Differences in the Cross-Linking Activities of Native and Recombinant *Erythrina corallodendron* Lectin with Asialofetuin. Evidence for Carbohydrate–Carbohydrate Interactions in Lectin–Glycoprotein Complexes†

Dipti Gupta,‡ Rafael Arango,§ Nathan Sharon,§ and Curtis Fred Brewer*,‡

Department of Molecular Pharmacology and Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York 10461, and Department of Membrane Research and Biophysics, The Weizmann Institute of Science, Rehovot 76100, Israel

Received September 15, 1993; Revised Manuscript Received November 24, 1993®

ABSTRACT: A previous study showed that several multivalent galactose-specific lectins including the 14-kDa lectin from calf spleen and the lectins from *Erythrina indica*, *Erythrina cristagalli*, and soybean agglutinin formed specific cross-linked complexes with the glycoprotein asialofetuin (ASF) [Mandal, D. K., & Brewer, C. F. (1992) *Biochemistry 31*, 8465–8472]. In the present study, we have used quantitative precipitation analysis to compare the cross-linking activities of the Gal/GalNAc-specific lectin from *Erythrina corallodendron* (ECorL) and the recombinant protein (rECorL) which lacks the covalently linked heptasaccharide chains of the native lectin, with ASF. At low concentrations of ASF relative to the lectin, native dimeric ECorL binds to each of the three terminal Gal residues of the three N-linked triantennary chains of ASF and precipitates as a cross-linked complex at a ratio of 1:9 ASF/lectin (monomer). With increasing concentrations of ASF, the 1:9 complex changes to a 1:3 ASF/lectin complex, and at higher ASF concentrations, a 1:1 cross-linked complex forms. However, rECorL, which possesses the same specificity and binding affinity as the native lectin, forms only the 1:9 and 1:3 ASF/lectin complexes. Other *Erythrina* lectins examined, all of which have covalently attached carbohydrate and are structurally similar to ECorL, show the same cross-linking behavior as native ECorL. On the other hand, the dimeric 14-kDa calf spleen lectin which lacks covalently attached carbohydrate forms only 1:9 and 1:3 cross-linked complexes with ASF [Mandal, D. K., & Brewer, C. F. (1992) *Biochemistry 31*, 8465–8472]. SBA, which is a tetrameric lectin with one Man9 oligomannose chain per monomer, formed 1:3 and 1:2 ASF/lectin (monomer) cross-linking complexes. Peanut agglutinin, which is a tetrameric Gal-specific lectin lacking covalently linked carbohydrate, formed only a 1:3 ASF/lectin cross-linked complex. These results indicate that lectins with covalently attached carbohydrates form specific ASF/lectin cross-linked complexes which are not formed by nonglycosylated lectins. This suggests that interactions occur between the carbohydrate chains of the glycoprotein lectins and the carbohydrate chains of ASF which stabilize the formation of certain ASF/lectin cross-linked complexes.

Lectins are carbohydrate binding proteins of nonimmune origin that are widely distributed in nature (Goldstein & Poretz, 1986). Their interactions with oligosaccharide chains of glycoconjugates have been implicated in a wide variety of biological recognition processes including adhesion, signal transduction, and metastasis (Brandley & Schnaar, 1986; Lennarz, 1980; Monsigny, 1984; Sharon & Lis, 1993). Binding of lectins to the glycoconjugate receptors often leads to aggregation, patching, and capping of receptors (Nicolson, 1976a,b) which in many cases is associated with concomitant biological signal transduction events such as the induction of mating reactions in fungi (Kojima & Hakomori, 1989), the arrest of bulk transport in ganglion cell axons (Edmonds & Koenig, 1990), and changes in the cytoskeletal proteins of cells (Edelman, 1976).

Previous studies have shown that multivalent lectins with a wide range of binding specificities can bind and cross-link (precipitate) branched-chain oligosaccharides (Bhattacharyya et al., 1987a,b, 1988a, 1989a, 1990). These interactions lead to a new source of lectin specificity, namely, the formation of homogeneous (homopolymeric) cross-linked complexes, even in mixtures of different carbohydrates and lectins (Bhattacharyya et al., 1988b, 1989b, 1990; Bhattacharyya & Brewer, 1992). Specific cross-linked complexes have also been demonstrated to be formed between multivalent plant and animal lectins with glycoproteins (Khan et al., 1991; Mandal & Brewer, 1992b), including asialofetuin (ASF)[1] (Mandal & Brewer, 1992a).

ASF is a monomeric glycoprotein of molecular weight 48 000 (Spiro, 1960) containing three N-linked triantennary complex-type carbohydrate chains each terminating in LacNAc (Figure 1A, approximately 74%), an isomer with a Galβ-(1,3) linkage in the outer LacNAc residue of the Manα(1,3)

† This work was supported, in part, by Grant CA-16054 from the National Cancer Institute, by Department of Health, Education and Welfare Core Grant P30 CA-13330 from the same agency (C.F.B.), and by The Basic Research Foundation of the Israel Academy of Sciences and Humanities (N.S.). The NMR facility at AECOM was supported by Instrumentation Grant I-S10-RR02309 from the National Institutes of Health and by Grant DMB-8413723 from the National Science Foundation.

* To whom correspondence should be addressed at the Department of Molecular Pharmacology, Albert Einstein College of Medicine.

‡ Albert Einstein College of Medicine.

§ The Weizmann Institute of Science.

® Abstract published in *Advance ACS Abstracts,* February 1, 1994.

[1] Abbreviations: ECorL, lectin from *Erythrina corallodendron*; rECorL, recombinant ECorL from *Escherichia coli*; EIL, lectin from *Erythrina indica*; ECL, lectin from *Erythrina cristagalli*; EFL, lectin from *Erythrina flabelliformis*; ECaL, lectin from *Erythrina caffra*; ELL, lectin from *Erythrina lysistemon*; PNA, peanut agglutinin; ConA, concanavalin A; SBA, soybean agglutinin; ASF, asialofetuin; LacNAc, *N*-acetyllactosamine; PBS, 0.02 M sodium phosphate buffer with 0.90 M NaCl, pH 7.2. All sugars are in the D configuration with the exception of L-Fuc.

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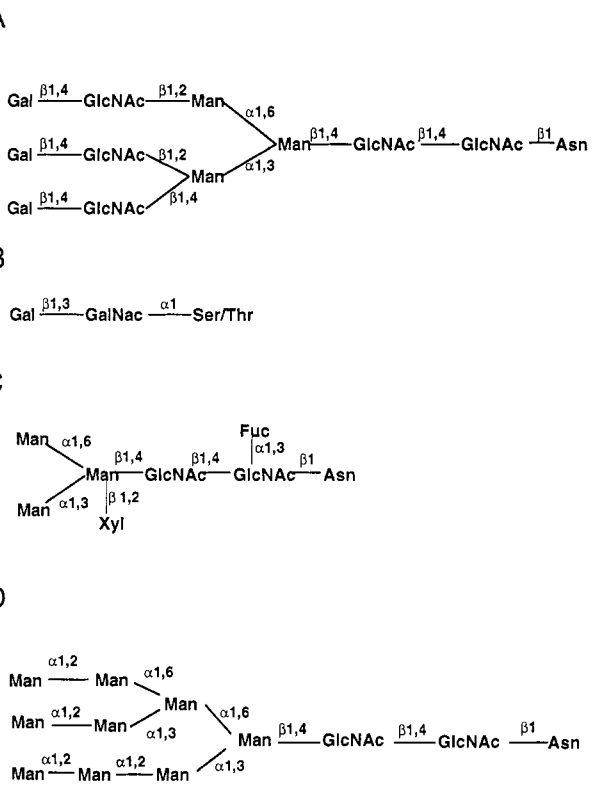

FIGURE 1: Structures of (A) the asialo N-linked triantennary complex-type glycopeptide from ASF, (B) the asialo O-linked glycopeptide from ASF, (C) the fucosyl heptasaccharide from the *Erythrina* lectins, and (D) the Man_9 oligomannose-type glycopeptide from SBA.

arm (9%), a biantennary chain with terminal LacNAc residues (17%) (Green et al., 1988), and three O-linked disaccharide chains [Galβ(1–3)GalNAc] (Figure 1B) (Nilsson et al., 1979). At relatively low concentrations of ASF, the dimeric 14-kDa Gal-specific calf spleen lectin forms a 1:9 ASF/lectin (monomer) cross-linked complex, in which all nine terminal Gal residues on each ASF molecule are bound by the lectin (Mandal & Brewer, 1992a). At higher concentrations of ASF, a 1:3 cross-linked complex forms. Two dimeric *Erythrina* lectins, ECL and EIL, also formed 1:9 and 1:3 ASF/lectin complexes, but at higher ASF concentrations went on to form a lower ratio complex described at 1:2. Thus, the two *Erythrina* lectins showed similar but distinct cross-linking activities from the 14-kDa animal lectin.

Erythrina corallodendron (ECorL) is similar to EIL and ECL in its physicochemical, structural, and carbohydrate binding properties (Goldstein & Poretz, 1986). The X-ray crystal structure of ECorL has recently been described at 2.0-Å resolution with bound lactose (Shaanan et al., 1991). Although only one oligosaccharide, Manα6(Manα3)(Xylβ2)-Manβ4GlcNacβ4(Fucα3)GlcNAcβ (Figure 1C), per subunit linked to Asn-17 was observed by X-ray, recent evidence demonstrates the presence of another such oligosaccharide linked to Asn-113 (unpublished results). Recently, recombinant lectin (rECorL) was expressed in *Escherichia coli* and shown to possess similar binding activity as the native lectin (Arango et al., 1992, 1993). In studying the interaction of rECorL with ASF, we observed that the recombinant lectin formed the 1:9 and 1:3 cross-linked complexes as native ECorL, but failed to form the lower ratio complex found for the native lectin and other two *Erythrina* lectins. Additional studies of other lectins with and without covalently linked oligosaccharide chains have provided evidence that the carbohydrate chains of the lectins interact with the carbohydrate chains of glycoproteins to stabilize specific glycoprotein/lectin cross-linked complexes.

MATERIALS AND METHODS

ECorL, ELL, ECL, EFL, ECaL (Bhattacharyya et al., 1981; Iglesias et al., 1982; Lis et al., 1985), PNA (Lotan et al., 1975), and SBA (Bhattacharyya et al., 1988a) were purified as described earlier. rECorL was prepared as described (Arango et al., 1992). Protein concentrations were determined spectrophotometrically at 280 nm using an extinction coefficient ($A^{1\%,1cm}$) of 13.4 for EIL (Bhattacharyya et al., 1981), 15.9 for the other *Erythrina* lectins, 7.7 for PNA (Lotan et al., 1975), and 12.8 for SBA (Lotan et al., 1974). Monomer molecular masses of the lectins are 29 kDa for ECorL, 28 kDa for rECorL, 34 kDa for EIL, 28 kDa for ELL, ECL, and EFL, 30 kDa for ECaL and SBA, and 27.5 kDa for PNA.

ASF was prepared from fetuin (Sigma Chemicals) and purified by FPLC on a Superdex 75 column, as described (Spiro & Bhoyroo, 1974). Its concentration was determined by the phenol–sulfuric acid method (Dubois et al., 1956) with mannose as standard using 21 mol of hexose (a mixture of 9 Man/12 Gal) per mole of protein (Nilsson et al., 1979; Spiro, 1960). Structures of the oligosaccharides were confirmed by 500 MHz ^{1}H NMR.

Radiolabeling of Proteins. All *Erythrina* lectins and SBA were radiolabeled with [^{14}C]formaldehyde in 0.1 M Hepes buffer, pH 7.2, containing 1 mM $CaCl_2$ and 1 mM $MnCl_2$, as previously described (Mandal & Brewer, 1992a). ASF was radiolabeled in 0.1 M sodium phosphate buffer, pH 7.2, with [^{3}H]formaldehyde. PNA was labeled with [^{14}C]formaldehyde in 20 mM PBS containing 1 mM $CaCl_2$.

Quantitative Precipitation Assay. Quantitative precipitation profiles were performed in 100 μL of 0.1 M Hepes buffer, pH 7.2, containing 0.9 M NaCl, 1 mM $CaCl_2$, and 1 mM $MnCl_2$ as described previously (Mandal & Brewer, 1992a). The exception was PNA, which was determined in 20 mM PBS buffer at pH 7.2 containing 0.9 M NaCl with no divalent metal ions. Precipitates were inhibited from forming or dissolved by addition of 0.1 M lactose. Nonspecific sugars such as Glc or Fuc had no effect.

RESULTS AND DISCUSSION

Properties of the Lectins. ECorL is a Gal-specific dimeric lectin composed of two identical N-glycosylated subunits of M_r 30 000. Each subunit contains one carbohydrate binding site, as well as Ca^{2+} and Mn^{2+} binding sites (Shaanan et al., 1991). The N-linked carbohydrate is the heptasaccharide Manα6(Manα3)(Xylβ2)Manβ4GlcNAcβ4-(Fucα3)GlcNAcβ (Figure 1C) which is present on both subunits (Shaanan et al., 1991). rECorL from *E. coli* consists of two subunits of M_r 28 000 with no carbohydrate chains on either subunit. The binding activity of the recombinant lectin is essentially the same as that of the native protein (Arango et al., 1992, 1993). The secondary structure of rECorL is also the same as that of the native lectin, as determined by circular dichroism measurements (data not shown).

EIL, ECL, ECaL, EFL, and ELL have close physiochemical, structural, and sugar binding properties (Goldstein & Poretz, 1986). They are Gal-specific dimeric proteins, like ECorL, consisting of two similar subunits with M_r values ranging from 28 000 to 34 000 per monomer. Each subunit contains one carbohydrate binding site as well as Ca^{2+} and Mn^{2+} binding sites which are essential for binding activity. Evidence indicates the presence of the heptasaccharide chain in Figure

Table 1: Stoichiometries of Cross-Linked Complexes of ASF with Various Gal-Specific Lectins at 22 °C

lectin	covalent-linked carbohydrate	no. of cross-linked complexes	lectin:ASF stoichiometry
ECorL	+	3	9:1
			3:1
			1:2
rECorL	–	2	9:1
			3:1
ELL	+	3	9:1
			3:1
			1:1
EFL	+	3	9:1
			3:1
			1:1
ECaL	+	3	9:1
			3:1
			1:1
EIL	+	3	9:1
			3:1
			1:1
SBA	+	2	3:1
			2:1
PNA	–	1	3:1

1C on the subunits of the lectins (Bhattacharyya et al., 1981; Iglesias et al., 1982; Lis et al., 1985).

SBA is a tetrameric Gal/GalNAc-specific glycoprotein with four equal subunits of M_r 30 000 (Shaanan et al., 1984). Each subunit possesses one carbohydrate binding site (De Boeck et al., 1984), one site each for Mn^{2+} and Ca^{2+}, and one N-linked Man_9 oligomannose-type chain (Figure 1D) (Lis & Sharon, 1978).

PNA is a specific tetrameric nonglycosylated lectin of molecular weight 110 000, composed of four identical subunits of M_r 27 500 (Goldstein & Poretz, 1986). It is a metalloprotein like SBA and possesses one carbohydrate binding site per subunit (Lotan et al., 1975). PNA is specific for the T-antigen disaccharide Galβ(1–3)GalNAc, which binds with 30-fold higher affinity than lactose (Lotan et al., 1975).

Lectin Binding Properties of ASF. The predominant triantennary N-linked glycopeptide from ASF (Figure 1A) binds to *Erythrina* lectins with approximately 180-fold higher affinity than the O-linked chains of ASF (Mandal & Brewer, 1992a). The N-linked chains of ASF also dominate binding to SBA (Mandal & Brewer, 1992a). However, the O-linked units of ASF [Galβ(1–3)GalNAc] bind to PNA with 30-fold higher affinity than lactose (Lotan et al., 1975) and with even greater affinity than LacNAc (Table 1). In addition, hemagglutination–inhibition experiments (Table 1) show that bi- and trianntennary synthetic clustered lactose glycosides (Lee, 1978) bind to PNA only as well as lactose, and thus there is no "clustered" glycoside effect [cf. Lee and Lee (1987)]. The present study shows that ASF inhibits PNA hemagglutination at 96.5 μM whereas the N-linked glycopeptide from ASF inhibits PNA hemagglutination at 3.7 mM. This indicates that the O-linked chains of ASF bind to the lectin with ~40-fold higher affinity than the N-linked chains.

Precipitation of rECorL and ECorL with ASF. Figure 2A shows the quantitative precipitation profile of ^{14}C-labeled rECorL (90 μM) in the presence of increasing concentrations of ^{3}H-labeled ASF in pH 7.2 Hepes buffer. The profile for total precipitated protein increases up to an ASF concentration of 18 μM, and then continues with a lower slope until a concentration of 54 μM ASF is reached, after which the profile decreases with increasing ASF concentration. The profile for precipitated ^{14}C-rECorL shows a maximum between concentrations of 18 and 54 μM ASF, and then decreases

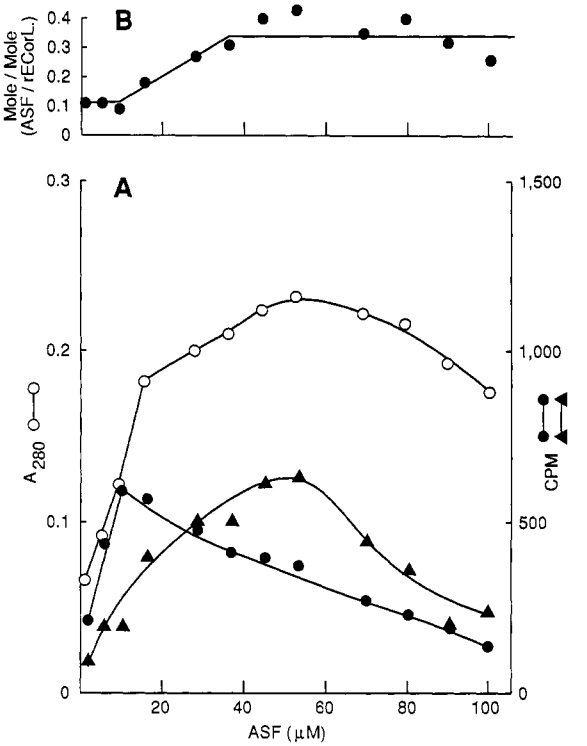

FIGURE 2: Quantitative precipitation profile of rECorL with ASF at 22 °C. (A) Total protein precipitated (○) and cpm of ^{14}C-rECorL (●) and ^{3}H-ASF (▲) in the precipitates. (B) Ratio (●) of moles of ASF precipitated per mole of rECorL monomer. The buffer was 0.1 M Hepes at pH 7.2. The concentration of rECorL was 90 μM. The specific activities of radiolabeled rECorL and ASF were 1.2×10^3 and 4.6×10^3 cpm/nmol, respectively.

with increasing concentration of the glycoprotein. Figure 2B shows that the ASF:rECorL (monomer) mole ratio is 1:9 up to an ASF concentration of 18 μM and then decreases to a 1:3 ratio with increasing concentrations of ASF.

These results indicate that rECorL forms two types of cross-linked complexes with ASF depending on the relative concentrations of the two proteins. In the 1:9 complex, nine lectin molecules bind to the three N-linked triantennary chains of ASF, as represented in Figure 2A. In this case, the valency of each triantennary chain is 3, which is similar to that of the free triantennary glycopeptide in cross-linked complexes with ECorL and several other *Erythrina* lectins (Bhattacharyya et al., 1989a). In the 1:3 complex, three lectin molecules bind to the three triantenary chains of ASF, as shown in Figure 7B. In this case, the valency of each triantennary chain is 1. Formation of the 1:9 and 1:3 ASF/rECorL cross-linked complexes is similar to that observed for the dimeric Gal-specific 14-kDa calf spleen lectin cross-linked with ASF (Mandal & Brewer, 1992a).

The quantitative precipitation profile of ^{14}C-labeled native ECorL (90 μM) (Figure 3A) in the presence of increasing concentrations of ^{3}H-ASF shows a maximum at an ASF concentration of 18 μM and then decreases. The profile for ^{14}C-ECorL has a maximum between 20 and 30 μM ASF and then decreases, while the profile for ^{3}H-ASF reaches a maximum near 35 μM of the glycoprotein and then decreases. Figure 3B shows that a 1:9 ASF/ECorL (monomer) cross-linked complex occurs up to an ASF concentration of 10 μM, and then a 1:3 cross-linked complex forms until a concentration of 46 μM ASF is reached, after which a 1:1 cross-linked complex forms. The results for native ECorL indicate that a third cross-linked complex forms with ASF. Since the 1:1 complex is not observed with the recombinant lectin, the

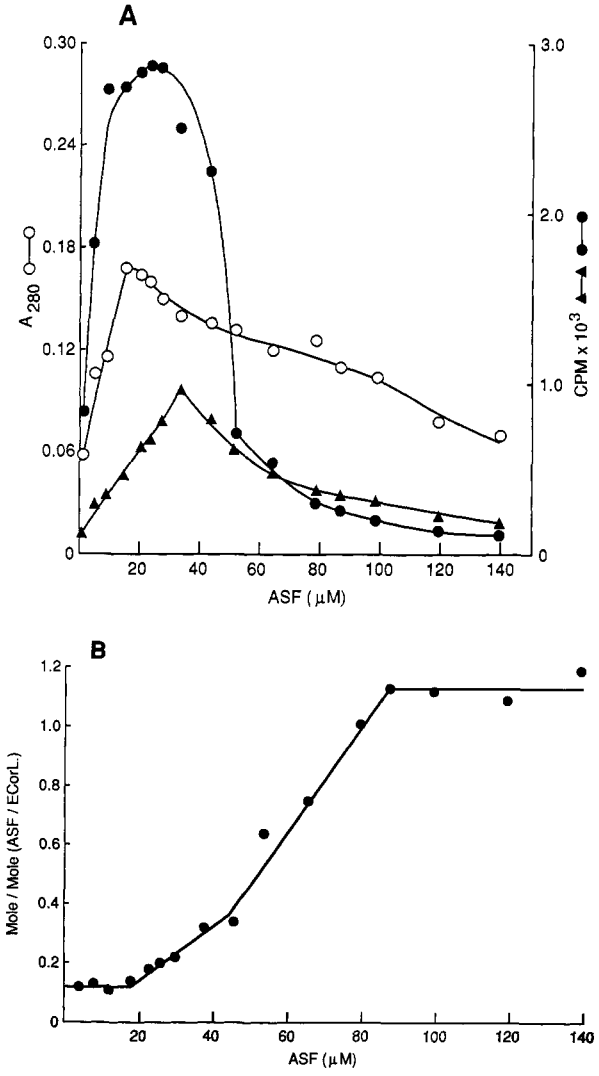

FIGURE 3: Quantitative precipitation profile of ECorL with ASF at 22 °C. (A) Total protein precipitated (○) and cpm of ^{14}C-ECorL (●) and ^{3}H-ASF (▲) in the precipitates. (B) Ratio (●) of moles of ASF precipitated per mole of ECorL monomer. The buffer was 0.1 M Hepes at pH 7.2. The concentration of ECorL was 90 μM. The specific activities of radiolabeled ECorL and ASF were 4.1 × 10^3 and 4.8 × 10^3 cpm/nmol, respectively.

carbohydrate chains of native ECorL are most likely involved in formation of this cross-linked complex with ASF.

Precipitation of EIL, ELL, EFL, and ECaL with ASF. The quantitative precipitation profile of EIL with ASF has previously been reported to show formation of 1:9, 1:3, and 1:2 ASF/lectin (monomer) cross-linked complexes (Mandal & Brewer, 1992a). However, extending the range of ASF concentrations reveals that the reported 1:2 cross-linked complex is actually a 1:1 complex (Figure 4). Similar quantitative precipitation profiles of EFL, ELL, and ECaL also indicate initial formation of 1:9 ASF/lectin cross-linked complexes which eventually convert to 1:1 cross-linked complexes with increasing ASF concentrations (data not shown).

Precipitation of SBA and PNA with ASF. SBA has previously been reported to form a 1:3 ASF/SBA (monomer) cross-linked complex (Mandal & Brewer, 1992a). However, extending the range of concentrations of ASF shows that SBA after forming the 1:3 cross-linked complex forms a 1:2 ASF/SBA cross-linked complex at higher ASF concentrations (Figure 5). PNA, on the other hand, which binds to the O-linked chains of ASF, forms only a 1:3 ASF/lectin

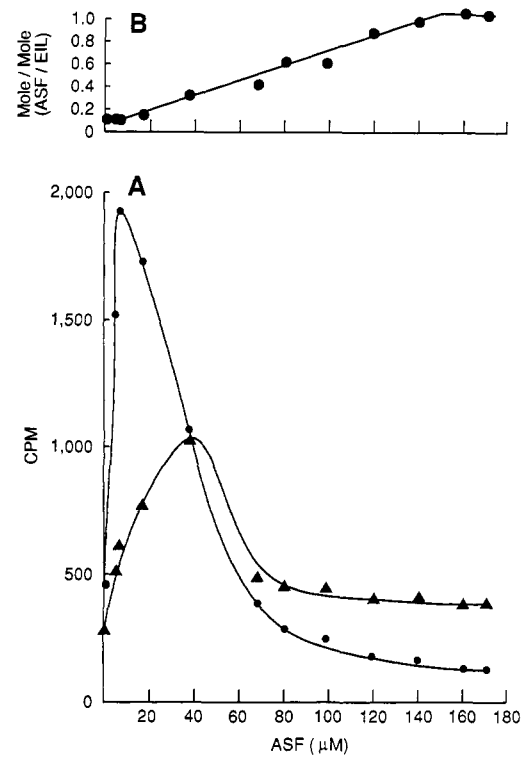

FIGURE 4: Quantitative precipitation profile of EIL with ASF at 22 °C. (A) cpm of ^{14}C-EIL (●) and ^{3}H-ASF (▲) in the precipitates. (B) Ratio (●) of moles of ASF precipitated per mole of ECorL monomer. The buffer was 0.1 M Hepes at pH 7.2. The concentration of EIL was 95 μM. The specific activities of radiolabeled EIL and ASF were 0.9 × 10^3 and 2.8 × 10^3 cpm/nmol, respectively.

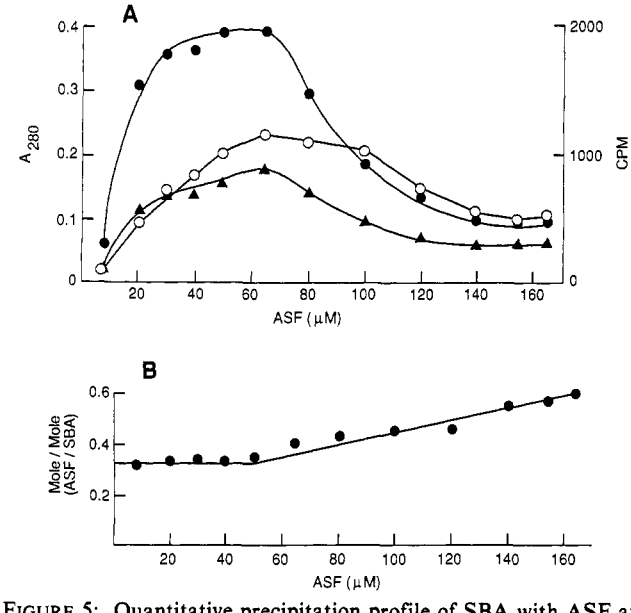

FIGURE 5: Quantitative precipitation profile of SBA with ASF at 22 °C. (A) Total protein precipitated (○) and cpm of ^{14}C-SBA (●) and ^{3}H-ASF (▲) in the precipitate. (B) Ratio (●) of moles of ASF precipitated per mole of SBA monomer. The buffer used was 0.1 M Hepes at pH 7.2. The concentration of the SBA was 95 μM. The specific activities of radiolabeled SBA and ASF were 0.88 × 10^3 and 3.0 × 10^3 cpm/nmol, respectively.

(monomer) cross-linked complex over the same concentration range (Figure 6). (The pronounced peak in the middle of the quantitative precipitation profile of PNA and ASF is as yet unexplained.) This lectin forms a well-defined cross-linked complex via the three O-linked chains of ASF, but does not form a 1:2 cross-linked complex as SBA does. An important

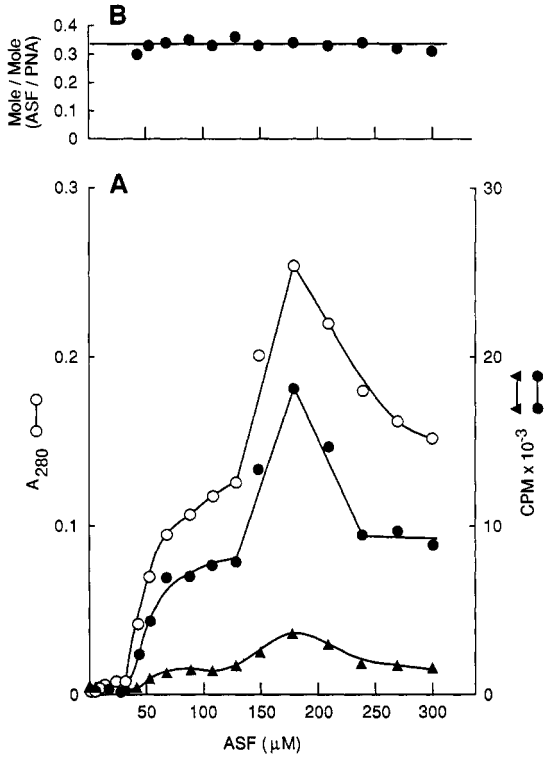

FIGURE 6: Quantitative precipitation profile of PNA with ASF at 22 °C. (A) Total protein precipitated (○) and cpm of ^{14}C-PNA (●) and ^{3}H-ASF (▲) in the precipitates. (B) Ratio (●) of moles of ASF precipitated per mole of ECorL lectin monomer. The buffer was 20 mM PBS at pH 7.2. The concentration of PNA was 100 μM. The specific activities of radiolabeled PNA and ASF were 3.2×10^3 and 1.8×10^3 cpm/nmol, respectively.

difference in the properties of the two lectins is the lack of covalently linked carbohydrate chains on PNA and the presence of four Man_9 oligomannose chains on SBA (Goldstein & Poretz, 1986). These results suggest the involvement of the carbohydrate chains of SBA in the formation of the 1:2 ASF/SBA cross-linked complex.

The present results show that additional cross-linked complexes are formed between ASF and Gal-specific glycoprotein lectins such as ECorL and other *Erythrina* lectins (ECL, EIL, EFL, and ECaL), and SBA, but not with rECorL, PNA, or the 14-kDa calf spleen lectin that lack covalently attached carbohydrate. The formation of the initial 9:1 and 3:1 cross-linked complexes between all these lectins and ASF is due to specific carbohydrate–protein interactions since they all can be inhibited from forming by 0.1 M LacNAc but not by unrelated oligosaccharides such as maltose. Thus, the 1:9 and 1:3 cross-linked complexes formed by ASF with the lectins can be understood in terms of specific carbohydrate–protein interactions involving the carbohydrate binding valencies of the lectins (usually the number of monomers and the size of the lectin) and the number of Gal or GalNAc epitopes on ASF (Mandal & Brewer, 1992a). This is also the case for the formation of specific cross-linked complexes between the Glc/Man binding lectin ConA and five glycoproteins containing Man epitopes (Mandal & Brewer, 1992b). However, formation of the 1:1 ASF/lectin cross-linked complexes with the *Erythrina* lectins and the 1:2 cross-linked complexes with SBA is not easily explained by the valencies of the lectins and the number of carbohydrate epitopes of ASF. Instead, it appears that these complexes may arise from further binding of ASF to the existing ASF/lectin cross-linked complexes mediated by the carbohydrate chains of the bound glycoprotein lectins, shown in Figure 7C. In this model, the stability of

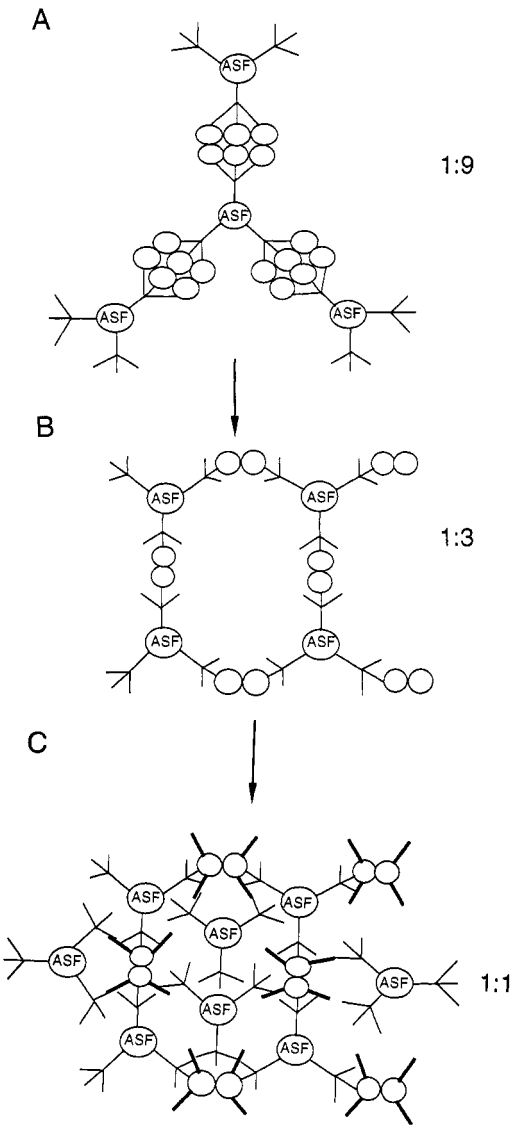

FIGURE 7: Schematic representations of the (A) 1:9 and (B) 1:3 ASF/rECorL cross-linked complexes and of the (C) 1:1 ASF/ECorL cross-linked complex. The paired circles represent dimeric rECorL, and ASF indicates the glycoprotein molecules in (A) and (B). The dark lines connected to the ECorL dimers in (C) represent the heptasaccharide chains of native lectin.

these complexes is due to binding of the three triantennary chains (or O-linked chains) of ASF to two or more chains of individual cross-linked lectin molecules. In this manner, weak carbohydrate–carbohydrate interactions are strengthened by multiple binding interactions of each ASF to the existing cross-linked complex. The apparent carbohydrate–carbohydrate interactions are heterotypic since the structures of the carbohydrate chains on ASF and the lectins are different (i.e., *Erythrina* lectins and SBA). Furthermore, the formation of these complexes depends on the existence of specific carbohydrate–protein mediated cross-links since all the cross-linked complexes can be prevented from forming or dissolved upon addition of LacNAc. Attempts at adding high concentrations of other saccharides such as maltose failed to prevent formation of the 1:1 ASF/*Erythrina* lectin complexes or the 1:2 ASF/SBA complex. It is possible, however, that the carbohydrate chains of the glycoprotein lectins bind to the protein matrix of ASF, but this possibility is deemed less likely.

Evidence that carbohydrate–carbohydrate interactions occur between oligosaccharides in solution has been reported by Hakomori and co-workers. Homotypic and heterotypic

interactions have been documented in the cases of Le^x–Le^x and G_{M3}–G_{g3} glycolipid interactions which require Ca^{2+} (Eggens et al., 1989a,b; Kojima & Hakomori, 1989). The requirement of Ca^{2+} for the apparent carbohydrate–carbohydrate interactions in the present study could not be determined since Ca^{2+} was required in the buffer to maintain the activities of the lectins. The possible involvement of carbohydrate–carbohydrate interactions in the high-affinity binding of N-linked oligosaccharides of cell-surface or soluble glycoproteins to the 205-kDa porcine large granular lymphocyte lectin, a member of the leukocyte common antigen family, has also been recently proposed (Bezouska et al., 1993).

Summary. The present study provides evidence that the carbohydrate chains of certain Gal/GalNAc-specific glycoprotein lectins interact with the carbohydrate chains of ASF in stabilizing ASF/lectin cross-linked complexes. These interactions are heterotypic and thought to be of relatively low affinity. However, through multiple chain interactions, they can result in the formation of stable cross-linked complexes. The present study demonstrates that a recombinant lectin, as a consequence of the lack of covalent carbohydrate, possesses different cross-linking activities from that of the native lectin. These results thus suggest that the absence of covalent carbohydrate (or modified carbohydrate) on recombinant lectins (and proteins) may affect their binding and cross-linking properties with other glycoconjugate molecules.

REFERENCES

Arango, R., Adar, R., Rozenblatt, S., & Sharon, N. (1992) *Eur. J. Biochem. 205*, 575–581.

Arango, R., Esperanza, R.-A., Adar, R., Belenky, D., Loontiens, F. G., Rozenblatt, S., & Sharon, N. (1993) *FEBS Lett. 330*, 133–136.

Bezouska, K., Krajhanzl, A., Pospisil, M., Kubrycht, J., Stajner, K., Felsberg, J., & Kocourek, J. (1993) *Eur. J. Biochem. 213*, 1303–1313.

Bhattacharyya, L., & Brewer, C. F. (1992) *Eur. J. Biochem. 208*, 179–185.

Bhattacharyya, L., Das, P. K., & Sen, A. (1981) *Arch. Biochem. Biophys. 211*, 459–470.

Bhattacharyya, L., Ceccarini, C., Lorenzoni, P., & Brewer, C. F. (1987a) *J. Biol. Chem. 262*, 1288–1293.

Bhattacharyya, L., Haraldsson, M., & Brewer, C. F. (1987b) *J. Biol. Chem. 262*, 1294–1299.

Bhattacharyya, L., Haraldsson, M., & Brewer, C. F. (1988a) *Biochemistry 27*, 1034–1041.

Bhattacharyya, L., Khan, M. I., & Brewer, C. F. (1988b) *Biochemistry 27*, 8762–8767.

Bhattacharyya, L., Haraldsson, M., Sharon, N., Lis, H., & Brewer, F. (1989a) *Glycoconjugate J. 6*, 141–150.

Bhattacharyya, L., Khan, M. I., Fant, J., & Brewer, C. F. (1989b) *J. Biol. Chem. 264*, 11543–11545.

Bhattacharyya, L., Fant, J., Lonn, H., & Brewer, C. F. (1990) *Biochemistry 29*, 7523–7530.

Brandley, B. K., & Schnaar, R. L. (1986) *J. Leukocyte Biol. 261*, 7306–7310.

De Boeck, H., Lis, H., van Tilbeurgh, H., Sharon, N., & Loontiens, F. G. (1984) *J. Biol. Chem. 259*, 7067–7074.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956) *Anal. Chem. 28*, 350–356.

Edelman, G. M. (1976) *Science 192*, 218–226.

Edmonds, B. T., & Koenig, E. (1990) *Cell Motil. Cytoskeleton 17*, 106–117.

Eggens, E., Fenderson, B. A., Toyokuni, T., Dean, B., Stroud, M., & Hakomori, S. I. (1989a) *J. Biol. Chem. 264*, 9476–9484.

Eggens, I., Fenderson, B. A., Toyokuni, T., & Hakomori, S. I. (1989b) *Biochem. Biophys. Res. Commun. 158*, 913–920.

Goldstein, I. J., & Poretz, R. D. (1986) in *The Lectins* (Liener, I. E., Sharon, N., & Goldstein, I. J., Eds.) pp 35–244, Academic Press, Inc., New York.

Green, E. D., Adelt, G., Baenziger, J. U., Wilson, S., & Van Halbeek, H. (1988) *J. Biol. Chem. 263*, 18253–18268.

Iglesias, J. L., Lis, H., & Sharon, N. (1982) *Eur. J. Biochem. 123*, 247–252.

Khan, M. I., Mandal, D. K., & Brewer, C. F. (1991) *Carbohydr. Res. 213*, 69–77.

Kojima, N., & Hakomori, S. I. (1989) *J. Biol. Chem. 264*, 20159–20162.

Lee, R., & Lee, Y. C. (1987) *Glycoconjugate J. 4*, 317–328.

Lee, Y. C. (1978) *Carbohydr. Res. 67*, 509–514.

Lennarz, W. J. (1980) *The Biochemistry of Glycoproteins and Proteoglycans*, Plenum Press, New York.

Lis, H., & Sharon, N. (1978) *J. Biol. Chem. 253*, 3468–3476.

Lis, H. Joubert, F. J., & Sharon, N. (1985) *Phytochemistry 24*, 1–7.

Lotan, R., Siegelman, H. W., Lis, H., & Sharon, N. (1974) *J. Biol. Chem. 249*, 1219–1224.

Lotan, R., Skutelsky, E., Danon, D., & Sharon, N. (1975) *J. Biol. Chem. 250*, 8518–8523.

Mandal, D. K., & Brewer, C. F. (1992a) *Biochemistry 31*, 8465–8472.

Mandal, D. K., & Brewer, C. F. (1992b) *Biochemistry 31*, 12602–12609.

Monsigny, M. (1984) *Biol. Cell 51*, 113–294.

Nicolson, G. L. (1976a) *Biochim. Biophys. Acta 457*, 57–108.

Nicolson, G. L. (1976b) *Biochim. Biophys. Acta 458*, 1–72.

Nilsson, B., Norden, N. E., & Svensson, S. (1979) *J. Biol. Chem. 254*, 4545–4553.

Shaanan, B., Lis, H., & Sharon, N. (1991) *Science 254*, 862–866.

Shaanan, B., Shoham, M., Yonath, A., Lis, H., & Sharon, N. (1984) *J. Mol. Biol. 174*, 723–725.

Sharon, N., & Lis, H. (1993) *Sci. Am. 268*, 82–89.

Spiro, R. G. (1960) *J. Biol. Chem. 235*, 2860–2869.

Spiro, R. G., & Bhoyroo, V. (1974) *J. Biol. Chem. 249*, 5704–5717.