Interactions of Concanavalin A with Glycoproteins: Formation of Homogeneous Glycoprotein–Lectin Cross-Linked Complexes in Mixed Precipitation Systems†

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ABSTRACT: We have previously demonstrated that the interactions between branched chain oligosaccharides and glycopeptides isolated from glycoproteins and glycolipids with specific lectins lead to the formation of homopolymeric carbohydrate–protein cross-linked complexes, even in the presence of mixtures of the carbohydrates or lectins [cf. Bhattacharyya, L., Fant, J., Lonn, H., & Brewer, C. F. (1990) *Biochemistry 29*, 7523–7530]. Recently, we have shown that highly ordered cross-linked lattices are formed between the tetrameric glycoprotein soybean agglutinin (SBA), which possesses a Man9 oligomannose chain per monomer, and the Glc/Man-specific plant lectin concanavalin A (Con A) [Khan, M. I., Mandal, D. K., & Brewer, C. F. (1991) *Carbohydr. Res. 213*, 69–77]. Using radiolabeling and quantitative precipitation techniques, we show in the present study that Con A binds and forms unique cross-linked complexes with four different glycoproteins having different numbers and types of carbohydrate chains as well as different quaternary structures. The glycoproteins include quail ovalbumin, *Lotus tetragonolobus* isolectin A (LTL-A), *Erythrina cristagalli* lectin (ECL), and *Erythrina corallodendron* lectin (EcorL). The results show that a preparation of quail ovalbumin containing either one Man7 or Man8 oligomannose chain per molecule forms a 1:2 cross-linked complex with tetrameric Con A, thereby demonstrating bivalency of the single carbohydrate chain(s) on the glycoprotein. Tetrameric LTL-A and dimeric ECL, which possess two xylose-containing carbohydrate chains per monomer, both form 1:2 and 1:1 cross-linked complexes (per monomer) of glycoprotein to lectin, depending on their relative ratios in solution. However, dimeric EcorL, which has the same carbohydrate structure and number of chains as ECL, forms only a 1:2 cross-linked complex with tetrameric Con A. Quail ovalbumin does not precipitate with dimeric acetyl- or succinyl-Con A, while LTL-A, ECL, and EcorL form 1:2 complexes with acetyl-Con A but fail to precipitate with succinyl-Con A. Mixed quantitative precipitation studies show that Con A forms the same unique stoichiometry cross-linked complexes with the above glycoproteins including SBA in the presence of binary mixtures of the glycoproteins. These results provide evidence that each glycoprotein forms a unique homopolymeric cross-linked lattice(s) with Con A which excludes the lattices of other glycoproteins. The present findings thus represent a new source of binding specificity between lectins and glycoproteins, namely, the formation of homogeneous aggregated complexes. The results are discussed in terms of the possible biological recognition properties of lectins and glycoconjugates as receptors.

Oligosaccharides attached to proteins and lipids 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). Interest has recently increased in the binding interactions of these carbohydrate receptors with lectins which are carbohydrate-binding proteins. Lectins have been isolated from a variety of sources including plants (Goldstein & Poretz, 1986) and vertebrate tissues (Drickamer, 1988). The importance of lectin–carbohydrate interactions has been demonstrated by the observation that oligosaccharide recognition by selectins is an important first step in the adhesion of leukocytes to the endothelium during inflammation (Stoolman, 1989). The majority of the best-characterized lectins, however, are those from plants, and it is these proteins that have been most widely used to explore the membrane structures and properties of both normal and transformed cells (Atkinson & Hakimi, 1980; Lis & Sharon, 1984).

Binding of multivalent lectins to the surface of cells often leads to cross-linking and aggregation of specific glycoprotein and glycolipid receptors, which, in turn, is associated with a variety of biological responses. For example, cross-linking of glycoconjugates on the surface of cells has been implicated in the mitogenic activities of lectins such as Con A[1] and SBA (cf. Nicolson, 1976), in the molecular sorting of glycoproteins in the secretory pathway of cells (Chung et al., 1989), in signal transduction mechanism of certain glycoprotein hormones (Sairam, 1989), 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). Furthermore, lectin-induced cross-linking of transmembrane glycoproteins results in changes in their interactions with cytoskeletal proteins and alterations in the mobility and

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[1] Abbreviations: Con A, concanavalin A, lectin from jack bean; SBA, lectin from *Glycine max*; LTL-A, isolectin A from *Lotus tetragonolobus*; ECL, lectin from *Erythrina cristagalli*; EcorL, lectin from *Erythrina corallodendron*; FPLC, fast-protein liquid chromatography; NMR, nuclear magnetic resonance; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; αMM, methyl α-mannopyranoside. Fucose is in the L configuration; all other sugars are in the D configuration.

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aggregation of other surface receptors (cf. Carraway & Carraway, 1989; Edelman, 1976). Thus, lectin–glycoconjugate cross-linking interactions are associated with a variety of biological signal transduction processes.

We have previously shown that plant lectins with a wide range of carbohydrate-binding specificities can bind and cross-link a variety of branched chain oligosaccharides isolated from glycoproteins and glycolipids (cf. Bhattacharyya et al., 1988a, 1990). For example, certain N-linked oligomannose, bisected hybrid, and bisected complex type glycopeptides were shown to be bivalent and to precipitate with the tetrameric Glc/Man-specific lectin Con A (Bhattacharyya et al., 1987a,b). Further studies have shown that these cross-linking interactions lead to the formation of unique homopolymeric cross-linked lattices between branched chain carbohydrates and lectins, even in the presence of mixtures of the molecules (Bhattacharyya et al., 1988b, 1990; Bhattacharyya & Brewer, 1992). In fact, several lectin–carbohydrate complexes have been shown to be crystalline and suitable for structural analysis by X-ray diffraction (Makowski and Brewer, unpublished results) and electron microscopic techniques (Bhattacharyya et al., 1990). Thus, the formation of oligosaccharide– and glycopeptide–lectin cross-linked complexes represents a new dimension of binding specificity between carbohydrates and proteins.

We have also shown that certain glycoproteins can form specific cross-linked complexes with lectins. For example, SBA, which is a tetrameric glycoprotein that possesses a single Man9 oligomannose type chain per monomer, forms two types of highly organized cross-linked lattices with Con A, depending on the relative ratio of the molecules in solution (Khan et al., 1991). More recently, we have demonstrated that the β-galactoside-specific 14-kDa lectin from calf spleen, an S-type animal lectin, binds to the three N-linked triantennary complex type oligosaccharide chains of asialofetuin and forms well-defined cross-linked complexes (Mandal & Brewer, 1992).

In the present study, we have examined the ability of Con A to form specific cross-linked complexes with a variety of glycoproteins having different types and numbers of carbohydrate chains as well as different quaternary structures. The results show that each glycoprotein forms a homopolymeric cross-linked lattice(s) with Con A, even in mixtures of the molecules. These findings are discussed in terms of the structure–function properties of lectins and glycoproteins as receptors.

MATERIALS AND METHODS

Materials. Seeds of *Lotus tetragonolobus* (Syn. *Tetragonolobus purpureas*), *Erythrina cristagalli*, and *Erythrina corallodendron* were purchased from Schumacher & Co., Sandwich, MA. Seeds of jack bean were obtained from Sigma Chemical Co. The Superdex 75 column and Sepharose 4B were obtained from Pharmacia. Con A–Sepharose and asialofetuin–Sepharose were prepared following a published procedure (Parikh et al., 1974). Monosaccharides were obtained 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. [*carbonyl*-^{14}C]Acetic anhydride (10 mCi/mmol) was obtained from Sigma Chemical Co. All other materials used were of analytical grade. Purity and structural analysis of the carbohydrates were determined by ^{1}H NMR at 500 MHz on a Varian VXR-500 spectrometer.

Preparation of Proteins. Con A was prepared according to the method of Agrawal and Goldstein (1967). The concentration of Con A was determined spectrophotometrically using $A^{1\%,1cm}$ = 12.4 at 280 nm and expressed in terms of monomer (M_r = 27 000) (Wang et al., 1971; Yariv et al., 1968). Acetyl- and succinyl-Con A were prepared as described (Khan et al., 1991) and were separated from tetrameric Con A by FPLC on Superdex 75.

The native lectin mixture from the seeds of *L. tetragonolobus* was purified from the crude extract [prepared according to Yariv et al. (1967)] by affinity chromatography as described previously (Allen & Johnson, 1977). The major isolectin A (LTL-A) was obtained by DEAE-cellulose chromatography as described (Kalb, 1968). The purified LTL-A thus obtained was applied to a Mono S (HR 5/5) cation-exchange column in Pharmacia FPLC system in 50 mM HEPES buffer, pH 6.5, containing 1 mM Mn^{2+} and 1 mM Ca^{2+}. The protein was eluted as a single symmetrical peak by using a linear gradient (0–0.43 M) of NaCl in the same buffer in 20 min at a flow rate of 1 mL/min. The concentration of LTL-A was measured spectrophotometrically by using $A^{1\%,1cm}$ = 17.4 at 280 nm and expressed in terms of monomer (M_r = 28 000) (Goldstein & Poretz, 1986).

The ovalbumin from quail egg white was prepared and fractionated on a Con A–Sepharose column according to the previously described procedure (Iwase et al., 1983) by using a linear gradient (0–0.2 M) of methyl α-glucopyranoside. The quail ovalbumin that was bound to Con A–Sepharose was obtained as bound fraction I (less tightly bound) and bound fraction II (more tightly bound). The concentration of the ovalbumin was determined by using an absorbance of $A^{1\%,1cm}$ = 7.5 at 280 nm (Shepherd & Montgomery, 1978) and M_r = 45 000.

ECL and EcorL were prepared from the respective seeds as described (Iglesias et al., 1982; Lis et al., 1985) using asialofetuin–Sepharose as an affinity matrix. The protein concentrations were determined spectrophotometrically by using $A^{1\%,1cm}$ = 15.3 at 280 nm and expressed in terms of subunit (M_r = 28 000) (Goldstein & Poretz, 1986).

SBA was purified according to Bhattacharyya et al. (1988a) except that either cross-linked guar gum (Appukuttan et al., 1977) or asialofetuin–Sepharose was used as affinity matrix. Protein concentration was determined spectrophotometrically using $A^{1\%,1cm}$ = 12.8 at 280 nm and expressed in terms of subunit (M_r = 30 000) (Lotan et al., 1974).

The neutral sugar contents of LTL-A, ECL, and EcorL were determined by the phenol–sulfuric acid method (Dubois et al., 1956) using appropriate mixtures of mannose, xylose, and fucose as standards.

Radiolabeling of Proteins. Con A, LTL-A, ECL, EcorL, and SBA were radiolabeled with ^{3}H or ^{14}C by reductive methylation as described previously (Khan et al., 1991). In the final step, the radiolabeled proteins were chromatographed on respective affinity columns. ^{14}C-Labeled acetyl-Con A was prepared by acetylation of Con A with [*carbonyl*-^{14}C]acetic anhydride according to the procedure described (Khan et al., 1991) and was separated from tetrameric Con A by gel filtration on Superdex 75. Radiolabeled or N-dimethylated ovalbumin showed a decrease in its precipitating activity (approximately a factor of 3) compared to unmodified ovalbumin.

Quantitative Precipitation Assays. Assays with individual glycoproteins were performed in a final volume of either 400 μL or 200 μL by using either 0.1 M Tris-HCl buffer, pH 7.2, containing 0.9 M NaCl, 1 mM Mn^{2+}, and 1 mM Ca^{2+} or 0.1 M HEPES buffer, pH 7.2, containing 0.9 M NaCl, 1 mM Mn^{2+}, and 1 mM Ca^{2+} as described previously (Mandal &

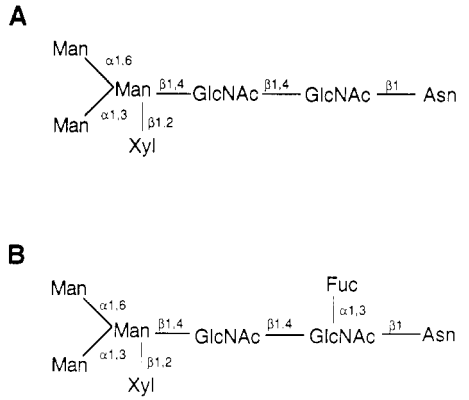

FIGURE 1: Structures of (A) the N-linked xylose-containing hexasaccharide chain from LTL-A and (B) the fucosyl heptasaccharide chain from LTL-A, ECL, or EcorL. Man, Xyl, Fuc, GlcNAc, and Asn represent mannose, xylose, fucose, *N*-acetylglucosamine, and asparagine residues, respectively.

Brewer, 1992). Assays with a binary mixture of glycoproteins were carried out in a final volume of 200 μL in two parallel experiments, each containing radiolabeled Con A and the two glycoproteins in which one was radiolabeled and the other N-dimethylated. In these assays, the concentrations of both Con A and the glycoprotein that forms a single type of cross-linked complex with Con A were kept fixed and the concentration of the other glycoprotein was varied.

RESULTS AND DISCUSSION

Properties of the Glycoproteins and Con A. Ovalbumin isolated from different species is a monomeric glycoprotein of M_r 45 000 and exhibits microheterogeneity with respect to its N-linked carbohydrate chain which is composed of high mannose and hybrid type oligosaccharides (Huang & Montgomery, 1972). In the case of quail ovalbumin, only high mannose type chains are associated with the protein (Iwase et al., 1983). Fraction I of quail ovalbumin obtained from Con A–Sepharose chromatography possesses Man5 and Man6 oligomannose type chains (Tai et al., 1975). Fraction II of quail ovalbumin from the column possesses Man7 and Man8 oligomannose type chains, as determined by high-resolution ^{1}H NMR spectroscopy at 500 MHz of the glycopeptide(s) isolated from a pronase digest of the glycoprotein (data not shown).

LTL-A is a tetrameric glycoprotein of subunit M_r 28 000 (Goldstein & Poretz, 1986) and possesses the N-linked xylose-containing oligosaccharide shown in Figure 1A as the major component (75%) and the fucosylated analog shown in Figure 1B as the minor component (25%) (Mandal and Brewer, unpublished data). ECL and EcorL are dimeric glycoproteins of subunit M_r 28 000 (Goldstein & Poretz, 1986) and both possess the N-linked fucosyl heptasaccharide in Figure 1B (Ashford et al., 1987). The neutral sugar content of each glycoprotein as determined by the phenol–sulfuric acid method was 5.4% for LTL-A, 5.9% for ECL, and 5.9% for EcorL, which in the latter case is similar to that reported by Arango et al. (1992). These results indicate that there are two oligosaccharide chains per monomer on each glycoprotein. SBA is a tetrameric glycoprotein that possesses a single Man9 oligomannose type chain per monomer of M_r 30 000 (Goldstein & Poretz, 1986).

Con A is a tetramer at physiological pH and consists of monomeric subunits of M_r 27 000 that possess one Glc/Man binding site (Agrawal & Goldstein, 1967; Yariv et al., 1968). Thus, the lectin is tetravalent in its carbohydrate binding activity. Acetyl- and succinyl-Con A are dimeric forms of

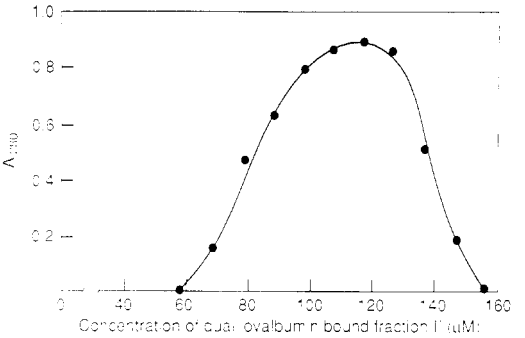

FIGURE 2: Precipitation profile of Con A in the presence of the quail ovalbumin bound fraction II at 22 °C. The buffer was 0.1 M Tris-HCl containing 0.9 M NaCl, 1 mM Mn^{2+}, and 1 mM Ca^{2+} at pH 7.2. Con A monomer concentration was 210 μM in a total volume of 200 μL.

Table I: Stoichiometries of Cross-Linked Complexes of Con A and Acetyl-Con A with Various Glycoproteins at 22 °C

glycoprotein	no. of cross-linked complexes	stoichiometry (glycoprotein:lectin)
	Con A	
quail ovalbumin	1	1:1.8
LTL-A	2	1:2.0
		1:1.1
ECL	2	1:2.0
		1:1.1
EcorL	1	1:1.9
SBA[a]	2	1:1.0
		2:1.0
	Acetyl-Con A	
LTL-A	1	1:1.8
ECL	1	1:2.0
EcorL	1	1:1.8

[a] From Khan et al. (1991).

the lectin which possess the same binding specificity (Goldstein & Poretz, 1986).

The relative affinity of the xylose-containing oligosaccharide isolated from LTL-A (Figure 1A) for Con A, as determined by hemagglutination inhibition measurements, is similar to that of αMM, while the corresponding fucosyl glycopeptide (Figure 1B) binds somewhat less tightly than αMM (data not shown). This contrasts with the relatively high affinity of the oligomannose type glycopeptides found on quail ovalbumin for Con A (100-fold or more greater than αMM) (Bhattacharyya & Brewer, 1989). Both xylose-containing free oligosaccharide chains bind to Con A as univalent ligands (Mandal and Brewer, unpublished results) in contrast to the bivalency of oligomannose type chains (Bhattacharyya & Brewer, 1989).

Quantitative Precipitation Profiles of Con A with Quail Ovalbumin. The quantitative precipitation profile of Con A (210 μM) in the presence of increasing amounts of quail ovalbumin fraction II shows a bell-shaped profile (Figure 2) similar to that observed for oligomannose type glycopeptides and the lectin (cf. Bhattacharyya et al., 1987a). The ratio of the concentration of ovalbumin to Con A monomer at the equivalence point (point of maximum precipitation) is 1:1.8 (Table I) which shows that the Man7 and Man8 oligomannose type chains present on ovalbumin fraction II are bivalent for Con A binding (Figure 9A), as are the corresponding free glycopeptides (Bhattacharyya & Brewer, 1989). Similar results are obtained with a mixture containing both fraction I and II of ovalbumin (data not shown); however, fraction I which contains a Man5 or Man6 chain did not precipitate with Con A, unlike the free glycopeptide (Bhattacharyya et

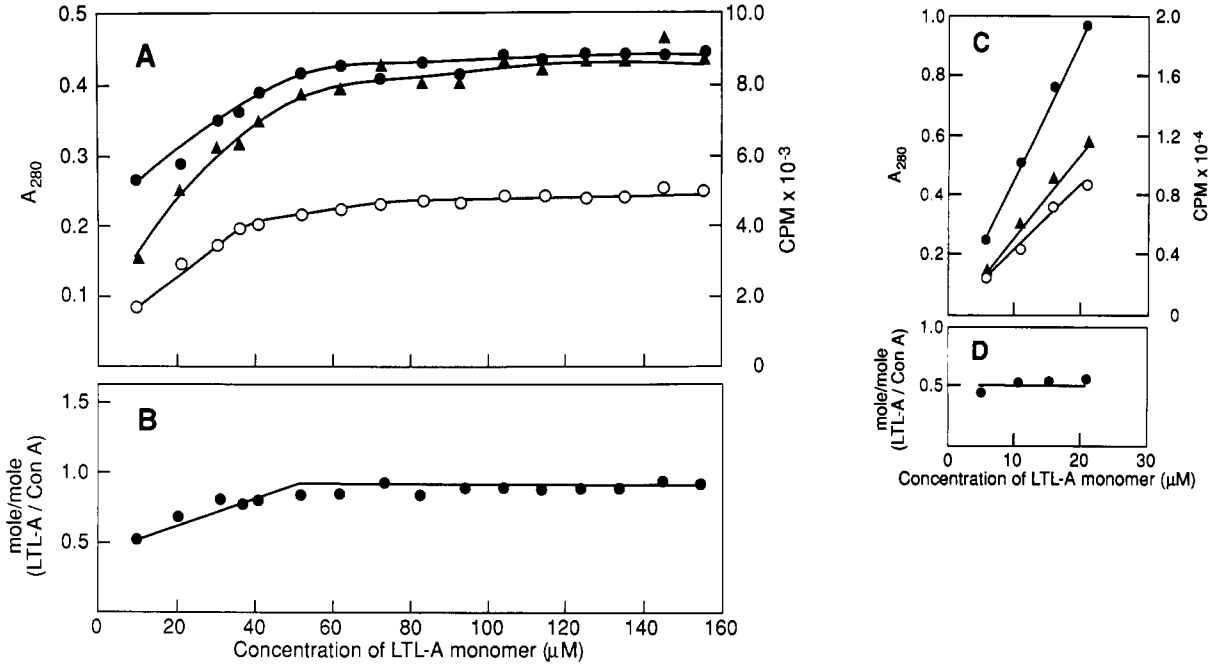

FIGURE 3: Precipitin curves for the quantitative precipitation of Con A by LTL-A at 22 °C: (A and C) profile of the total protein (A_{280}) precipitated (○) and counts per minute of [^{14}C]Con A (●) and [^{3}H]LTL-A (▲) in the precipitate; and (B and D), ratio (●) of moles of LTL-A monomer precipitated per mole of Con A monomer. The buffer was the same as that in Figure 2. [^{14}C]Con A monomer concentration was fixed at 44 μM in a total volume of 200 μL in parts A and B and 88 μM in a total volume of 400 μL in parts C and D. The specific activities of the radiolabeled Con A and LTL-A are 1320 and 1450 cpm/nmol, respectively.

al., 1987a). None of the quail ovalbumin preparations precipitate with dimeric acetyl- or succinyl-Con A, which is consistent with previous results suggesting that divalent lectins do not form stable cross-linked complexes with divalent carbohydrates (Bhattacharyya et al., 1990).

Quantitative Precipitation Profiles of Con A and Acetyl-Con A with LTL-A. Tetrameric LTL-A forms two different types of cross-linked complexes with Con A depending on the relative ratio of the two proteins in solution (Figure 3). Titration of [^{14}C]Con A (44 μM) with increasing amounts of [^{3}H]LTL-A (Figure 3A) shows increasing total protein precipitated (A_{280} profile) as well as increasing amounts of LTL-A in the precipitate (^{3}H profile) until a broad plateau is reached. The radioactive profile of [^{14}C]Con A, however, shows a small initial slope which becomes flat, at which point about 80% Con A is precipitated. Figure 3B shows that the mole ratio of LTL-A to Con A (per monomer) in the precipitates increases from 0.5 (1:2) to 0.9 (1:1.1) (Table I), after which it remains constant. These results indicate that a 1:2 LTL-A–Con A cross-linked complex is transformed into a 1:1 complex which shows no further change even in the presence of very high concentrations of LTL-A.

Data obtained in the region of low concentrations of LTL-A relative to Con A (88 μM) show that the A_{280} profile and the radioactive profiles are very similar (Figure 3C) and that the mole ratio of LTL-A to Con A remains constant at 0.5 (Figure 3D). These results confirm that LTL-A initially forms a 1:2 cross-linked complex with Con A which spontaneously transforms to a 1:1 cross-linked complex with increasing concentrations of LTL-A.

The initial formation of the 1:2 LTL-A–Con A cross-linked complex indicates that each xylose-containing carbohydrate chain on LTL-A functions as a univalent binding site for Con A and that both N-linked chains on each monomer of LTL-A are occupied in the complex (Figure 9B). The total carbohydrate valency of LTL-A in the 1:2 complex is eight. At higher concentrations of LTL-A, a 1:1 cross-linked complex forms (Table I) which indicates that only one N-linked oligosaccharide chain per LTL-A monomer is bound to Con A under these conditions (Figure 9B). The total carbohydrate valency of LTL-A in the 1:1 complex is four. (In theory, Con A could be binding to four carbohydrate chains of two monomers of the LTL-A tetramer; however, this is considered to be less likely than the complex shown in Figure 9B.) 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 transforms to a 2:1 complex in the presence of increasing SBA concentration (Khan et al., 1991).

The quantitative precipitation profile for [^{14}C]acetyl-Con A (81 μM) in the presence of ^{3}H-labeled LTL-A shows increasing precipitation of total protein (A_{280} profile) until a broad plateau is reached (not shown). The radioactive profiles are parallel with the total protein precipitation curve. In the region of maximum precipitation, about 65% of acetyl-Con A is precipitated. The ratio of moles of LTL-A to acetyl-Con A (per monomer) in the precipitates shows that it remains constant at 1:2 over the entire profile (Table I). Dimeric succinyl-Con A, however, does not precipitate with LTL-A, possibly due to unfavorable electrostatic interactions. This demonstrates that the quaternary structure and charge state of Con A influence the expressed valency of the carbohydrates on LTL-A. These results are consistent with our earlier observations that SBA forms a weaker cross-linked lattice with succinyl-Con A than with acetyl-Con A (Khan et al., 1991).

Quantitative Precipitation Profiles of Con A and Acetyl-Con A with ECL. The quantitative precipitation profile of ^{3}H-labeled Con A (64 μM) with increasing amounts of ^{14}C-labeled ECL is shown in Figure 4. Figure 4A shows that the total protein curve (A_{280}) and ^{14}C curve (ECL) are almost parallel over the entire profile, whereas the ^{3}H data (Con A) show a region of maximum precipitation (75%) and then a decline. The mole ratio of ECL:Con A (per monomer) as a

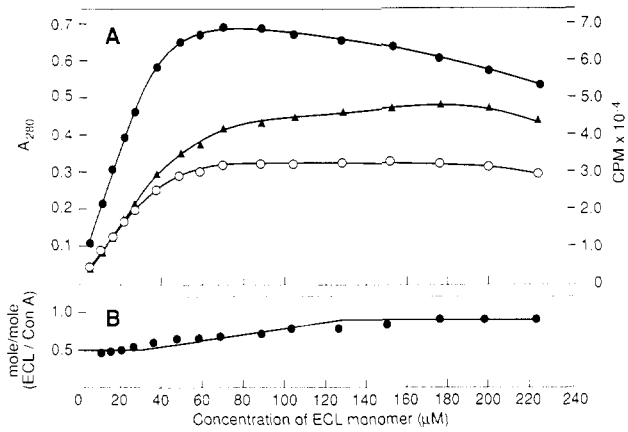

FIGURE 4: Precipitin curves for the quantitative precipitation of Con A by ECL at 22 °C: (A) profile of the total protein (A_{280}) precipitated (○) and counts per minute of [^{3}H]Con A (●) and [^{14}C]ECL (▲) in the precipitate; and (B) ratio (●) of moles of ECL monomer precipitated per mole of Con A monomer. The buffer was 0.1 M HEPES containing 0.9 M NaCl, 1 mM Mn^{2+}, and 1 mM Ca^{2+} at pH 7.2. [^{3}H]Con A monomer concentration was fixed at 64 μM in a total volume of 200 μL. The specific activities of the radiolabeled Con A and ECL are 7400 and 6300 cpm/nmol, respectively.

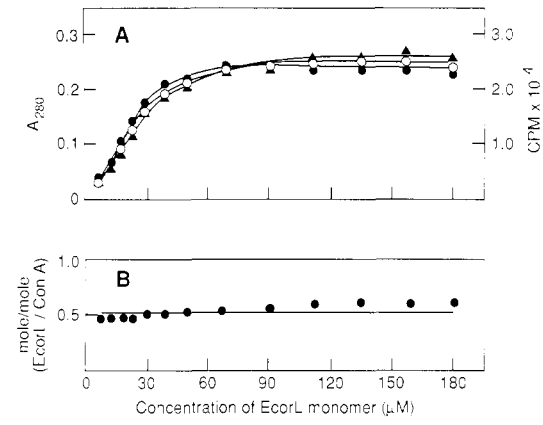

FIGURE 5: Precipitin curves for the quantitative precipitation of Con A by EcorL at 22 °C: (A) profile of the total protein (A_{280}) precipitated (○) and counts per minute of [^{3}H]Con A (●) and [^{14}C]-EcorL (▲) in the precipitate; and (B) ratio (●) of moles of EcorL monomer precipitated per mole of Con A monomer. The buffer was the same as that in Figure 2. [^{3}H]Con A monomer concentration was fixed at 66 μM in a total volume of 200 μL. The specific activities of the radiolabeled Con A and EcorL are 3030 and 5480 cpm/nmol, respectively.

function of the concentration of ECL (Figure 4B) shows the initial formation of 1:2 cross-linked complex which transforms into a 1:1 complex in the presence of a relatively high concentration of ECL in solution. The formation of the 1:2 cross-linked complexes indicates that the binding interactions are mediated by the two xylose-containing heptasaccharide chains on each monomer of ECL (Figure 9C). Thus, the total carbohydrate valency of ECL in the 1:2 complex is four. In the 1:1 complex, only half of the carbohydrate chains of ECL are bound, and therefore the total carbohydrate valency is two (Figure 9C) (Table I). With acetyl-Con A, ECL forms only the 1:2 cross-linked complex, as shown in Figure 9D (Table I) (data not shown). ECL failed to precipitate with succinyl-Con A. These results demonstrate that the quaternary structure and charge state of Con A influence the expressed valency of the carbohydrates on ECL, similar to the above results for LTL-A.

Quantitative Precipitation Profiles of Con A and Acetyl-Con A with EcorL. Corresponding quantitative precipitation data for EcorL with Con A are shown in Figure 5. In the case of EcorL, a similar 1:2 complex forms; however, unlike ECL, no further transformation of this complex occurs with increasing concentrations of the glycoprotein (Table I). Furthermore, the maximum percentage of Con A precipitated with EcorL is smaller (60%) as compared to ECL (75%). With acetyl-Con A, EcorL also forms only the 1:2 cross-linked complex (Table I) (data not shown). EcorL also failed to precipitate with succinyl Con A.

The results suggest that although the carbohydrate chains of ECL and EcorL are the same, the overall protein quaternary structures of the two glycoproteins influence the stability and formation of their respective cross-linked complexes with Con A. Although the tertiary structure of the EcorL monomer is similar to that of Con A and other legume lectins, X-ray diffraction studies have shown that the quaternary structure of EcorL is drastically different from that of the Con A dimer due to the position of a carbohydrate chain of EcorL near the dimer interface (Shaanan et al., 1991). Furthermore, only one of the two carbohydrate chains of EcorL is observed in the crystal structure, even though the same group has reported a neutral carbohydrate content (6%) (Arango et al., 1992) which is the same as that obtained in the present study (5.9%), consistent with two carbohydrate chains per monomer. (This may be due to selective crystallization of a subset of EcorL molecules.) Although the quaternary structure of ECL is not known, its N-terminal sequence is similar to that of other *Erythrina* species of the lectin including EcorL, as are its physicochemical properties (Bhattacharyya et al., 1986; Lis & Sharon, 1987; Arango et al., 1992). It is therefore possible that the difference in the cross-linked complexes of ECL and EcorL with Con A may be due to differences in the quaternary structure of the two dimeric *Erythrina* proteins and/or the positioning of their carbohydrate chains. This possibility is being investigated.

In all of the above cases, inhibition of formation and dissolution of the precipitates of acetyl-Con A and native Con A with the glycoproteins occurred in the presence of 0.1 M αMM, a specific competing monosaccharide, but not by monosaccharides such as Gal or Fuc which do not bind to the lectin. This demonstrates that formation of the precipitates occurs by specific carbohydrate–protein interactions.

Precipitation of a Binary Mixture of Glycoproteins with Con A. We have previously demonstrated that quantitative precipitation profiles of Con A in the presence of binary mixtures of oligomannose type glycopeptides provide evidence for the formation of homogeneous cross-linked lattices between each glycopeptide and the lectin (cf. Bhattacharyya et al., 1988b). We now show similar evidence for the formation of unique, homogeneous cross-linked lattices between different glycoproteins and the jack bean lectin. The present strategy is to prepare binary mixtures of the glycoproteins in the present study including SBA in which one of the two is known to form a single stoichiometric cross-linked complex with Con A (i.e., quail ovalbumin or EcorL; Table I) and the other is known to form two types of stoichiometric cross-linked complexes with the lectin (i.e., SBA, LTL-A, or ECL). The concentration of the former glycoprotein is held constant while the concentration of the latter glycoprotein is increased. Since each glycoprotein is separately radiolabeled in parallel experiments with radiolabeled Con A, the amount of Con A precipitated with the first glycoprotein can be calculated, assuming formation of the single stoichiometric cross-linked complex listed in Table I. The difference between the amount of Con A precipitated by both glycoproteins and the amount precipitated with the first glycoprotein permits calculation of the

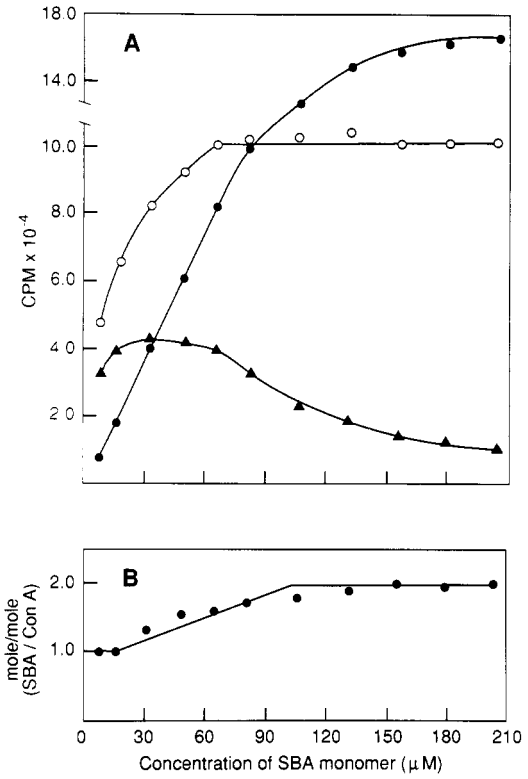

FIGURE 6: Precipitation profile of Con A in presence of a mixture of quail ovalbumin and SBA at 22 °C: (A) ○, [^{3}H]Con A; ▲, [^{14}C]-quail ovalbumin; ●, [^{14}C]SBA; and (B) mole/mole ratio (per monomer) (●) of SBA to Con A in the precipitate. The buffer was the same as that in Figure 2. The monomeric concentrations of Con A and quail ovalbumin were kept fixed at 86 μM and 44 μM, respectively. For details, see Materials and Methods. The specific activities of the radiolabeled Con A, quail ovalbumin, and SBA are 7300, 12 500, and 6540 cpm/nmol, respectively.

ratio of the second glycoprotein–Con A cross-linked complex in the mixture. If the same two stoichiometric cross-linked complexes listed in Table I are observed for the second glycoprotein with Con A, this is taken as evidence for the formation of homogeneous cross-linked complexes between Con A and the two glycoproteins in the mixture.

Mixed Quantitative Precipitation Profiles of Con A in the Presence of Quail Ovalbumin and SBA. Figure 6 shows the quantitative precipitation profile of Con A (86 μM) in the presence of quail ovalbumin (44 μM) and increasing amounts of SBA. The counts per minute for [^{3}H]Con A and [^{14}C]-SBA in the precipitates in Figure 6A were obtained from an assay in which N-dimethylated quail ovalbumin was used, and the counts per minute for [^{14}C]quail ovalbumin were obtained from a parallel assay in which N-dimethylated SBA was used. The precipitation profile of quail ovalbumin with Con A shows a small initial increase and then declines as the concentration of SBA is increased. Assuming that quail ovalbumin forms a 1:2 cross-linked complex with Con A as demonstrated in the individual precipitation profile (Table I), the mole ratio of SBA to Con A in the mixed precipitates clearly shows the initial formation of a 1:1 and then 2:1 SBA–Con A cross-linked complex (Figure 6B), as observed in the precipitation assay of Con A with SBA alone (Table I) (Khan et al., 1991). Thus, the data shown in Figure 6 demonstrate that in the presence of a mixture of quail ovalbumin and SBA Con A forms unique cross-linked complexes with both glycoproteins.

Mixed Quantitative Precipitation Profiles of Con A in the Presence of Quail Ovalbumin and LTL-A. Figure 7 shows the quantitative precipitation profile of Con A (141 μM) in

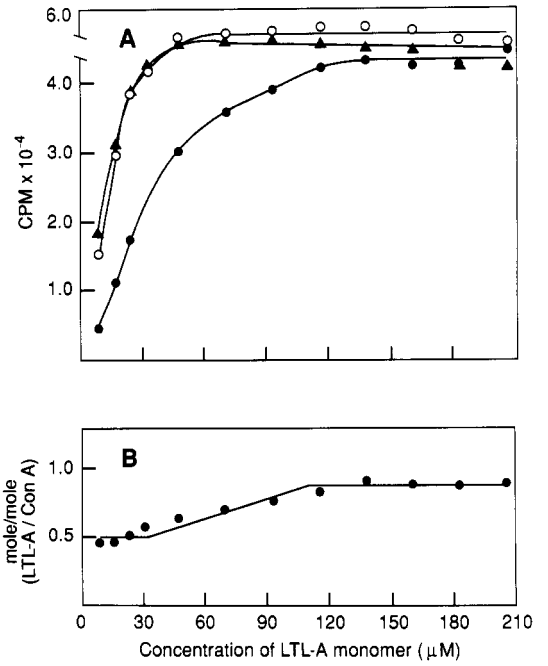

FIGURE 7: Precipitation profile of Con A in presence of a mixture of quail ovalbumin and LTL-A at 22 °C: (A) ○, [^{3}H]Con A; ▲, [^{14}C]quail ovalbumin; ●, [^{14}C]LTL-A; and (B) mole/mole ratio (per monomer) (●) of LTL-A to Con A in the precipitate. The buffer was the same as that in Figure 2. The monomeric concentrations of Con A and quail ovalbumin were kept fixed at 141 μM and 37 μM, respectively. For details, see Materials and Methods. The specific activities of the radiolabeled Con A, quail ovalbumin, and LTL-A are 3100, 12 300, and 5600 cpm/nmol, respectively.

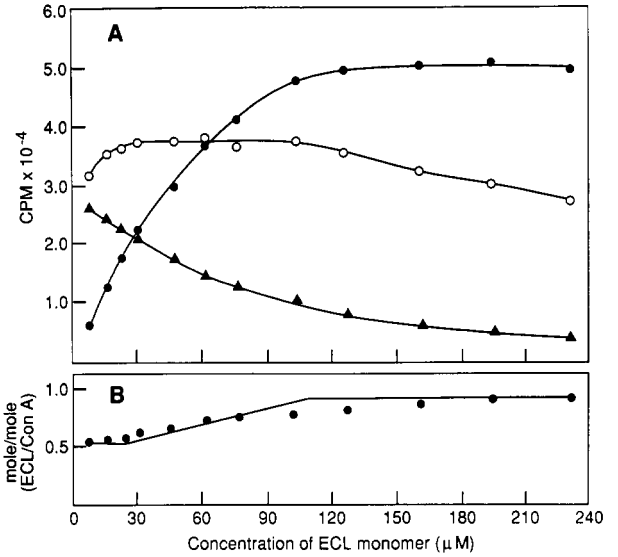

FIGURE 8: Precipitation profile of Con A in presence of a mixture of EcorL and ECL at 22 °C: (A) ○, [^{3}H]Con A; ▲, [^{14}C]EcorL; ●, [^{14}C]ECL; and (B) mole/mole ratio (per monomer) (●) of ECL to Con A in the precipitate. The buffer was the same as that in Figure 2. The monomeric concentrations of Con A and EcorL were kept fixed at 83 μM and 42 μM, respectively. For details, see Materials and Methods. The specific activities of the radiolabeled Con A, EcorL, and ECL are 3030, 5300, and 6680 cpm/nmol, respectively.

the presence of a mixture of glycoproteins containing quail ovalbumin (37 μM) and increasing amounts of LTL-A. In this case, the precipitation of quail ovalbumin initially increases and then becomes flat (Figure 7A). (It should be noted that the increase in quail ovalbumin precipitated in Figure 7A is due to the decreasing amount of free Con A present due to increasing concentrations of LTL-A. This has the effect of climbing the ascending side of the bell-shaped profile of the

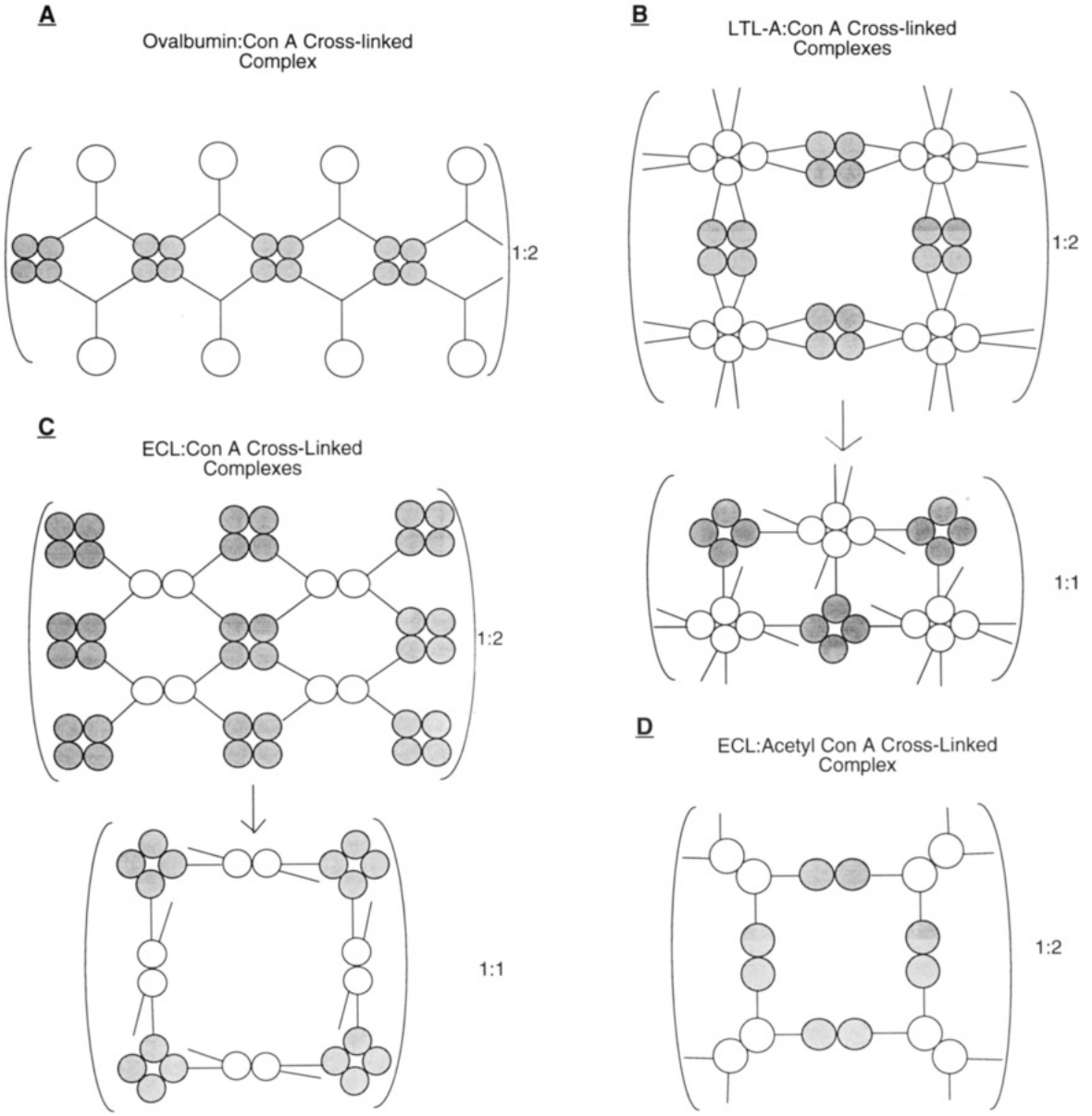

FIGURE 9: Schematic representations of (A) 1:2 ovalbumin–Con A cross-linked complex; (B) 1:2 and 1:1 LTL-A–Con A cross-linked complexes; (C) 1:2 and 1:1 ECL–Con A cross-linked complexes; and (D) 1:2 ECL(EcorL)–acetyl-Con A cross-linked complex. The arrows indicate transformation of one cross-linked complex to another complex. The hatched circles represent Con A tetramers in parts A, B, and C and acetyl-Con A dimers in part D. The open circles and connected line structures represent the protein and carbohydrate moieties, respectively, of the different glycoproteins in parts A, B, C, and D.

quail ovalbumin–Con A complex in Figure 2. This effect is also noted to a less extent in Figure 6A since the amount of Con A initially present is less than in Figure 7A.) Assuming that quail ovalbumin forms a 1:2 cross-linked complex with Con A, the mole ratio of LTL-A to Con A in the precipitates shows the initial formation of 1:2 and then 1:1 LTL-A–Con A cross-linked complexes, similar to that observed in the individual precipitation assay of Con A with LTL-A (Table I). Thus, Con A forms unique cross-linked complexes with quail ovalbumin and LTL-A in mixtures of the two glycoproteins.

Interestingly, the precipitation profile of quail ovalbumin in the mixture shows an initial increase and then becomes flat (Figure 7A), in contrast to that observed for binary mixtures of SBA and quail ovalbumin in which the profile of the latter decreases with increasing SBA concentration (Figure 6A). This indicates that although LTL-A is a tetrameric glycoprotein like SBA, the lower affinity of the xylose-containing oligosaccharide chains of LTL-A relative to the high affinity oligomannose chain of ovalbumin does not readily lead to displacement of quail ovalbumin by LTL-A in the respective cross-linked complexes with Con A.

Mixed Quantitative Precipitation Profiles of Con A in the Presence of ECL and EcorL. The quantitative precipitation profile of Con A (83 μM) in the presence of EcorL (42 μM) and increasing amounts of ECL is shown in Figure 8. The precipitation profile of EcorL gradually declines while that of ECL gradually increases and finally becomes flat (Figure 8A). Assuming that EcorL forms a 1:2 cross-linked complex with Con A (Table I), the mole ratio data shows the initial formation of a 1:2 and then a 1:1 cross-linked complex between ECL and Con A (Figure 8B), which is the same as that for the individual precipitation assay (Table I). These results demonstrate that Con A forms unique cross-linked complexes with ECL and ECorL, even in the presence of mixtures of the two glycoproteins which possess the same number of identical carbohydrate chains (Figure 1B) and

similar physicochemical properties (Goldstein & Poretz, 1986; Ashford et al., 1987).

Summary. Present findings provide evidence that five different glycoproteins form unique homogeneous cross-linked lattices with Con A, even in the presence of mixtures of the molecules (Table I). These results are similar to those previously observed for cross-linked complexes formed between branched chain glycopeptides and oligosaccharides with lectins (Bhattacharyya et al., 1988b, 1990; Bhattacharyya & Brewer, 1992). In fact, unique, highly ordered lattice patterns have been observed by electron microscopy for several oligosaccharide–lectin cross-linked complexes (Bhattacharyya et al., 1989, 1990). These lattice patterns appear to be due to the unique molecular packing interactions that occur between the individual proteins and oligosaccharides which, in turn, stabilize the long-range order of the cross-linked complexes and appear to be the basis for their specificity of formation (Makowski and Brewer, unpublished data). Heterogeneous complexes involving two different carbohydrates cross-linked to a lectin do not form presumably due to the lack of long-range order in such complexes. Although we have not observed the formation of patterns for lectin–glycoprotein cross-linked complexes by electron microscopy, the present results suggest that the stability of these homogeneous cross-linked complexes must also be due to similar close molecular packing interactions and long-range order in the lattices.

The present results thus suggest a new source of specificity in glycoprotein–lectin interactions, namely, the formation of unique homogeneous cross-linked lattices. These interactions may be important in lectin–glycoprotein receptor-mediated aggregation events involving cellular recognition and signal transduction processes. These studies thus provide a model for the spontaneous formation of homogeneous aggregates between specific glycoproteins and lectins in biological systems.

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