Cross-Linking Activity of the 14-Kilodalton β-Galactoside-Specific Vertebrate Lectin with Asialofetuin: Comparison with Several Galactose-Specific Plant Lectins†

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ABSTRACT: We have previously shown that plant lectins with a wide range of carbohydrate binding specificities can bind and cross-link (precipitate) specific multiantennary oligosaccharides and glycopeptides [cf. Bhattacharyya, L., Fant, J., Lonn, H., & Brewer, C. F. (1990) *Biochemistry 29*, 7523–7530]. This leads to a new source of binding specificity: namely, the formation of homogeneous cross-linked lattices between lectins and carbohydrates. Recently, we have demonstrated the existence of highly ordered cross-linked lattices that form between the D-Man/D-Glc-specific plant lectin concanavalin A and the soybean agglutinin which is a tetrameric glycoprotein possessing a single Man9 oligomannose chain per monomer [Khan, M. I., Mandal, D. K., & Brewer, C. F. (1991) *Carbohydr. Res. 213*, 69–77]. In the present study, we have compared the ability of the 14-kDa β-galactoside-specific lectin from calf spleen, a dimeric S-type animal lectin, and several galactose-specific plant lectins from *Erythrina indica*, *Erythrina cristagalli*, and *Glycine max* (soybean agglutinin) to form specific cross-linked complexes with asialofetuin (ASF), a 48-kDa monomeric glycoprotein, using quantitative precipitation analyses. The results show the formation of 1:9 and 1:3 stoichiometric cross-linked complexes (per monomer) of ASF to the 14-kDa lectin, depending on their relative ratio in solution. Evidence indicates that the three triantennary N-linked complex-type oligosaccharide chains of ASF mediate the cross-linking interactions and that each chain expresses either trivalency in the 1:9 cross-linked complex or univalency in the 1:3 complex. The two dimeric *Erythrina* lectins also form 1:9 and 1:3 ASF–lectin cross-linked complexes as well as a lower ratio complex at high relative concentrations of ASF. In the presence of tetrameric soybean agglutinin, only a 1:3 ASF–lectin cross-linked complex is formed, presumably due to the larger size of the agglutinin. Unlike the plant lectins, the 14-kDa lectin fails to precipitate with the free triantennary glycopeptide or oligosaccharide from ASF, or with other related branched-chain carbohydrates, which suggests that this may be an important difference in the cross-linking activities of the animal lectin compared to the plant lectins. Insight has also been obtained into the conformational properties of the triantennary oligosaccharide in cross-linked complexes, and the factors affecting the valency of the oligosaccharide chain attached to a protein matrix. The present results thus demonstrate that the 14-kDa animal lectin possesses similar but distinct cross-linking activities from several Gal-specific plant lectins toward a glycoprotein with well-defined carbohydrate epitopes. The findings are discussed in relation to the biological properties of lectins and their corresponding glycoconjugate receptors.

Lectins are proteins which bind to the carbohydrate moieties of glycoproteins and glycolipids which, in turn, have been implicated as receptors in a variety of biological recognition processes including cellular recognition, adhesion, signal transduction, and metastasis (Brandley & Schnaar, 1986; Lennarz, 1980; Monsigny, 1984). Lectins are widely distributed in nature, and are found in plants, microorganisms, bacteria, and animals (Kobata, 1984; Kornfeld & Kornfeld, 1985; Nicolson, 1976; Snider, 1984; Lis & Sharon, 1986). Because of their abundance and ease of isolation (Goldstein & Poretz, 1986), plant lectins have been widely used to investigate the binding epitopes of a variety of carbohydrates isolated from both normal and transformed cells (Lis & Sharon, 1986).

Binding of lectins to cells often leads to cross-linking and aggregation of specific glycoproteins and glycolipids, which, in many cases, is associated with concomitant biological responses. For example, cross-linking of glycoconjugates on the surface of cells have been implicated in the mitogenic activities of lectins including concanavalin A (Con A)[1] and SBA [cf. Nicolson (1976)], in the arrest of bulk transport in ganglion cell axons (Edmonds & Koenig, 1990), and in the induction of mating reactions in fungi (Kooijman et al., 1989). Lectin-induced cross-linking of cellular transmembrane gly-

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[1] Abbreviations: 14-kDa lectin, β-galactoside binding vertebrate lectin with subunit M_r 14 000; EIL, lectin from the seeds of *Erythrina indica*; ECL, lectin from the seeds of *Erythrina cristagalli*; SBA, lectin from the seeds of *Glycine max*; Con A, concanavalin A, lectin from jack bean; BSA, bovine serum albumin; ASF, asialofetuin; Meβ Gal, methyl β-galactopyranoside; LacNAc, Galβ(1,4)GlcNAC; FPLC, fast-protein liquid chromatography; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; PBS, 0.02 M sodium phosphate buffered with 0.15 M sodium chloride, pH 7.4; PMSF, phenylmethanesulfonyl fluoride; EDTA, ethylenediaminetetraacetic acid disodium salt; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; DTT, dithiothreitol; fucose is in the L configuration; all other sugars are in the D configuration.

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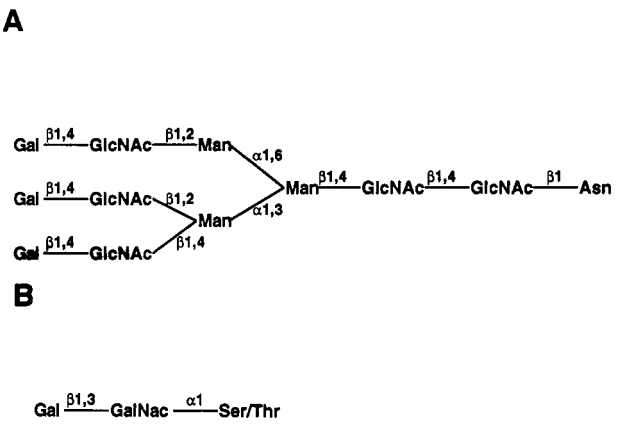

FIGURE 1: Structures of (A) the triantennary N-linked complex-type glycopeptide and (B) the O-linked glycopeptide from ASF. Gal, Man, GlcNAc, GalNAc, Asn, Ser, and Thr represent galactose, mannose, *N*-acetylglucosamine, *N*-acetylgalactosamine, asparagine, serine, and threonine residues, respectively.

coproteins leads to changes in their interactions with cytoskeletal proteins, and alterations in the mobility and aggregation of other surface receptors [cf. Carraway and Carraway (1989) and Edelman (1976)]. Thus, it is clear that lectin–glycoconjugate cross-linking interactions play an important role in the biological properties of these molecules.

Recently, we have shown that plant lectins with a wide range of carbohydrate binding specificities can bind and cross-link (precipitate) a variety of carbohydrate chains from glycoproteins and glycolipids (Bhattacharyya & Brewer, 1989; Bhattacharyya et al., 1987a,b, 1988a,b, 1990). For example, the triantennary complex-type glycopeptide isolated from asialofetuin (Figure 1A) was shown to be a trivalent ligand for several Gal-specific *Erythrina* lectins, including *E. indica* (EIL)[1] and *E. cristagalli* (ECL), and to precipitate with these dimeric proteins (Bhattacharyya et al., 1989a). In addition, certain oligomannose, bisected hybrid, and bisected complex-type oligosaccharides were shown to be divalent and to precipitate with dimeric and tetrameric forms of the Glc/Man-specific lectin Con A. Further studies have shown that these cross-linking interactions lead to the formation of homogeneous cross-linked lattices between lectins and oligosaccharides and glycopeptides, even in the presence of mixtures of the molecules (Bhattacharyya et al., 1988b, 1989a, 1990). In fact, several lectin–carbohydrate cross-linked complexes have been shown to be crystalline and suitable for structural analysis by electron microscopy (Bhattacharyya et al., 1989a, 1990) and X-ray diffraction techniques (Makowski and Brewer, unpublished results). These results indicate a new dimension of binding specificity exists in the formation of protein–carbohydrate cross-linked complexes. These studies have also been extended to examining the cross-linking interactions of plant lectins with glycoproteins. It has been shown, for example, that highly organized cross-linked lattices are also formed between the tetrameric glycoprotein SBA, which possesses a single Man9 oligomannose-type chain per monomer, and Con A (Khan et al., 1991). Thus, certain plant lectins also appear to form highly organized cross-linked lattices with specific glycoproteins.

Certain animal lectins also possess carbohydrate binding specificities and multisubunit structures similar to plant lectins, and therefore have the potential for specific cross-linking interactions with glycoconjugates. Indeed, interest has increased in the roles of animal lectins in a variety of biological processes including cellular recognition and metastasis (Brandley & Schnaar, 1986; Barondes et al., 1988; Monsigny et al., 1988). On the basis of structural and functional studies, animal lectins have been classified into two main types (Drickamer, 1988): the "C-type", which is characterized by their dependence on calcium ions, and the "S-type", which is cation independent but thiol dependent. A family of soluble S-type lectins that bind β-Gal residues constitutes the developmentally regulated class of dimeric proteins with 14-kDa subunits (Barondes, 1984). The 14-kDa family of lectins has been found in mammalian tissues including muscle, lung, and brain, and evidence suggests that it may be involved in the metastasis of certain tumor cells [cf. Raz and Lotan (1987)]. Although a specific function of these lectins has not been demonstrated, it appears related to their ability to bind to specific cellular glycoconjugates (Feizi & Childs, 1987; Solomon et al., 1991). Toward this end, binding specificity studies of the 14-kDa lectin have been reported (Abbott et al., 1988; Childs & Feizi, 1979; Lee et al., 1990; Leffler & Barondes, 1986; Sparrow et al., 1987; Solomon et al., 1991) which have clearly shown a preference for Galβ(1,4)Glc(GlcNAc) sequences. A recent report has indicated that the complete polypeptide chain of the bovine 14-kDa lectin is necessary for the integrity of the carbohydrate recognition domain (Abbott & Feizi, 1991).

In order to gain further insight into the molecular binding properties of the 14-kDa lectins, we have examined the ability of the 14-kDa lectin from calf spleen to form specific cross-linked complexes with ASF, a monomeric glycoprotein with carbohydrate chains containing LacNAc residues, using quantitative precipitation analyses. We have compared the cross-linking activities of the 14-kDa lectin with those of several galactose-specific plant lectins, namely, EIL, ECL, and SBA. The findings are discussed in terms of the cross-linking activities of plant and animal lectins, the factors affecting the valency of oligosaccharde chains present on a protein matrix, and the effects of the quaternary structure of lectins on their cross-linking activities.

MATERIALS AND METHODS

Materials. Calf spleen was purchased from Pel-Freez. Bovine fetuin and BSA were obtained from Sigma Chemical Co. Superdex 75 and Sepharose 4B were from Pharmacia. ASF–Sepharose was prepared following a published procedure (Parikh et al., 1974). The triantennary N-linked glycopeptide (Figure 1A) and the corresponding oligosaccharide analog containing one core *N*-acetylglucosamine residue as well as the O-linked glycopeptide (Figure 1B) were isolated from the Pronase digest of ASF as described (Bhattacharyya et al., 1988a). The tetraantennary complex-type oligosaccharide with the nonreducing terminal LacNAc structures was obtained from BioCarb Chemicals, Sweden. The triantennary synthetic cluster glycoside with nonreducing terminal lactose residues was a gift from Dr. Y. C. Lee, The John Hopkins University, Baltimore, MD (Lee, 1978). The concentrations of the oligosaccharides and the glycopeptide were determined by the phenol–sulfuric acid method using Man as the standard (Dubois et al., 1956). Monosaccharides were purchased from Sigma Chemical Co. [^{3}H]- and [^{14}C]formaldehyde were purchased from New England Nuclear (25 mCi/mmol) and Sigma Chemical Co. (10 mCi/mmol), respectively. All other materials used were of analytical grade.

Protein Purification. The 14-kDa lectin was purified from calf spleen by modification of previously described procedures (Allen et al., 1987; Briles et al., 1979). Briefly, frozen spleen (450 g) was cut into small pieces and homogenized in 10 volumes of acetone containing 1 mM DTT at 0 °C. Acetone powder (200 g) was then extracted overnight with PBS (1:8 w/v) in the presence of 0.02% sodium azide, 2 mM EDTA,

3 mM DTT, 0.1 M lactose, and 0.25 mM PMSF at 4 °C. The supernatant, obtained by centrifugation at 17000*g* for 30 min, was adjusted to pH 4.5 with 2 N HCl and centrifuged at 12000*g* for 15 min. The clear supernatant was made pH 7.4 with 2 N NaOH, concentrated by ultrafiltration, and dialyzed extensively against PBS containing 0.02% sodium azide, 2 mM EDTA, and 1 mM DTT. The lectin was purified by affinity chromatography on an ASF–Sepharose column (2.8 × 90 cm) at 4 °C. After the unabsorbed proteins were removed by extensive washing with PBS containing 1 M NaCl and 1 mM DTT, 1 bed volume of PBS with 1 mM DTT was percolated through the column, and the lectin was eluted with 0.1 M lactose in the same buffer. The purity of the preparation was checked by SDS–PAGE at pH 7.2 (Weber & Osborn, 1969) on a 13.5% gel. Hemagglutinating activity was assayed in PBS containing 2 mM DTT using a 3% (v/v) suspension of rabbit erythrocytes as described (Osawa & Matsumoto, 1972). Protein concentration was determined either by the method of Lowry et al. (1951) on samples in which DTT was diluted using BSA as a standard or by spectrophotometric methods using $A^{1\%,1cm} = 5.5$ at 280 nm (Sharma et al., 1990) and reported in terms of monomer concentration. The purified 14-kDa calf spleen lectin used in the precipitation studies was homogeneous on SDS–PAGE and had a minimum hemagglutinating activity of 10 μg/mL against rabbit erythrocytes.

The *Erythrina* lectins EIL and ECL were prepared as described (Bhattacharyya et al., 1981; Iglesias et al., 1982). Purification of SBA was done according to Bhattacharyya et al. (1988a). Protein concentrations were determined spectrophotometrically with the respective extinction coefficients and expressed in terms of subunit. $A^{1\%,1cm}$ values at 280 nm for EIL, ECL, and SBA are 13.4 (Bhattacharyya et al., 1981), 15.3 (Iglesias et al., 1982), and 12.8 (Lotan et al., 1974), respectively, with subunit molecular weights of 34 000 for EIL, 28 000 for ECL, and 30 000 for SBA.

ASF was prepared by desialylating fetuin (Sigma) as described (Spiro & Bhoyroo, 1974) and purified by FPLC on Superdex 75. The concentration of ASF was determined by the phenol–sulfuric acid method (Dubois et al., 1956) with Man as the standard using 21 mol of hexoses (Man/Gal) per mole of protein (Spiro, 1960; Nilsson et al., 1979).

Radiolabeling of Proteins. The 14-kDa lectin was radiolabeled with ^{14}C by reductive methylation (Jentoft & Dearborn, 1983) in 0.1 M sodium phosphate buffer, pH 7.1, containing 1 mM DTT and 0.1 M lactose. Sodium cyanoborohydride (10 mg) was added to the protein solution (12 mg, 5 mL) followed by 15-μL aliquots of [^{14}C]formaldehyde (3.7% v/v in water) at 10-min intervals over a period of 1 h. The reaction mixture was further kept for 6 h at room temperature, dialyzed extensively against PBS containing 2 mM DTT, and finally chromatographed on ASF–Sepharose column.

The plant lectins EIL, ECL, and SBA were similarly radiolabeled with ^{14}C in 0.1 M HEPES buffer, pH 7.0, containing 0.2 mM Ca^{2+} and 0.2 mM Mn^{2+}. ASF was radiolabeled with [^{3}H]formaldehyde in an analogous manner.

Quantitative Precipitation Assays. The assays with the 14-kDa lectin were performed at 22 °C in a volume of 200 μL of either 0.1 M sodium phosphate buffer, pH 7.4, containing 0.9 M NaCl and 2 mM DTT or PBS containing 2 mM DTT. No significant differences in the amount of precipitated lectin were observed as a function of salt concentration. Quantitative precipitation assays with the plant lectins were performed at 22 °C in a volume of 200 μL of 0.1 M HEPES buffer, pH 7.4, containing 0.9 M NaCl, 1 mM Ca^{2+}, and 1 mM Mn^{2+}, as described previously (Khan et al., 1991). Inhibition of

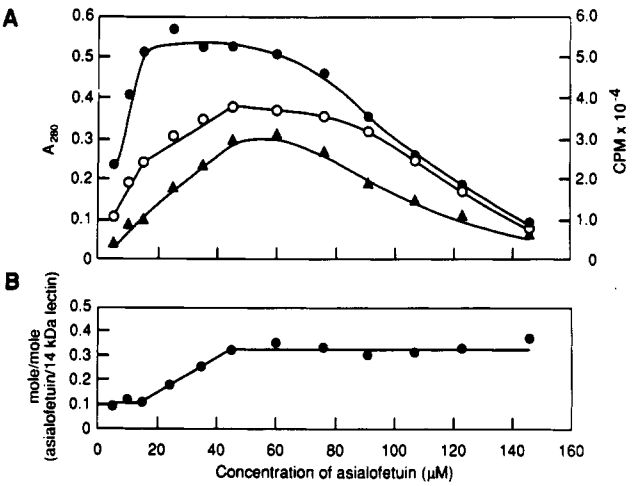

FIGURE 2: Precipitin curves for the quantitative precipitation of the 14-kDa lectin from calf spleen by ASF at 22 °C: (A) profile of the total protein precipitated (○) and cpm of ^{14}C-14-kDa lectin (●) and ^{3}H-ASF (▲) in the precipitate; (B) ratio (●) of moles of ASF precipitated per mole of 14-kDa lectin monomer. The buffer was 0.1 M sodium phosphate containing 0.9 M NaCl and 2 mM DTT at pH 7.4. The ^{14}C-14-kDa lectin concentration was fixed at 130 μM. The specific activities of the radiolabeled 14-kDa lectin and ASF are 3.3 × 10^3 and 5.8 × 10^3 cpm/nmol, respectively.

formation and dissolution of the precipitates of the 14-kDa and other plant lectins with ASF occurred in the presence of 0.2 M lactose, a specific competing saccharide, but not by nonspecific sugars such as Glc or Fuc.

RESULTS AND DISCUSSION

Precipitation of the 14-kDa Calf Spleen Lectin with ASF. The 14-kDa β-Gal-specific lectin from calf spleen is a dimeric protein with a molecular weight of approximately 30 000 [cf. Allen et al. (1987)]. Although the number of combining sites per molecule has not been determined, the present results together with a recent report (Abbott & Feizi, 1991) suggest the presence of one carbohydrate binding site per monomer. Binding specificity studies of the 14-kDa lectin from other sources reveal a preference for the Galβ(1,4)GlcNAc (LacNAc) sequence, and clustering of LacNAc residues in multiantennary glycopeptides does not improve their affinity for the lectin (Lee et al., 1990).

ASF is a monomer of molecular weight 48 000 (Spiro, 1960) that contains three N-linked complex-type oligosaccharide chains comprised predominantly of the triantennary chain shown in Figure 1A (approximately 74%), a small amount of isomer with a Galβ(1,3) linkage in the outer LacNAc residue of the Manα(1,3) arm (9%), a biantennary chain with terminal LacNAc residues (17%) (Green et al., 1988), and three O-linked disaccharide chains (Figure 1B) (Nilsson et al., 1979). Lee and co-workers (Lee et al., 1990) have shown that the 14-kDa lectin from human spleen exhibits similar affinities for the two isomeric triantennary glycopeptides and the biantennary glycopeptide, which are nearly 200-fold higher than that of the O-type chains of ASF (Ahmed et al., 1990). The quantitative precipitation data for the 14-kDa calf spleen lectin with ASF support preferential binding of the lectin to the three N-linked carbohydrate chains of the glycoprotein.

Figure 2A shows the quantitative precipitation data of ^{14}C-labeled 14-kDa calf spleen lectin in the presence of increasing concentrations of ^{3}H-labeled ASF in 0.1 M sodium phosphate buffer, pH 7.4, containing 0.9 M NaCl and 2 mM DTT. The profile for the total protein precipitated (A_{280}) and the radioactivity profiles show several characteristic features. Titration of the 14-kDa lectin (130 μM) with an increasing

concentration of ASF leads to an increase in the total amount of protein precipitated (A_{280} profile) with a constant slope up to an ASF concentration of 15 μM. A break then occurs with a decrease in slope until the concentration of ASF becomes 45 μM. The total protein profile and the profile for precipitated ^{3}H-labeled ASF are parallel over this concentration range while the radioactive profile for the ^{14}C-labeled 14-kDa lectin shows that the lectin is precipitated to the greatest extent (60%) between ASF concentrations of 15 and 45 μM. At higher concentrations of ASF, the precipitation profile of the 14-kDa lectin declines as well as the other two profiles. As shown in Figure 2B, the ASF:14-kDa lectin monomer mole ratio in the precipitates is 1:9 up to an ASF concentration of 15 μM and then decreases with further addition of ASF to a value of 1:3. Similar results were obtained using lower salt concentrations in the precipitation assay [PBS buffer containing 2 mM DTT (data not shown)], which demonstrated that the precipitation reaction is not significantly influenced by salt concentration.

The results indicate that the 14-kDa lectin forms two different types of cross-linked complexes with ASF, depending on the relative ratio of the two proteins in solution. The binding interactions also appear to be mediated by the three N-linked carbohydrate chains and not the O-linked chains of ASF which have much lower affinity. Since the triantennary carbohydrate chain of ASF is trivalent as a glycopeptide for binding and precipitating with EIL and ECL which are also dimeric Gal-specific lectins (Bhattacharyya et al., 1989a), formation of a 1:9 ASF–14-kDa lectin cross-linked complex (per monomer) (1:8.5 ratio when taking into account the amount of biantennary chain present on ASF) indicates that each N-linked chain on ASF also functions as a trivalent binding site for the smaller animal lectin and that all three N-linked chains are occupied in the complex (Figure 6A). At higher concentrations of ASF, the 1:9 complex converts to a 1:3 cross-linked complex which indicates that the three N-linked oligosaccharide chains of ASF exhibit univalent binding activity under these conditions (Figure 6A). Thus, the effects of the protein matrix on the valency of the N-linked carbohydrate of ASF are apparent from this transition. This transition is similar to that observed for SBA cross-linked with Con A in which an initial 1:1 SBA–Con A cross-linked complex (per monomer) converts to a 2:1 complex in the presence of increasing SBA concentration (Khan et al., 1991). Thus, the 14-kDa calf spleen lectin is capable of forming two well-defined cross-linked complexes with ASF.

Precipitation of EIL and ECL with ASF. The plant lectins EIL and ECL have close physicochemical, structural, and carbohydrate binding properties (Goldstein & Poretz, 1986). Each protein is a dimer consisting of two similar subunits of 34 and 28 kDa, respectively, each possesses one saccharide binding site per monomer, and both lectins are specific for Gal (Bhattacharyya et al., 1981; Iglesias et al., 1982). As previously mentioned, earlier studies have shown that the triantennary glycopeptide from ASF binds and precipitates with EIL and ECL as a trivalent ligand (Bhattacharyya et al., 1989a).

Hemagglutination inhibition measurements in the present study (data not shown) indicates that N-linked triantennary glycopeptide(s) from ASF bind(s) to EIL with 180-fold higher affinity than the O-linked glycopeptide, which binds 4-fold weaker than MeβGal. These results are similar to those reported for binding of the 14-kDa lectin to the two types of carbohydrate chains from ASF (Ahmed et al., 1990). Affinity data for the O-linked glycopeptide binding to ECL (and SBA, below) were not obtained because of the high concentration

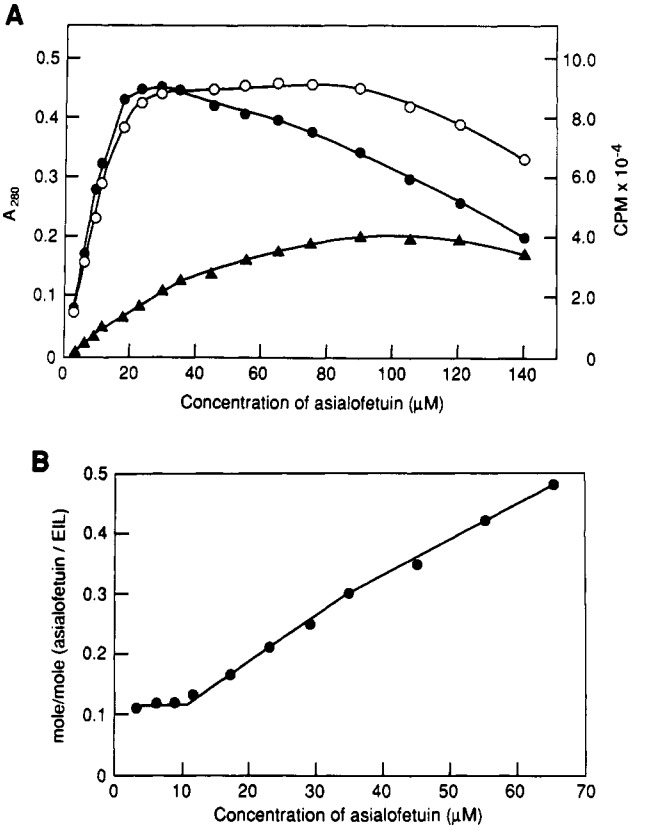

FIGURE 3: Precipitin curves for the quantitative precipitation of EIL by ASF at 22 °C: (A) profile of the total protein precipitated (○) and cpm of ^{14}C-EIL (●) and ^{3}H-ASF (▲) in the precipitate; (B) ratio (●) of moles of ASF precipitated per mole of EIL monomer (see text). The buffer was 0.1 M HEPES containing 0.9 M NaCl, 1 mM Ca^{2+}, and 1 mM Mn^{2+} at pH 7.4. The ^{14}C-EIL concentration was fixed at 87 μM. The specific activities of the radiolabeled EIL and ASF are 5.6 × 10^3 and 5.3 × 10^3 cpm/nmol, respectively.

required for inhibition and the limited amount of this compound. However, on the basis of the similarity in binding activities of EIL and ECL (Bhattacharyya et al. 1989a), ECL is also expected to preferentially bind to the N-linked chains of ASF.

The quantitative precipitation profiles for EIL and ECL in the presence of ASF are shown in Figures 3A and 4A, respectively, and are similar for the two lectins. The A_{280} profile for the total protein precipitated denotes the sum of the two radioactive profiles for ^{14}C-labeled lectin and ^{3}H-labeled ASF. The profile for ^{3}H-labeled ASF shows two break points at concentrations of 11 and 34 μM, after which a plateau is formed in which the amount of ASF in the precipitate first increases and then decreases. The radioactive profiles for the ^{14}C-labeled lectins show that both are precipitated to the greatest extent (90–94%) at ASF concentration between 11 and 34 μM, after which the precipitation of lectin gradually declines. The mole ratio (per monomer) of ASF to lectin in the precipitates of both *Erythrina* lectins (Figures 3B and 4B) is 1:9 up to the ASF concentration of 11 μM. Further addition of ASF decreases the ratio to 1:3 up to a concentration of 34 μM ASF. However, unlike the 14-kDa lectin, a further increase in ASF concentration leads to a change in this ratio to nearly 1:1 for both plant lectins (not shown in Figures 3B and 4B).

These results are similar to those for the 14-kDa lectin in many ways. The valency of the N-linked chains of ASF exhibits trivalency in the 1:9 cross-linked complexes with EIL and ECL, and univalency in the 1:3 complexes, respectively. However, unlike the 14-kDa lectin, a further increase in the

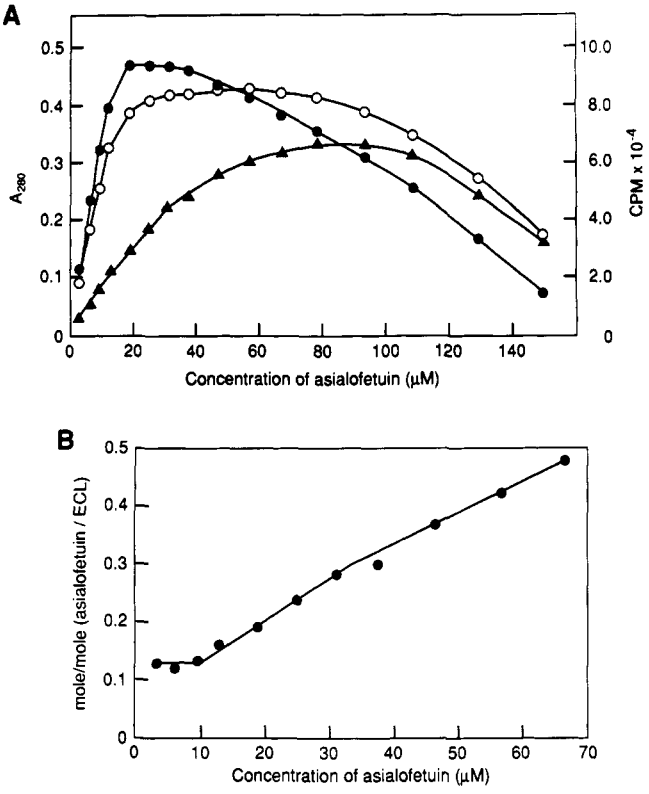

FIGURE 4: Precipitin curves for the quantitative precipitation of ECL by ASF at 22 °C: (A) profile of the total protein precipitated (○) and cpm of ^{14}C-ECL (●) and ^{3}H-ASF (▲) in the precipitate; (B) ratio (●) of moles of ASF precipitated per mole of ECL monomer (see text). The buffer was the same as that in Figure 3. The ^{14}C-ECL concentration was fixed at 87 μM. The specific activities of the radiolabeled ECL and ASF are 6.2×10^3 and 1.1×10^4 cpm/nmol, respectively.

relative ratio of ASF to the plant lectins in solution leads to a lower ratio of the two in the precipitates, indicating probable formation of at least 1:2 ASF–lectin cross-linked complexes which would be the minimal valency of ASF required for precipitation. It is not, however, clear why the ASF:lectin ratio approaches 1:1 in the precipitates. All of these cross-linked complexes (Figure 6B) are readily reversible upon addition of a competing monosaccharide such as Gal, thus demonstrating that carbohydrate–lectin interactions are responsible for the formation of the complexes. Interestingly, maximum precipitation of two plant lectins as well as the 14-kDa lectin occurs upon formation of their respective 1:3 cross-linked complexes with ASF. Under these conditions, the percentage of the 14-kDa lectin precipitated is smaller (60%) as compared to the *Erythrina* lectins (90–94%), though the concentration of the 14-kDa lectin is higher. This is presumably due to the lower affinity of the 14-kDa lectin for ASF relative to the *Erythrina* lectins. In any case, the larger dimeric plant lectins form 1:9 and 1:3 cross-linked complexes with ASF as does the 14-kDa lectin. Thus, the structures of these two cross-linked complexes of the two plant lectins with ASF are similar to the corresponding cross-linked complexes of the animal lectin.

Precipitation of SBA with ASF. SBA is a Gal/GalNAc-specific tetrameric lectin with four equal subunits of molecular weight 30 000 (Shaanan et al., 1984), and one carbohydrate binding site per monomer (De Boeck et al., 1984). Previous studies have shown that the triantennary oligosaccharide chain from ASF precipitates with SBA as a trivalent ligand (Bhattacharyya et al., 1988a).

The quantitative precipitation profile for ^{14}C-labeled SBA in the presence of increasing amounts of ^{3}H-labeled ASF

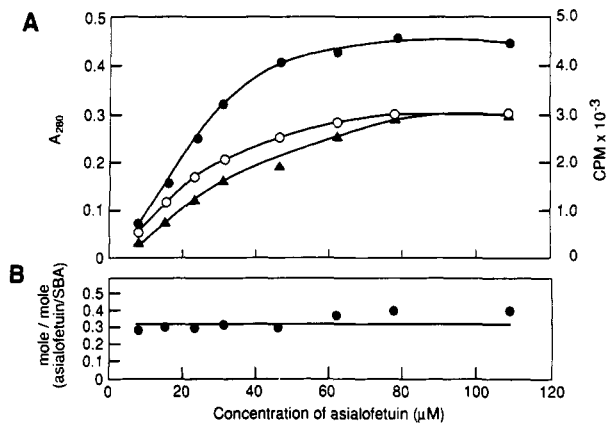

FIGURE 5: Precipitin curves for the quantitative precipitation of SBA by ASF at 22 °C: (A) Profile of the total protein precipitated (○) and cpm of ^{14}C-SBA (●) and ^{3}H-ASF (▲) in the precipitate; (B) ratio (●) of moles of ASF precipitated per mole of SBA monomer. The buffer was the same as that in Figure 3. The ^{14}C-SBA concentration was fixed at 87 μM. The specific activities of the radiolabeled SBA and ASF are 6.2×10^2 and 1.0×10^3 cpm/nmol, respectively.

(Figure 5A) shows increasing total protein precipitated (A_{280}) until a broad plateau is reached. The radioactive profiles are parallel with the total protein precipitation curve. In the region of maximum precipitation, approximately 55% of the lectin is precipitated. The ratio of moles of ASF to SBA (per monomer) shows that it remains constant at 1:3 over the entire profile (Figure 5B). Thus, SBA, which is a tetramer, forms only a 1:3 cross-linked complex with ASF. The schematic representation of the cross-linked complex is shown in Figure 6C. The larger size of SBA compared to EIL, ECL, and the 14-kDa lectin suggests that steric factors determine the structure of the ASF–SBA cross-linked complex, preventing formation of the 1:9 complex. Thus, the N-linked chains of ASF appear to exhibit univalent interactions with SBA.

Lectin Interactions with Branched-Chain Oligosaccharides. Previous studies have shown that EIL, ECL, and SBA precipitate with branched-chain oligosaccharides and glycopeptides possessing terminal Gal residues, including the triantennary N-linked oligosaccharide and glycopeptide from ASF, and a tetraantennary complex-type oligosaccharide with terminal LacNAc residues (Bhattacharyya et al., 1988a, 1989a). EIL and ECL also precipitate with a synthetic triantennary "cluster" glycoside with terminal lactose residues (Bhattacharyya et al., 1989a). However, the 14-kDa lectin shows no precipitation activity with any of these carbohydrates (present findings), even at a lectin concentration of 230 μM at 4 °C. This can be compared to the ability of the 14-kDa lectin at a concentration of 130 μM to precipitate appreciably with ASF at 22 °C, as do the other lectins, although the relative affinities of the 14-kDa lectin for ASF and the N-linked glycopeptide from ASF are essentially the same and nearly equal to LacNAc (Ahmed et al., 1990; Lee et al., 1990). By comparison, the affinities of EIL and ECL for the N-linked glycopeptide of ASF (and by extension to ASF) are nearly 7-fold greater than LacNAc (Bhattacharyya et al., 1989a). Thus, under conditions where the 14-kDa lectin binds to the N-linked carbohydrate chains of ASF (since it precipitates with ASF), the lectin shows no ability to precipitate with the triantennary oligosaccharide or glycopeptide, nor with the tetraantennary complex-type oligosaccharide or synthetic triantennary "cluster" glycoside with terminal lactose residues.

The ability of the 14-kDa lectin to cross-link ASF, but not the corresponding glycopeptide or oligosaccharide(s), suggests that this may be an important difference in the cross-linking

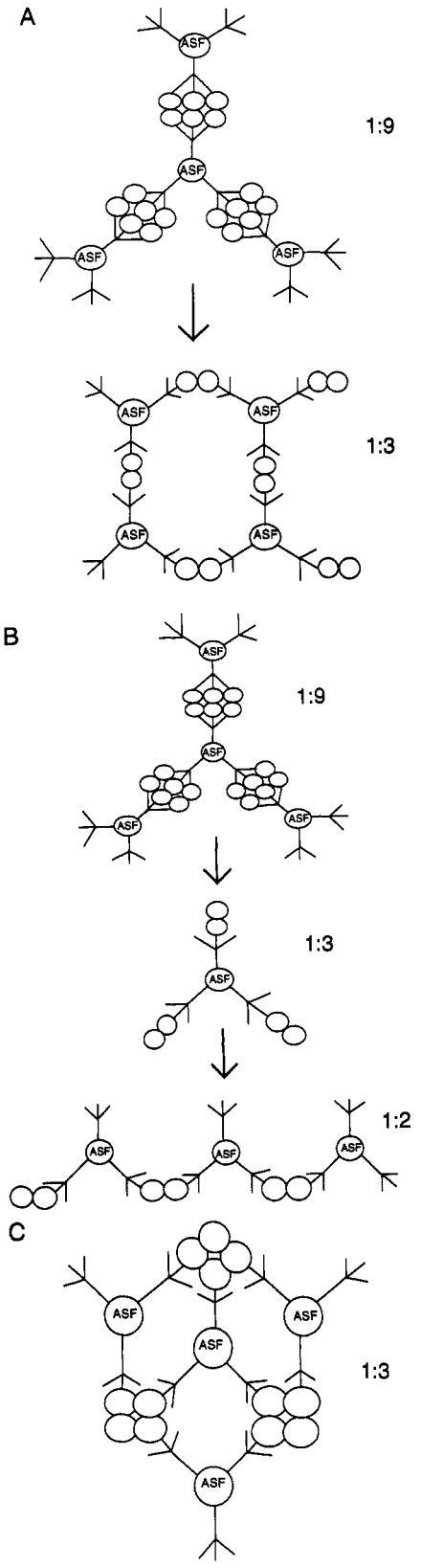

FIGURE 6: Schematic representations of the cross-linked complexes of ASF with β-galactoside-specific animal and plant lectins: (A) the 1:9 and 1:3 stoichiometric cross-linked complexes (per monomer) of ASF and 14-kDa lectin from calf spleen; (B) the 1:9, 1:3, and 1:2 stoichiometric cross-linked complexes (per monomer) of ASF and *Erythrina* lectin, EIL, or ECL; (C) the 1:3 stoichiometric cross-linked complexes (per monomer) of ASF and SBA. The circles with ASF, a pair of circles, and four circles clustered together represent ASF, dimeric 14-kDa lectin or *Erythrina* lectin, and tetrameric SBA, respectively. The connecting lines between ASF and lectin molecules are the triantennary complex-type oligosaccharide chains of ASF (Figure 1A). The arrows indicate transformation of one cross-linked complex to another complex.

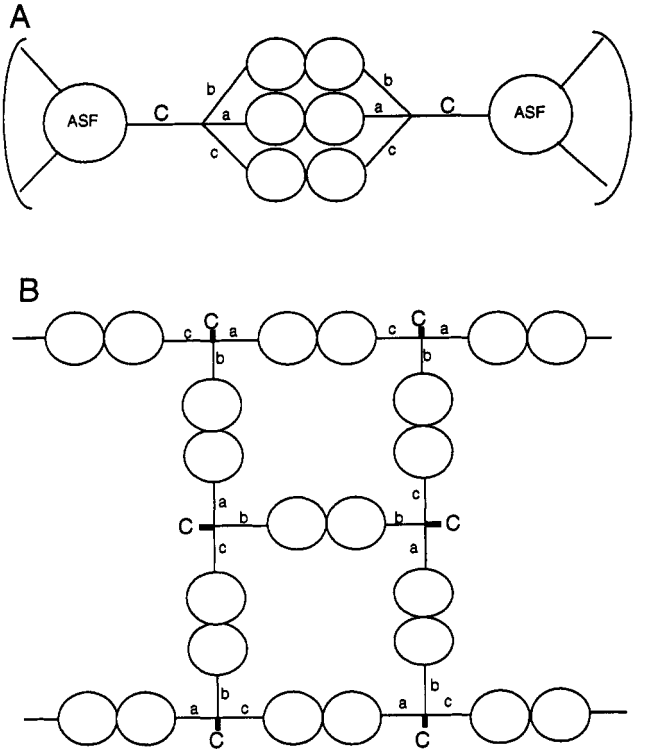

FIGURE 7: Schematic representations of cross-linked complex(es) of (A) the N-linked triantennary oligosaccharide chains of ASF in the 1:9 ASF–lectin (14-kDa lectin, EIL, or ECL) cross-linked complexes and (B) the free triantennary oligosaccharide chain in the 1:3 carbohydrate–lectin (EIL or ECL) cross-linked complexes (Bhattacharyya et al., 1988a, 1989a). The stoichiometric ratios are expressed in terms of lectin monomer. The three arms of the oligosaccharide are designated as a, b, and c. The core chitobiose residues in (A) and the core Man residue in (B) are represented by C. Lectin dimers are represented as pairs of circles.

activities of this animal lectin compared to the above plant lectins. This difference may limit the cross-linking activities of the 14-kDa lectin to multivalent glycoproteins, as opposed to branched-chain glycolipids with internal and/or terminal LacNAc residues [cf. Wolfe et al. (1974)]. This possibility is under investigation.

Conformational Flexibility of the Triantennary N-Linked Oligosaccharide Chain(s) of ASF. All three arms of individual N-linked oligosaccharide chains on ASF are bound in the respective 1:9 cross-linked complexes with the 14-kDa lectin, EIL, and ECL, which is similar to that of the free triantennary oligosaccharide cross-linked with EIL and ECL (Bhattacharyya et al., 1989a). However, the structures of the ASF–lectin and the oligosaccharide–lectin complexes require different conformations of the triantennary chains in the respective cross-linked lattices. In the ASF cross-linked complex(es), the three arms on each triantennary oligosaccharide (a, b, and c in Figure 7A) are cross-linked via protein to three arms of another covalently bound oligosaccharide molecule of ASF. In this case, the three arms of each oligosaccharide must be directed toward the adjacent cross-linked carbohydrate. However, in the free oligosaccharide cross-linked complexes, at least one of the three arms of the oligosaccharide must be directed away from the other two arms in order to provide an alternating carbohydrate/protein cross-linked lattice (shown in Figure 7B with all three arms providing independent cross-links to adjoining molecules). The conformation of the oligosaccharide in this case must be different from that in the former case, assuming that the carbohydrate binding sites on each lectin are fixed.

Lee and co-workers have shown that the α(1–6) arm of the triantennary glycopeptide chain is flexible and exists in at

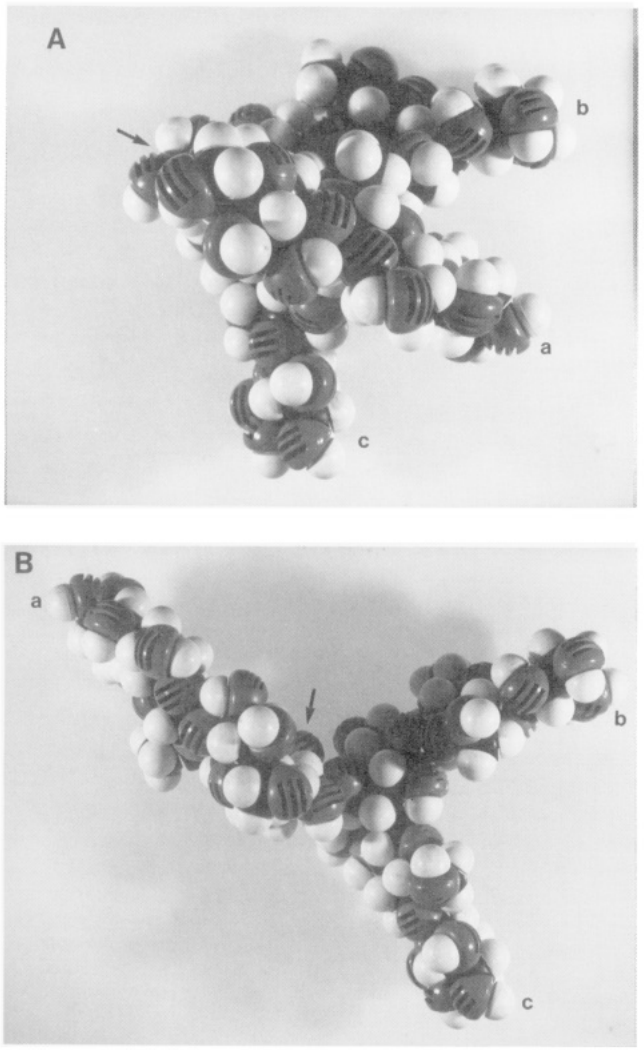

FIGURE 8: Corey–Pauling–Koltun (CPK) space-filling model of the triantennary complex-type oligosaccharide in Figure 1A, without the core chitobiose moiety, with its α(1–6) arm in (A) the $\omega = 180°$ rotamer conformation and (B) the $\omega = -60°$ rotamer conformation. The rotation angle ω is the dihedral angle formed by the H-5, C-5, C-6, and O-6 atoms of the core Man. The conformation of the α-(1–3) arm in (A) is somewhat different from that in (B) (Brisson & Carver, 1983). The Galβ(1–4)GlcNAcβ(1–2) arm connected to the α(1–6) Man residue of the carbohydrate is designated as a. The Galβ(1–4)GlcNAcβ(1–4)- and Galβ(1–4)GlcNAcβ(1–2)- arms connected to the α(1–3) Man residue are designated as b and c, respectively. The arrow in both (A) and (B) shows the location of the core Man residue.

least two major conformations (Rice et al., 1991). Figure 8A,B shows a CPK model of the oligosaccharide (without the core residues, for simplicity) in two possible conformations: (1) with the α(1–6) arm in the $\omega = 180°$ conformation (ω is the dihedral angle formed by H-5, C-5, C-6, and O-6); and (2) with the α(1–6) arm in the $\omega = -60°$ conformation, respectively. The conformation of the oligosaccharide in Figure 8A also possesses a small but significantly different rotation angle about the core α(1–3) arm as compared to that shown in Figure 8B (Brisson & Carver, 1983). [The outer branched chains on the α(1–3) arm are relatively rigid as compared to the α(1–6) arm (Brisson & Carver, 1983).] In the $\omega = 180°$ conformation (Figure 8A), all three arms of the oligosaccharide (a, b, and c) can be aligned around a common axis, as must be found in the 1:9 ASF–lectin cross-linked complexes. In the $\omega = -60°$ conformation (Figure 8B), the α(1–6) arm extends away from the other two arms, which would satisfy the geometry of the free 1:3 oligosaccharide–lectin cross-linked complexes (Bhattacharyya et al., 1989a). Thus, the triantennary oligosaccharide appears to be able to exist in either bound conformation.

Summary. The present results demonstrate that an S-type dimeric animal lectin with LacNAc binding specificity forms highly ordered cross-linked lattices with a glycoprotein possessing multiple N-linked carbohydrate chains with terminal LacNAc residues. The structures of these cross-linked complexes are similar to those of two dimeric Gal-specific plant lectins. Thus, both plant and animal lectins are capable of forming highly ordered cross-linked lattices with specific glycoproteins. It appears then that the geometry of the binding sites of this class of S-type animal lectin resembles that of the plant lectin Con A and many other homologous lectins, where the carbohydrate binding sites are located at the far ends of peanut-shaped dimers (Becker et al., 1976; Lemieux, 1989) which facilitates cross-linking of specific glycoconjugates. This contrasts with C-type animal lectins where two or more subunits of the lectin appear to face in the same direction which often leads to multiple, high-affinity interactions with a carbohydrate ligand [cf. Lee et al. (1991)]. In view of the possible role of this class of β-Gal-specific 14-kDa vertebrate lectins in cell adhesion and other cellular phenomena (Catt & Harrison, 1985; Regan et al., 1986; Zalik & Milos, 1986), the implications of the present findings are intriguing. The results suggest that lectin-mediated aggregation events involved in the cellular recognition processes and signal transduction processes could be regulated by unique cross-linking interactions with specific glycoprotein receptors.

The present findings also provide new observations on the specificity of cross-linking interactions between lectins and glycoproteins. The results indicate that the total valency of the carbohydrate chains of ASF for a series of Gal-specific plant lectins and an animal lectin is determined by (1) the number and composition of the carbohydrate chains on ASF, (2) the quaternary structure and size of the lectins, and (3) the relative ratio of the glycoprotein to lectin in solution. The trivalency of the free triantennary oligosaccharide is also exhibited in the 1:9 ASF–lectin cross-linked complexes (per monomer) (1:8.5 ratio when taking into account the amount of N-linked biantennary chain present on ASF) with the 14-kDa lectin, EIL, and ECL. This contrasts with the individual N-linked Man9 oligomannose-type chains on SBA which are either univalent or half-valent in cross-linked complexes with Con A (Khan et al., 1991), even though the free glycopeptide is bivalent for binding to Con A (Bhattacharyya et al., 1987a).

Lastly, the present findings also suggest that the triantennary oligosaccharide exists in different conformations when cross-linked as a free oligosaccharide with EIL or ECL as compared to that of the covalently attached oligosaccharide in the corresponding 1:9 ASF–lectin cross-linked complex(es). This demonstrates the flexible cross-linking activity of the N-linked oligosaccharide in its interactions with a variety of lectins, which may be related to its functions as a receptor in biological systems.

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