is related to many biological signal transduction events and endocytosis (Brandley & Schnaar, 1986; Fishman & Atikkan, 1980; Lennarz, 1980; Monsigny, 1984). Since the valencies of the carbohydrates as well as their affinities regulate their cross-linking efficiency in the present study, the relative amounts of these carbohydrates on the surface of cells would influence their ability to cross-link with exogenous binding proteins via similar mechanisms.

The present results and recent findings (Baenziger & Maynard, 1980; Bhattacharyya & Brewer, 1986a,b; Bhattacharyya et al., 1987a,b; Lee et al., 1983, 1984) also suggests that there are at least two classes of lectins which have different binding properties. One class, such as the hepatic galactose-specific lectins, possesses either extended binding sites or clustered subunits that lead to very high affinity binding to N-linked carbohydrates (Baenziger & Maynard, 1980; Lee et al., 1983, 1984). The other class, represented by Con A, RCA-I, EIL, EAL, and SBA, exhibits lower affinity interactions with N-linked carbohydrates but can cross-link the latter. The relationship between the binding mechanisms of these two apparent classes of lectins and their biological properties is under investigation.

Summary. The present results indicate that certain multiantennary complex type oligosaccharides and glycopeptides with nonreducing terminal galactose residues undergo bi-, tri-, and tetravlent interactions with a variety of galactose-specific lectins leading to precipitation of the complexes. The valency of a carbohydrate is generally equal to the number of chains with nonreducing terminal galactose residues, although the size of the lectin monomer also influences the valency of the oligosaccharide. In addition, a tetravalent lectin appears to be required to cross-link biantennary oligosaccharides, and the length of the chains in a biantennary molecule is a factor in determining whether a precipitate will form. Lastly, the presence or absence of a bisecting GlcNAc in biantennary complex type oligosaccharides has little effect on the binding activities and valencies of the carbohydrates, in contrast to the results found with Con A (Bhattacharyya et al., 1987b; Narasimhan et al., 1986).

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Asymmetry of Tyrosyl-tRNA Synthetase in Solution†

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ABSTRACT: The tyrosyl-tRNA synthetase from *Bacillus stearothermophilus* crystallizes as a symmetrical dimer with each subunit having a complete active site. The enzyme–substrate complexes, however, are known to be asymmetrical in solution because the enzyme exhibits half-of-the-sites activity by binding tightly only 1 mol of tyrosine or 1 mol of tyrosyl adenylate per mole of dimer. Evidence is now presented that the unligated enzyme is also asymmetrical in solution. Symmetry was investigated by construction of heterodimers containing one full-length subunit and one truncated subunit, allowing the introduction of different mutations into each monomer. Each dimer is active at only one site, but the site used is randomly distributed between the subunits. Each heterodimer thus consists of two equal populations, one activating tyrosine at a full-length subunit and the other at the truncated subunit. No detectable interconversion is found between active and inactive sites over several minutes either in the absence of substrates or when the enzyme is turning over in the steady state. Kinetic evidence implies that wild-type enzyme is inherently asymmetrical even in the absence of substrate.

Tyrosyl-tRNA synthetase catalyzes the aminoacylation of tRNA as a two-step reaction (eq 1 and 2). The enzyme from

$$E + Tyr + ATP \rightleftharpoons E\text{-}Tyr\text{-}AMP + PP_i \quad (1)$$

$$E\text{-}Tyr\text{-}AMP + tRNA^{Tyr} \rightarrow E + Tyr\text{-}tRNA^{Tyr} + AMP \quad (2)$$

Bacillus stearothermophilus is composed of two identical subunits (YTS/YTS).[1] Each subunit has a complete active site (Blow & Brick, 1985). However, the enzyme displays half-of-the-sites activity by binding tightly only 1 mol of tyrosine and forming only 1 mol of tyrosyl adenylate per two active sites (Fersht, 1975; Fersht et al., 1975a). The mech-

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[1] Abbreviations: (for subunits of tyrosyl-tRNA synthetase) YTS, wild type; ΔYTS, truncated wild type [see Waye et al. (1983)]; YTS(Asn-45), His → Asn mutation at position 45; ΔYTS(Asn-45), truncated with His → Asn mutation; (general) Bistris, [bis(2-hydroxyethyl)amino]tris(hydroxymethyl)methane; EDTA, ethylenediaminetetraacetic acid; FPLC, fast protein liquid chromatography; M-tRNA, modified *B. stearothermophilus* tRNA with the 3′-terminal adenosine chemically removed by the method of Fersht (1977); PMSF, phenylmethanesulfonyl fluoride; Tris, tris(hydroxymethyl)aminomethane.

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