Immunochemical Studies on the Interaction between Synthetic Glycoconjugates and α-L-Fucosyl Binding Lectins†

Jerzy Petryniak and Irwin J. Goldstein*
Department of Biological Chemistry, The University of Michigan, Ann Arbor, Michigan 48109
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ABSTRACT: *Evonymus europaea* lectin precipitated with αDGal(1→3)βDGal(1→4)βDGlcNAc–bovine serum albumin (BSA), αLFuc(1→2)βDGal(1→3)βDGlcNAc–BSA, αLFuc(1→2)βDGal(1→4)DGlcNAc, and αDGal(1→3)[αLFuc(1→2)]βDGal–BSA. However, the lectin neither precipitated with αLFuc(1→2)-βDGal–BSA, αDGal(1→3)βDGal–BSA, or βDGal(1→4)βDGlcNAc–BSA nor agglutinated erythrocytes of O_h phenotype having multiple terminal βDGal(1→4)βDGlcNAc residues. These results indicate that the minimal structural requirement for glycoprotein precipitation or cell agglutination by the lectin includes any of the three trisaccharides (fucosylated or nonfucosylated) derived from the blood group B tetrasaccharide. The monosaccharides linked to the β-D-galactosyl residue in the blood group B tetrasaccharide, namely, α-D-galactose, α-L-fucose, and *N*-acetyl-β-D-glucosamine, participate almost equally in binding to the lectin inasmuch as removal of any one of these sugars reduces the inhibiting potency of the resulting trisaccharide. αLFuc(1→2)βDGal(1→3)βDGlcNAc–BSA (H type 1) and αLFuc(1→2)βDGal(1→4)βDGlcNAc (H type 2) were precipitated to the same extent. The *E. europaea* lectin neither precipitated αDGal(1→4)-βDGal(1→4)βDGlcNAc–BSA, Le^a–BSA, Le^b–BSA, or βDGlcNAc(1→4)[αLFuc(1→6)]βDGlcNAc–BSA nor agglutinated O_h,Le^a and O_h,Le^b erythrocytes, demonstrating that terminal D-galactose linked α-(1→4) to subterminal β-D-galactose, or α-L-fucose linked to *N*-acetylglucosamine, prevents lectin binding. Corey–Pauling–Koltun molecular models, built on the basis of data from ^1H NMR and hard-sphere exo-anomeric (HSEA) calculations provided by Lemieux and co-workers [Lemieux, R. U., Bock, K., Delbaere, L. T. J., Koto, S., & Rao, V. S. (1980) *Can. J. Chem. 58*, 631–653], show that these α-D-galactosyl and α-L-fucosyl groups act to sterically hinder lectin binding to these oligosaccharides; these observations also suggest that the lectin binds to the β-side of these oligosaccharides. These sides, on both blood group H type 1 and blood group H type 2 oligosaccharides, provide a similar contour which can fully account for their equal reactivity with *E. europaea* lectin. The only difference found between *Lotus* and *Ulex* I lectins in precipitating ability was that only *Lotus* precipitated with βDGlcNAc(1→4)[αLFuc(1→6)]βDGlcNAc–BSA. Additionally, βDGlcNAc(1→4)[αLFuc(1→6)]βDGlcNAc and αLFuc(1→6)βDGlcNAc were good inhibitors of *Lotus* lectin but not of *Ulex* I lectin. *Griffonia simplicifolia* IV also did not precipitate βDGlcNAc(1→4)[αLFuc(1→-6)]βDGlcNAc–BSA. These results show that *E. europaea*, *Lotus tetragonolobus*, and *Ulex europaeus* I lectins, despite their reactivity with the same blood group H type 2 oligosaccharide, recognize different surface features of this oligosaccharide.

Lectins serve as probes for the detection of carbohydrate determinants on cells and glycoconjugates (Goldstein & Hayes, 1978; Lis & Sharon, 1977; Nicolson, 1974; Pereira & Kabat, 1979). *Evonymus europaea* lectin is blood group B and H specific (Krüpe, 1956; Pacák & Kocourek, 1975; Petryniak et al., 1977; Schmidt, 1954); it agglutinates stimulated (but not resident) macrophages from mice (Petryniak et al., 1983) and precipitates asialoglycophorin from O_h^A erythrocytes (Petryniak, 1981). This precipitating activity cannot be rationalized on the basis of the lectin's blood group B or H specificity. Conversely, the L-fucose-binding lectins from *Griffonia simplicifolia* (GS IV), *Lotus tetragonolobus*, and *Ulex europaeus* (I) neither agglutinate stimulated murine macrophages nor precipitate asialoglycophorin (Petryniak, 1981; Petryniak et al., 1983).

Despite extensive studies on the specificity of *E. europaea* (Petryniak et al., 1977) *G. simplicifolia* IV (Shibata et al., 1982), *L. tetragonolobus* (Allen et al., 1977; Debray et al., 1981; Pereira & Kabat, 1974), and *U. europaeus* I lectins (Allen et al., 1977; Debray et al., 1981; Hindsgaul et al., 1982; Pereira et al., 1978), the reactivity of these agglutinins with certain carbohydrate groupings is still an unsolved problem. To answer these questions and to compare the combining sites of the L-fucose-binding lectins mentioned above with the *Evonymus* lectin, we have carried out quantitative precipitation and hapten inhibition studies employing synthetic glycoproteins and oligosaccharides of known constitution.

Materials and Methods

Di- and Oligosaccharides. αDGal(1→3)DGal[1] and βDGal(1→4)DGlcNAc were synthesized by Nike Plessas of this laboratory. αLFuc(1→6)DGlcNAc was kindly provided by Dr. Arne Lundblad (Lundblad et al., 1978), University of Lund, Sweden. αDGal(1→3)βDGal(1→4)βDGlcNAc–O–$(CH_2)_8CO_2CH_3$ was a gift of Dr. Per Garegg from University of Stockholm. αDGal(1→3)[αLFuc(1→2)]βDGal–O–$(CH_2)_8CO_2CH_3$ and αLFuc(1→2)βDGal(1→3)[αLFuc(1→-4)]βDGlcNAc–O–$(CH_2)_8CO_2CH_3$ were obtained from

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[1] Abbreviations: BSA, bovine serum albumin; PBS, 0.01 M phosphate buffer, pH 7.1, with 0.15 M NaCl and 3 mM sodium azide; CPK, Corey–Pauling–Koltun; CETE, [(carboxyethyl)thio]ethyl; LFuc, L-fucose; DGal, D-galactopyranose; DGalNAc, 2-acetamido-2-deoxy-D-galactopyranose; DGlc, D-glucopyranose; DGlcNAc, 2-acetamido-2-deoxy-D-glucopyranose; Le^a, Lewis a; Le^b, Lewis b; R, $-(CH_2)_8CO-$.

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Chembiomed Ltd., Edmonton, Alberta, Canada. βDGalNAc(1→4)[αLFuc(1→6)]βDGlcNAc-1-*O*-CETE was purchased from Sockerbolaget, Arlöv, Sweden.

Blood Group, Milk, and Urine Oligosaccharides. αDGal(1→3)[αLFuc(1→2)]βDGal(1→4)βDGlcNAc(1→6)-3-hexenetetrol(s) was (were) the gift of Dr. Elvin A. Kabat (Lloyd et al., 1966; Lundblad et al., 1972). 2′-Fucosyllactose, lacto-*N*-fucopentaose I, and lacto-*N*-fucopentaose II were generous gifts of Dr. Victor Ginsburg (Ginsburg, 1972). αDGal(1→3)[αLFuc(1→2)]βDGal(1→4)[αLFuc(1→3)]DGlc was kindly provided by Dr. Arne Lundblad (Björndal & Lundblad, 1970).

Glycoconjugates. [αLFuc(1→2)βDGal(1→3)βDGlcNAc-1-*O*-R]$_{18}$–BSA, [αLFuc(1→2)βDGal(1→4)βDGlcNAc-1-*O*-R]$_{20}$–BSA, [αDGal(1→3)[αLFuc(1→2)]βDGal-11-*O*-R]$_{15}$–BSA, [βDGal(1→3)[αLFuc(1→4)]βDGlcNAc-*O*-R]$_{15}$–BSA, and [αLFuc(1→2)βDGal(1→3)[αLFuc(1→4)]βDGlcNAc-*O*-R]$_{18}$–BSA were from Chembiomed Ltd., Edmonton, Alberta, Canada. [αLFuc(1→2)βDGal-1-*O*-CETE]$_{47}$–BSA, [αDGal(1→4)βDGal(1→4)βDGlcNAc-1-*O*-CETE]$_{14}$–BSA, [βDGal(1→4)βDGlcNAc-1-*O*-CETE]$_{16}$–BSA, and [βDGlcNAc(1→4)[αLFuc(1→6)βDGlcNAc-*O*-CETE]$_{25-35}$–BSA were the gift of Dr. Göran Magnusson, Sockerbolaget, Arlöv, Sweden. [αDGal(1→3)βDGal-1-*O*-R]–BSA and [αDGal(1→3)βDGal(1→4)βDGlcNAc-1-*O*-R]$_{25}$–BSA were synthesized by Nike Plessas of this laboratory.

Glycoproteins. Human α_1-acid glycoprotein was kindly given by Dr. Gilbert Ashwell (Van Lenten & Ashwell, 1971). Blood group H substance was purified from mucin of porcine stomach (Sigma) as reported earlier (Morgan & King, 1943). Fetuin, purified according to Spiro (1960), was a generous gift of Dr. Hubert Krotkiewski, L. Hirszfeld Institute of Immunology and Experimental Therapy, Wroclaw, Poland. Glycophorin A from O,M erythrocytes was provided by Dr. Elwira Lisowska (Baranowski et al., 1959).

Lectins and Enzyme. Seeds of *E. europaea* were collected from wild-growing shrubs in the neighborhood of Wroclaw, Poland; *G. simplicifolia* seeds were obtained from Calbiochem, La Jolla, CA; *L. tetragonolobus* seeds were provided by Dr. Myron Leon from Wayne State University, Detroit, MI. *E. europaea* and *G. simplicifolia* IV lectin were purified as described earlier [Petryniak et al. (1981) and Shibata et al. (1982), respectively]. *L. tetragonolobus* lectin was purified according to Yariv et al. (1972).

U. europaeus I lectin was purchased from E. Y. Laboratories, Inc., San Mateo, CA. Neuraminidase from *Vibrio cholerae* was a product of Serva, Heidelberg, FRG.

Erythrocytes and Sera. Human erythrocytes of blood group phenotype "Bombay" (O_h; O_h,Le^a) and "Parabombay" (O_h,-Le^b) as well as goat sera anti-Le^a and anti-Le^b were obtained through the courtesy of John Judd, University of Michigan Medical Center.

Assay Procedures. Protein was determined by the method of Lowry et al. (1951) using ovalbumin (Sigma, St. Louis, MO) as standard. Sialic acid was estimated by the periodate–resorcinol method of Jourdian et al. (1971).

Precipitin Reactions. A modification of the quantitative microprecipitin technique described by Kabat (1961) was used. Reactions were carried out in 1.5-mL polypropylene microcentrifuge tubes with caps (obtained from Curtin Matheson Scientific, Inc., Houston, TX). Approximately 40 μg of lectin protein was mixed with varying amounts of glycoconjugates or glycoproteins in a final volume of 250 μL of PBS. The mixtures were incubated at 37 °C for 1 h and kept at 4 °C for 5–7 days. Precipitates were separated from supernatant solutions by centrifugation for 10 min at 15600*g* at 4 °C and decantation. The protein in the washed precipitates was determined by the procedure of Lowry et al. (1951).

Inhibition assays were carried out by adding varying quantitites of sugar to amounts of lectin and glycoprotein giving maximum precipitation in a total volume 250 μL. The extent of inhibition was expressed as the percentage of protein precipitated vs. that of control containing no inhibitor. The difference of the binding free energies $\Delta\Delta G°$ was calculated according to Pressman and Grossberg (1968). Inhibition studies on the *Evonymus* lectin were conducted by using asialoglycophorin as precipitant because of the high sensitivity of this system (Petryniak et al., 1980).

Hemagglutination assays were carried out in glass test tubes (7 × 0.8 cm); 50 μL of diluted lectin or serum was mixed with 25 μL of a 3% suspension of erythrocytes and incubated at room temperature for 1 h. Tubes were centrifuged for 15 s at 1000*g*, and the agglutination titer was recorded. The titration score of agglutinated erythrocytes was expressed by numbers corresponding to the following: 4+ = 10, 3+ = 7, 2+ = 5, and 1+ = 3.

Desialization. Glycophorin A preparations were desialized by hydrolysis with 0.025 M H_2SO_4 at 80 °C for 40 min. The reaction was stopped by cooling and neutralization with 0.1 M NaOH. Sialic acid and salts were removed by ultrafiltration in a Diaflo chamber on a PM-10 membrane (Amicon, Oosterhout, Holland). The retentate was lyophilized.

Fetuin and α_1-acid glycoprotein were desialyzed by neuraminidase. The reaction mixture in a volume of 3 mL of 0.05 M acetate buffer, pH 5.0, contained 1 mM $CaCl_2$, 3 mM sodium azide, 0.01 unit of neuraminidase, and up to 10 mg of glycoprotein. The reaction digest of each glycoprotein was placed in dialysis tubing and dialyzed against the same digestion buffer for 3 days at 37 °C, the buffer being changed each day. The desialized glycoprotein was dialyzed extensively against water and lyophilized. The buffer was changed each day. The desialized glycoprotein was dialyzed extensively against water and lyophilized, and the sialic acid content was determined. After desialization by both procedures, sialic acid was undetectable on all glycoproteins treated.

RESULTS

Quantitative Precipitin Studies on Evonymus europaea Lectin. Panels A and B of Figure 1 depict the precipitin reactions of *Evonymus europaea* lectin with nonfucosylated and fucosylated glycoconjugates, respectively. The structures of the carbohydrate portions of these glycoconjugates are shown in Table I. The lectin precipitated a nonfucosylated blood group B trisaccharide coupled to BSA, αDGal(1→3)-βDGal(1→4)βDGlcNAc–BSA. Of 62 μg of total protein in the reaction mixture, 34 μg (55%) was found in the precipitate. However, the lectin did not precipitate glycoconjugates containing the trisaccharide αDGal(1→4)βDGal(1→4)-βDGlcNAc–BSA or the disaccharides αDGal(1→3)βDGal–BSA or βDGal(1→4)βDGlcNAc–BSA.

Evonymus lectin precipitated, to the same extent, synthetic glycoconjugates of the fucosylated trisaccharides αLFuc(1→2)βDGal(1→3)βDGlcNAc–BSA (blood group H type 1), αLFuc(1→2)βDGal(1→4)DGlcNAc–BSA (blood group H type 2), and αDGal(1→3)[αLFuc(1→2)]βDGal–BSA. Lectin also bound to Synsorb-B, a matrix containing the B-active trisaccharide αDGal(1→3)[αLFuc(1→2)]βDGalβ (data not shown). However, it did not precipitate with the disaccharide conjugate αLFuc(1→2)βDGal–BSA. Nonfucosylated and fucosylated trisaccharide–BSA conjugates were equally active on a weight basis, and approximately 5 μg of each glyco-

Table I: Structure of Oligosaccharide Present in Synthetic and Natural Glycoproteins Used in Precipitation Studies

oligosaccharide (common name)	no. of oligosaccharides per molecule	structure
	Group I: Fucose Linked to Subterminal Galactose	
fucosylated blood group B trisaccharide	15	αLFuc ↓(1→2) αDGal(1→3)βDGal1→O-$(CH_2)_8$-CO-NH-BSA
blood group H		
type 1	18	αLFuc ↓(1→2) βDGal(1→3)βDGlcNAc1→O-$(CH_2)_8$-CO-NH-BSA
type 2	20	αLFuc ↓(1→2) βDGal(1→4)βDGlcNAc1→O-$(CH_2)_8$-CO-NH-BSA
	47	αLFuc ↓(1→2) βDGal1→O-CETE-BSA
	Group II: Fucose Linked to *N*-Acetylglucosamine	
blood group		
Le^a	15	αLFuc ↓(1→4) βDGal(1→3)βDGlcNAc1→O-$(CH_2)_8$-CO-NH-BSA
Le^b	19	αLFuc αLFuc ↓(1→2) ↓(1→4) βDGal(1→3)βDGlcNAc1→O-$(CH_2)_8$-CO-NH-BSA
fucosylated chitobiose	25–35	αLFuc ↓(1→6) βDGlcNAc(1→4)βDGlcNAc1→O-CETE-BSA
human asialo-α_1-acid glycoprotein	5	αLFuc ↓(1→3) βDGal(1→4)βDGlcNAc ↓(1→3) βDGal(1→4)βDGlcNAc(1→2)βDMan ↓(1→3) βDMan(1→4)βDGlcNAc(1→4)βDGlcNAc→Asn ↑(1→6) βDGal(1→4)βDGlcNAc(1→2)βDMan or ↑ (1→6) βDGal(1→4)βDGlcNAc
	Group III: Lacking Fucose	
blood group B		
disaccharide		αDGal(1→3)βDGal1→O-$(CH_2)_8$-CO-NH-BSA
trisaccharide	25	αDGal(1→3)βDGal(1→4)βDGlcNAc1→O-$(CH_2)_8$-CONH-BSA
	14	αDGal(1→4)βDGal(1→4)βDGlcNac1→O-CETE-BSA
N-acetyllactosamine	16	βDGal(1→4)βDGlcNAc1→O-CETE-BSA
human asialo-α_1-acid glycoprotein	5	βDGal(1→4)βDGlcNAc(1→2)αDMan ↓(1→3) βDMan(1→4)βDGlcNAc(1→4)βDGlcNAc→Asn ↑(1→6) βDGal(1→4)βDGlcNAc(1→2)αDMan
human asialo-α_1-acid glycoprotein	5	βDGlc(1→4)βDGlcNAc ↓(1→4) βDGal(1→4)βDGlcNAc(1→2)αDMan ↓(1→3) βDMan(1→4)βDGlcNAc(1→4)→Asn ↑(1→6) βDGal(1→4)βDGlcNAc(1→2)αDMan ↑(1→6) βDGal(1→4)βDGlcNAc
human asialo-α_1-acid glycoprotein and asialofetuin	5 and 3	βDGal(1→4)βDGlcNAc ↓ (1→3) βDGal(1→4)βDGlcNAc(1→2)αDMan ↓(1→3) βDMan(1→4)βDGlcNAc(1→4)βDGlcNAc→Asn ↑(1→6) βDGal(1→4)βDGlcNAc(1→2)αDMan
asialofetuin	3	βDGal(1→3)αDGalNAc1→O-[serine or threonine]

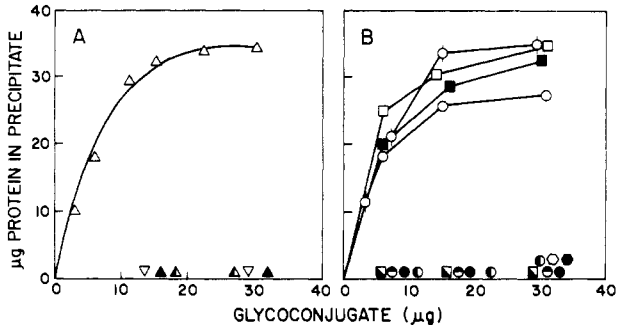


FIGURE 1: Quantitative precipitin curves of *E. europaea* lectin (36 μg of protein) with (A) nonfucosylated and (B) fucosylated glycoconjugates. Full structures and numbers of di- and oligosaccharides per molecule of BSA are listed in Table I. Symbols: blood group H substance from hog mucin, used as a reference (○); αDGal(1→3)βDGal(1→4)βDGlcNAc–BSA (△); αDGal(1→4)βDGal(1→4)βDGlcNAc–BSA (▲); αDGal(1→3)βDGal–BSA (▽); βDGal(1→4)βDGlcNAc–BSA (▲); αDGal(1→3)[αLFuc(1→2)]βDGal–BSA (◇); H type 1–BSA (□); H type 2–BSA (■); αLFuc(1→2)DGal–BSA (◪); Le^a–BSA (◓); Le^b–BSA (●); βDGlcNAc(1→4)[αLFuc(1→6)]-βDGlcNAc–BSA (◐); asialo-α_1-acid glycoprotein (○); asialofetuin (●).

conjugate was required for 50% precipitation of the lectin. *Evonymus* lectin precipitated neither Le^a–BSA, Le^b–BSA, or βDGlcNAc(1→4)[αLFuc(1→6)]βDGlcNAc–BSA conjugates nor glycoproteins with βDGal(1→4)[αLFuc(1→3)]DGlcNAc determinants (e.g., human asialo-α_1-acid glycoprotein).

These precipitation results demonstrate that the minimal determinant required for glycoprotein precipitation and cell agglutination by *Evonymus* lectin is any of the three trisaccharides (fucosylated or nonfucosylated), derived from the blood group B tetrasaccharide. The data also show that all three monosaccharides linked to β-D-galactose, α-D-galactose, α-L-fucose, and *N*-acetyl-β-D-glucosamine, participate in binding inasmuch as removal of any one of these sugars from the trisaccharide abolishes the precipitating ability of the resulting disaccharide (see Figure 2). The lack of precipitation of glycoconjugates by the *Evonymus* lectin in the presence of a terminal D-galactosyl groups linked α-(1→4) to a subterminal β-D-galactosyl residue [as in αDGal(1→4)βDGal(1→4)βDGlcNAc–BSA] as well as in the presence of a second α-L-fucosyl unit linked to GlcNAc [as in Le^b or Le^a substance or βDGal(1→4)[αLFuc(1→6)]βDGlcNAc units in which the sole α-L-fucosyl unit is linked to a GlcNAc residue] suggests that these terminal galactosyl and fucosyl units both hinder binding to the lectin.

Evonymus lectin precipitated blood group H type 1 and type 2 BSA conjugates to the same extent.

Agglutination of Erythrocytes of O_h,Le^a; O_h,Le^b; and O_h,-$Le^{a-,b-}$ by E. europaea Lectin. Results of agglutination studies are shown in Figure 3A. Erythrocytes of Bombay O_h,Le^a; O_h,$Le^{a-,b-}$; and Parabombay O_h,Le^b phenotypes were chosen because they lack the blood group H determinants (Race & Sanger, 1968) and therefore allow for evaluation of interaction between lectin and the *N*-acetyllactosaminyl unit as well as Lewis determinants. *Evonymus* lectin, at a concentration of 52 μg/mL, did not agglutinate O_h,Le^b erythrocytes and caused minimal (+) agglutination of O_h,Le^a erythrocytes. This represents a ≥32-fold increase in lectin concentration required for agglutination as compared with agglutination of O erythrocytes at ≤1.6 μg/mL (Figure 3A). *Evonymus* lectin also did not agglutinate O_h,$Le^{a-,b-}$ erythrocytes at a concentration of 52 μg/mL (not shown). As control reference, agglutination of O_h,Le^a and O_h,Le^b by anti-Le^a and anti-Le^b sera was carried out (Figure 3B,C). These agglutination results are in agreement with precipitation data and show that *Evonymus* lectin is unable to agglutinate cells with Le^a,Le^b and *N*-acetyllactosamine determinants.

Precipitation Inhibition Studies. The ability of several synthetic and natural disaccharides and oligosaccharides from milk, urine, and blood group substances to inhibit precipitation of purified *E. europaea* lectin by asialoglycophorin is shown in Figure 4. Table II gives the structures and activities of the carbohydrates tested.

As reference oligosaccharides, the tetrasaccharide from the P1 fraction of blood group B substance (Beach P1 R_L 0.44) and lacto-*N*-fucopentaose I were used (Petryniak et al., 1977). The nonfucosylated blood group B trisaccharide is as inhibitory as lacto-*N*-fucopentaose I and about 4 times less active than the fucosylated B trisaccharide. Thus removal of the (1→2)-linked α-L-fucosyl group from the nonreducing end of Beach P1 oligosaccharide caused substantial loss of inhibitory power, a loss equal to the loss caused by the removal of the (1→3)-linked α-D-galactosyl group from the nonreducing end, observed in lacto-*N*-fucopentaose I. From these results one may conclude that both terminal monosaccharide units,

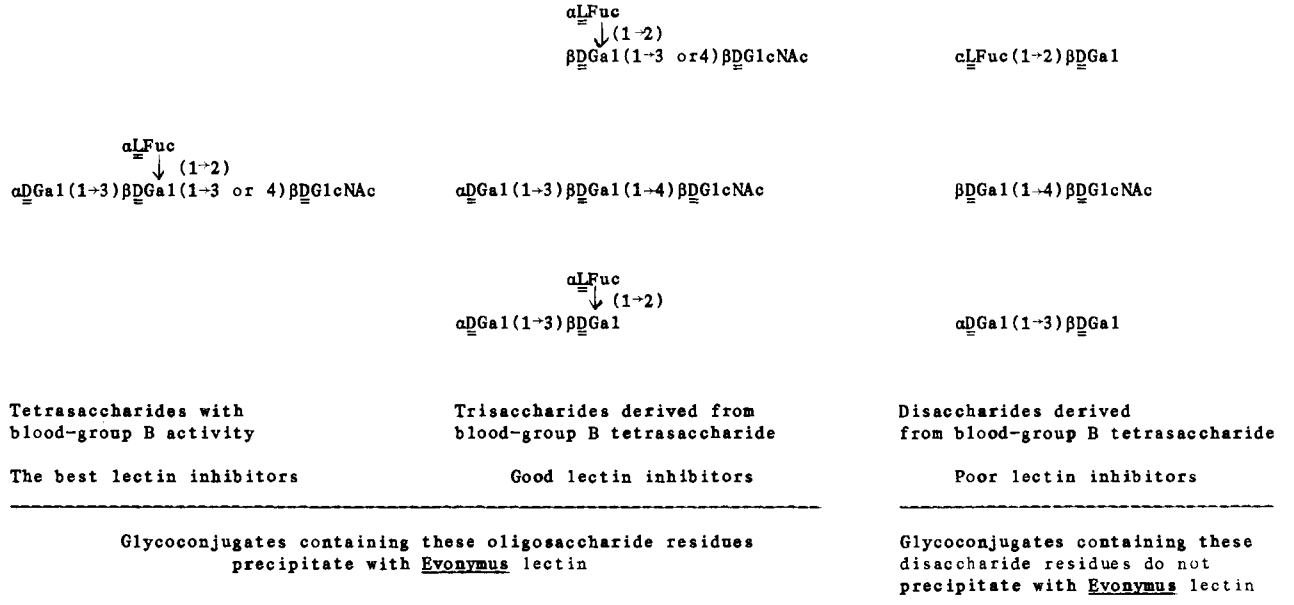


FIGURE 2: Structures of *Evonymus* lectin determinants. Data are compiled from the present and previous results (Petryniak et al., 1977).

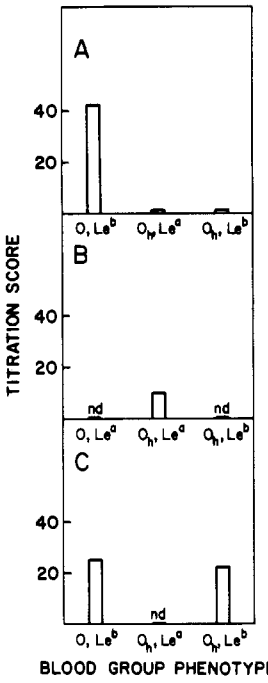


FIGURE 3: Hemagglutination of erythrocytes of O; O_h,Le^a; and O_h,Le^b blood group phenotypes by (A) purified *Evonymus* lectin, (B) anti-Le^a serum, or (C) anti-Le^b. Serum titration score was scored 4+ as 10, 3+ as 7, 2+ as 5, and 1+ as 3; nd = not determined.

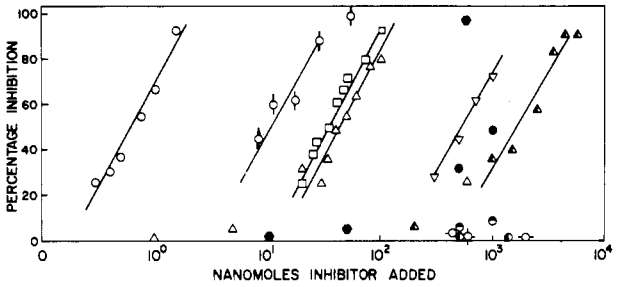


FIGURE 4: Inhibition of the precipitation reaction between *E. europaea* (39 μg of protein) and asialoglycophorin (34 μg of glycoprotein) by di- and oligosaccharides. Tetrasaccharide Beach P1 R_L 0.44 and lacto-*N*-fucopentaose I were used as reference compounds. Symbols are listed in Table II. Increasing amounts of carbohydrates in a total volume of 250 μL of PBS were used in inhibition studies.

αDGal(1→3)– and αLFuc(1→2)–, contribute to the binding energy to the same extent.

Removal of the β-linked *N*-acetylglucosamine unit from the reducing terminus of the B tetrasaccharide lowered the inhibitory power of the resulting trisaccharide 20-fold. However, it was approximately 3 and 4 times more inhibitory than lacto-*N*-fucopentaose I and αDGal(1→3)βDGal(1→4)-βDGlcNAc, respectively. This shows that the contribution of *N*-acetylglucosamine to the binding energy is approximately 3–4 times lower than that of the α-L-fucosyl and α-D-galactosyl units.

Urine blood group B pentasaccharide containing a second L-fucosyl group linked α-(1→4) to D-glucose is approximately 228 times less inhibitory than the B tetrasaccharide R_L 0.44. This great loss in inhibitory power was caused by the presence of a second α-L-fucosyl unit on D-glucose, and not by replacement of DGlcNAc by DGlc in the pentasaccharide, inasmuch as such a replacement resulted in only about a 3-fold decrease in inhibitory power (Petryniak et al., 1977).

The Le^b oligosaccharide, containing a second L-fucose unit α-linked to *N*-acetylglucosamine, is 22 times less active than the monofucosylated lacto-*N*-fucopentaose I (III, Table II). Lacto-*N*-fucopentaose II (IX, Table II) with a sole α-L-fucosyl group linked to *N*-aceylglucosamine was noninhibitory at a concentration of 1000 nmol. This was a 22-fold higher concentration than that of lacto-*N*-fucopentaose I. Comparison of the inhibitory power of the monofucosylated blood group B tetrasaccharide R_L 0.44 (I) and lacto-*N*-fucopentaose I (III, Table II) with their difucosylated derivatives (B pentasaccharide, Le^b) and with monofucosylated species with L-fucose linked α-(1→4) (lacto-*N*-fucopentaose II) to *N*-acetylglocosamine shows that this fucosyl unit substantially reduced or abolished the inhibitory power of the oligosaccharide and suggests that it results in steric hindrance to interaction with the lectin. These oligosaccharides were even poorer inhibitors than the disaccharides *N*-acetyllactosamine, lactose, and αDGal(1→3)DGal, which, despite some inhibitory power, were not able to precipitate with *Evonymus* lectin, when coupled to BSA. This poor inhibitory power of oligosaccharides with L-fucose linked to *N*-acetylglucosamine is in agreement with the lack of precipitation observed with glycoconjugates consisting of these oligosaccharides coupled to BSA.

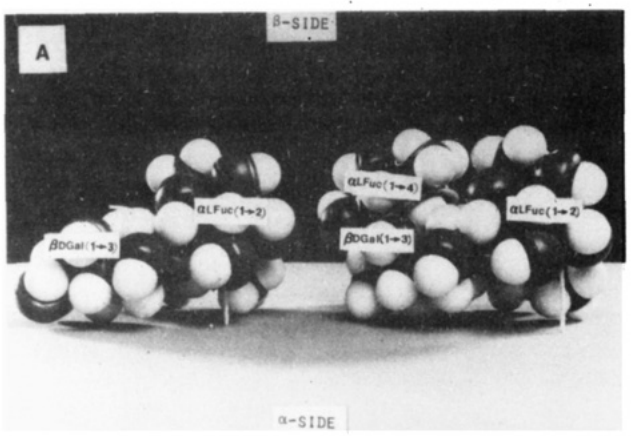
Disaccharide XI (Table II) and fucosylated *N*,*N*′-diacetylchitobiose (X, Table II) with L-fucose linked α-(1→6) to the reducing *N*-acetylglucosamine unit did not show any inhibitory activity at concentrations at which even the poor disaccharide inhibitors exhibited substantial inhibitory power. These results are also in agreement with the lack of precipitation of the *Evonymus* lectin by βDGlcNAc(1→4)[αLFuc-(1→6)]βDGlcNAc–BSA.

The disaccharides αDGal(1→3)βDGal and βDGal(1→4)-βDGlcNAc were 9 and 30 times less active, respectively, than αDGal(1→3)βDGal(1→4)βDGlcNAc, showing that both αDGal and βDGlcNAc units participate in binding; however, βDGlcNAc provides approximately 3 times less binding energy than terminal α-(1→3)-D-galactosyl groups.

Studies with CPK Molecular Models. To assist in determining the site of attachment of *Evonymus* lectin to oligosaccharides, CPK space-filling molecular models of blood group B tri- and tetrasaccharides type 1 and type 2, H trisaccharide type 1, Le^b tetrasaccharide, and αDGal(1→4)-βDGal(1→4)DGlcNAc were built. The mutual arrangement of monosaccharides and groupings in the models was based on the internuclear distances provided by Lemieux et al. (1980). Although these distances in the CPK models are approximate, they are nevertheless sufficient for these studies. The following argument suggests that the β-sides of blood group B tetra- and trisaccharides are sites of interaction for the *Evonymus* lectin.

According to Lemieux (1978) and Lemieux et al. (1980), L-fucose linked α-(1→4) to *N*-acetylglucosamine is on the β-side of the oligosaccharide. Our precipitation and inhibition data show that this α-L-fucosyl group prevents binding. One can see (Figure 5) that the second α-(1→4)-linked L-fucosyl unit covers a substantial area of the β-side of both the *N*-acetyl-β-D-glucosamine residue and terminal α-(1→3)-linked β-D-galactosyl end group. The α-(1→4)-linked fucosyl group also shields a portion of the α-(1→2)-linked fucosyl group. Thus, important portions of the determinant necessary for high-affinity binding to *Evonymus* lectin are no longer accessible.

In the trisaccharide αDGal(1→4)βDGal(1→4)βDGlcNAc, the terminal galactosyl group is linked via the axial hydroxyl group on C-4 of the subterminal galactosyl residue, which causes the terminal D-galactosyl group to extend above the β-plane of the oligosaccharide and profoundly changes the contour of its β-side (Figure 6, right). On the other hand, the



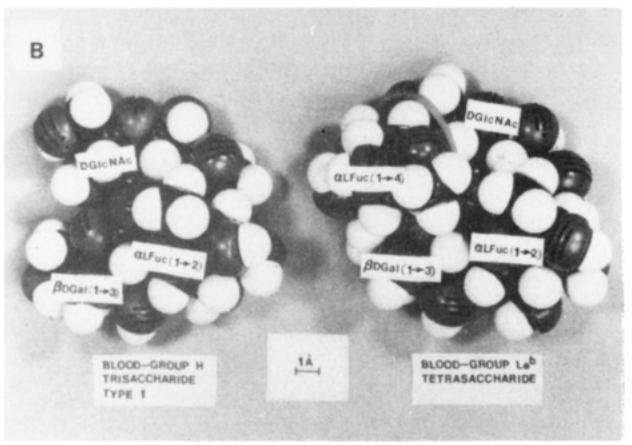


FIGURE 5: (A) CPK molecular models of blood group H type 1 trisaccharide αLFuc(1→2)βDGal(1→3)βDGlcNAc (left) and Le^b tetrasaccharide αLFuc(1→2)βDGal(1→3)[αLFuc(1→4)]βDGlcNAc (right). The photograph presents the front view from the nonreducing end. The upper surface is a view of the β-side; the lower surface is the α-side of the oligosaccharides. The second L-fucosyl group, linked α-(1→4) to βDGlcNAc, is outlined by the tubing. (B) Top view on the β-side of the same oligosaccharides as in (A). The second L-fucosyl group linked α-(1→4) to βDGlcNAc is outlined by the tubing.

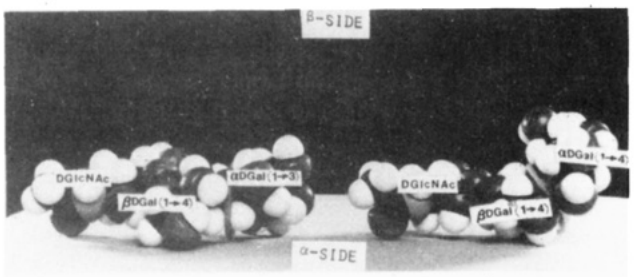


FIGURE 6: Side view of CPK molecular models of αDGal(1→3)-βDGal(1→4)βDGlcNAc (left) and αDGal(1→4)βDGal(1→4)-βDGlcNAc (right). Both terminal nonreducing αDGal units are outlined by the tubing.

terminal α-(1→3)-D-galactosyl group (as in the B trisaccharide) is in the plane of the oligosaccharide (Figure 6, left). The β-planes in both blood group B and blood group H tetra- and trisaccharides of type 2 and type 1 chains present similar shapes and contours and can fully account for equal reactivity of *Evonymus* lectin with both types of oligosaccharides (Figure 7).

Precipitation and Inhibition Studies on Lotus tetragonolobus and Ulex europaeus I. Figure 8A shows the precipitation reactions of *Lotus tetragonolobus* lectin with synthetic glycoconjugates. The blood group H (type 2)–BSA conjugate was used as a reference. Lectin precipitated αLFuc(1→2)-βDGal–BSA and βDGlcNAc(1→4)[αLFuc(α1→6)]-

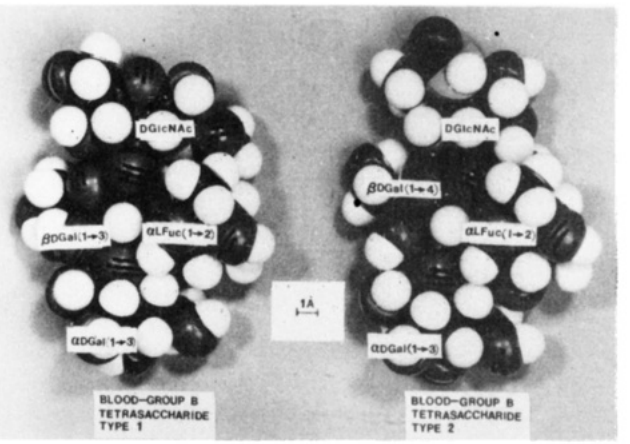


FIGURE 7: Top view, on β-side, of CPK molecular models of blood group B tetrasaccharides type 1 (left) and type 2 (right). Despite the fact that the reducing *N*-acetylglucosamine units are linked through either their C-3 or C-4 hydroxyl groups in type 1 and type 2 chains, respectively, they provide similar contours. The CH_2OH group in the type 1 chain is in a position similar to that of the CH_3 group of the acetamido group in the type 2 chain. The nitrogen of the acetamido group is in approximately the same position as the ring oxygen in βDGlcNAc of the type 1 chain. These groupings can provide similar interactions with the lectin binding site.

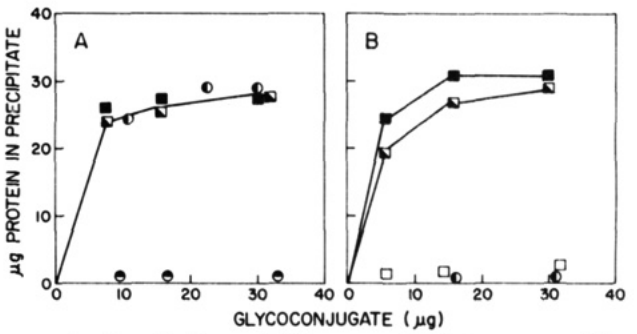


FIGURE 8: Quantitative precipitin curves of (A) *L. tetragonolobus* (36 μg of protein) and (B) *U. europaeus* I (35 μg of protein) lectins with synthetic glycoconjugates. Full structures and number of di- and oligosaccharides per molecule of BSA are listed in Table I. Blood group H type 2–BSA conjugate was used as a reference. Symbols are the same as in Figure 1.

βDGlcNAc–BSA. All three glycoconjugates were equally active on a weight basis, and approximately 5 μg of each was required for 50% of lectin precipitation. At the equivalence zone, 20 μg of lectin was precipitated out of a total of 36 μg of lectin protein used in the reaction. These results show that lectin can bind with high affinity to both fucosyl-D-galactose and fucosylchitobiose regardless of whether L-fucose is linked α-(1→2) to the subterminal D-galactose or α-(1→6) to the reducing *N*-acetylglucosamine unit of *N*,*N*′-diacetylchitobiose. Lectin did not precipitate with the Le^a–BSA conjugate.

The ability of di- and trisaccharide, with L-fucose linked α-(1→6) to the reducing *N*-acetylglucosamine unit, to inhibit *Lotus* lectin is shown in Figure 9A and Table III. Twenty-one nanomoles of αLFuc(1→6)DGlcNAc (I) was required for 50% inhibition of precipitation. This disaccharide was about 3 and 13 times more inhibitory, on a molar basis, than 2′-fucosyllactose (II) (reference compound) and fucosylated *N*,*N*′-diacetylchitobiose (III), respectively. These results show that oligosaccharides with L-fucose linked α-(1→6) to reducing *N*-acetylglucosamine are powerful *Lotus* lectin inhibitors. Extension of the disaccharide chain by addition of an *N*-acetylglucosamine unit (to the nonreducing end), as is the case of fucosylated *N*,*N*′-diacetylchitobiose, caused a 13-fold decrease in the inhibitory power of the derived oligosaccharide.

Table II: Structures and Activities of Synthetic and Natural Di- and Oligosaccharides (from Milk, Urine, and Blood Group Substances) Used in Inhibition Studies of *Evonymus* Lectin

symbol	no.	sugar	amount (nmol) for 50% inhibition	$\Delta\Delta G°$ (kcal mol^{-1})[a]
○	I	αLFuc ↓(1→2) αDGal(1→3)βDGal(1→4)βDGlcNAc(1→6)-3-hexenetetrol(s) Beach P1 R_L 0.44	0.7	−4.3
⚲	II	αLFuc ↓(1→2) αDGal(1→3)βDGal→O$(CH_2)_8CO_2CH_3$	14	−2.6
□	III	αLFuc ↓(1→2) βDGal(1→3)βDGlcNAc(1→3)βDGal(1→4)DGlc Lacto-N-fucopentaose I	45	−2.0
△	IV	αDGal(1→3)βDGal(1→4)βDGlcNAc1→O$(CH_2)_8CO_2CH_3$	54	−1.9
▽	V	αDGal(1→3)DGal	510	−0.6
▲	VI	βDGal(1→4)DGlcNAc N-acetyllactosamine	1650	0
●	VII	αLFuc αLFuc ↓(1→2) ↓(1→3) αDGal(1→3)βDGal(1→4)DGlc Urine B pentasaccharide	160	−1.2
●	VIII	αLFuc αLFuc ↓(1→2) ↓(1→4) βDGal(1→3)βDGlcNAc1→O−$(CH_2)_8CO_2CH_3$ Lewis b hapten	1000	−0.3
⊖	IX	αLFuc ↓(1→4) βDGal(1→3)βDGlcNAc(1→3)βDGal(1→4)DGlc Lacto-N-fucopentaose II	1000[b]	
◐	X	αLFuc ↓(1→6) βDGlcNAc(1→4)βDGlcNAc-O-CETE* Fucosylated chitobiose	1370[c]	
-○-	XI	αLFuc ↓(1→6) DGlcNAc	2000[c]	
-⊙-	XII	αLFuc ↓(1→6) DGlcNAc→Asn	498[c]	

[a] The relative inhibitory potency of sugars was calculated with reference to *N*-acetyllactosamine, which was assumed to be 1, and therefore $\Delta G° = 0$ for this disaccharide. [b] 9% inhibition. [c] 0% inhibition.

Table III: Structures and Activities of Synthetic and Natural Oligosaccharides Used for Inhibition Studies of *L. tetragonolobus* and *U. europaeus* I Lectins

			amount (nmol) for 50% inhibition		$\Delta\Delta G°$ (kcal mol^{-1})[a]	
symbol	no.	sugar	*L. tetragonolobus*	*U. europaeus*	*L. tetragonolobus*	*U. europaeus*
-○-	I	αLFuc(1→6)DGlcNAc	21	400 (0% inhibition)	−0.8	
○	II	αLFuc ↓(1→2) βDGal(1→4)DGlc 2′-Fucosyllactose	95	130	0	0
◐	III	αLFuc ↓(1→6) βDGlcNAc(1→4)βDGlcNAc-O-CETE Fucosylated chitobiose	280	300 (18% inhibition)	0.6	

[a] The relative inhibitory potency of sugars was calculated with reference to 2′-fucosyllactose, which was assumed to be 1; therefore, $\Delta\Delta G° = 0$ for this trisaccharide.

U. europaeus I lectin showed the same precipitating reactivity with synthetic glycoconjugates (Figure 8B) as *Lotus* lectin, with one exception: it did not precipitate with the fucosylated *N*,*N*′-diacetylchitobiose–BSA conjugate. For precipitation of 50% of the lectin, approximately 3 and 4 μg of blood group H type 2–BSA conjugate and αLFuc(1→2)-

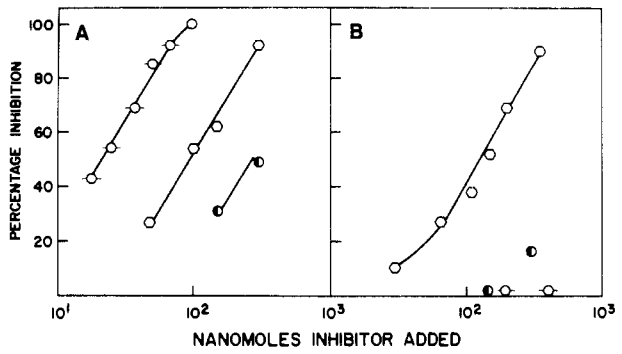


FIGURE 9: Inhibition of (A) *L. tetragonolobus* (52 μg of protein) and (B) *U. europaeus* I (40 μg of protein) precipitation with blood group H from hog mucin (20 and 12.6 μg, respectively) by di- and trisaccharides. Symbols are listed in Table III. Increasing amounts of carbohydrates in a total volume of 250 μL of PBS were used for inhibition.

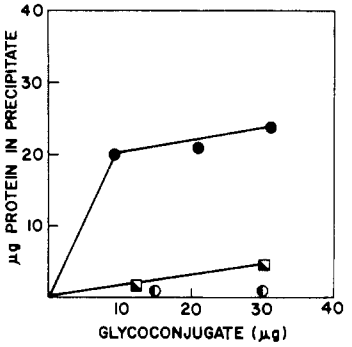


FIGURE 10: Quantitative precipitin curves of *G. simplicifolia* IV lectin (36 μg of protein) with synthetic glycoconjugates. Full structures and number of di- and oligosaccharides per molecule of BSA are listed in Table I. Symbols are the same as in Figure 1.

DGal–BSA, respectively, were required. Lectin, also, did not precipitate H type 1–BSA conjugate. These results are in good agreement with inhibition studies (Figure 9B, Table III). 2′-Fucosyllactose (reference compound) inhibited 50% of *Ulex* I lectin precipitation at a concentration of 130 nmol, whereas αLFuc(1→6)GlcNAc (I) and βDGlcNAc(1→4)[αLFuc(1→6)]DGlcNAc (III) were not inhibitory at concentrations of 400 and 300 nmol, respectively. These results show that both lectins, despite being essentially blood group H type 2 specific lectins, exhibit fine differences as in their combining site requirements.

Griffonia simplicifolia IV lectin, which is most specific for difucosylated tetrasaccharides of Le^b and Le^d (Shibata et al., 1982), did not precipitate with the fucosylated *N,N′*-diacetylchitobiose–BSA conjugate and showed very weak activity against αLFuc(1→2)βDGal–BSA (Figure 10).

DISCUSSION

The precipitation of *E. europaea* lectin by asialoglycophorin from Bombay O_h^A erythrocytes (Petryniak, 1981), laminin (unpublished data) and partially hydrolyzed blood group B substance (Beach P1 and horse 4 25% P1) with a reduced amount of α-L-fucosyl groups (Allen & Kabat, 1959; Petryniak et al., 1977) cannot be accounted for by the specificity of the lectin for blood group B and H determinants. These observations raised the question as to what other structures could be recognized by the *Evonymus* lectin.

Evonymus lectin is inhibited by several galactose-containing disaccharides (lactose, melibiose), and *N*-acetyllactosamine appears to be part of the determinant. The question arose as to whether antigens or cells with multiple, terminal *N*-acetyllactosamine units would be precipitated or agglutinated by *Evonymus* lectin, and what constituted the minimal determinant required for these reactions? Uncertainty also existed regarding the interaction of *Evonymus* lectin with determinants having L-fucose α-linked to an Asn-linked *N*-acetylglucosaminyl residue or to an "outer" terminal *N*-acetylglucosaminyl residue as occurs in the Le^a and Le^b determinants. Previous studies showed weak precipitation of *Evonymus* lectin with natural Le^a substance (N-1) and indicated that the fucose attached to *N*-acetyl-D-glucosamine or D-glucose caused steric hindrance or a conformational change in the resulting oligosaccharide; however, it was not known to what extent Le^a and Le^b haptens are inhibitory for the lectin (Petryniak et al., 1977). The present study was undertaken to answer some of these questions.

Oligosaccharide chains of blood group glycoproteins present a wide variety of heterogeneous structures (Rovis et al., 1973; Wu et al., 1984). Therefore, only limited conclusions can be drawn regarding the lectins' specificity from precipitation studies with natural glycoproteins. To answer these questions, the reactions of the above lectins with synthetic glycoproteins containing oligosaccharides of defined constitution, and cells containing some well-defined carbohydrate determinants (Bombay phenotype), were studied.

Recent developments in the chemical synthesis of oligosaccharides and glycoconjugates as well as in structural and conformational analysis of oligosaccharides (Biswas & Rao, 1982; Hindsgaul et al., 1982; Lemieux et al., 1975, 1980; Lemieux, 1977, 1978) provide glycoconjugates that are homogenous in terms of their carbohydrate moieties and therefore provide excellent tools for establishing carbohydrate binding specificity.

Our studies demonstrate that *Evonymus* lectin can precipitate glycoconjugates with both fucosylated and nonfucosylated trisaccharides derived from blood group B tetrasaccharide. The capability of the *Evonymus* lectin to precipitate with αDGal(1→3)βDGal(1→4)βDGlcNAc accounts fully for its ability to precipitate with two partially hydrolyzed blood group B substances: Beach P1 and horse 4 25% P1 (Petryniak et al., 1977).

In these studies it was also shown that the *Evonymus* lectin is unable to react with Le^a and Le^b determinants as studied by both precipitation and agglutination assays. Therefore, the precipitation of *Evonymus* lectin with natural blood group Le^a substance N-1 (Petryniak et al., 1977) cannot be due to the interaction between the lectin and the Le^a determinant. This also suggests that a determinant different from the Le^a determinant present on N-1 glycoprotein is responsible for this reaction. The similar observation that HLe^b blood group substance possesses more than one immunologically important determinant was also made by Lemieux (1978). The Le^b determinant also can be excluded in the precipitation reaction of blood group HLe^b substance (JS phenol insoluble) with *Evonymus* lectin (Petryniak et al., 1977).

Evonymus lectin precipitated precursor blood group substance with I and i activity (OG 10% 2×). However, a determinant other than terminal *N*-acetyllactosamine must be responsible for this reaction inasmuch as it was shown in the present study that the lectin neither precipitated βDGal(1→4)βDGlcNAc–BSA nor agglutinated erythrocytes of Bombay phenotypes having multiple terminal *N*-acetyllactosamine units.

The wedge-like hydrophobic region on the α-side of the type 2 oligosaccharide was suggested to be the site of attachment for *Ulex* I lectin (Lemieux, 1982). However, in the type 1 chain this region is profoundly changes because *N*-acetyl

groups occupy a major portion of this area (Lemieux, 1982; Hindsgaul et al., 1982). Because of this structural difference this hydrophobic region cannot be the site of attachment for *Evonymus* lectin, which does not discriminate between type 1 and type 2 chains. This consideration represents a further indication that the β-side of the oligosaccharides provides the site for *Evonymus* lectin attachment. These β-sides, on both blood group H type 1 and blood group H type 2 oligosaccharides, provide similar contours which can fully account for their identical reactivity with *E. europaea* lectin, as it was observed in the inhibition studies (Petryniak et al., 1977) and precipitation reactions. The β-side in both oligosaccharides, except for the hydrophobic CH_3 group of fucose and the CH groups of C-3, C-4, and C-5 of the α-D-galactosyl group, is strongly hydrophilic and contains many exposed –OH groups. Because three monosaccharides (αDGal, αLFuc, and βDGlcNAc) of the tetrasaccharide participate in binding, it appears very likely that some of the –OH groups might bind to the lectin through hydrogen bonds. Indeed, Wright (1984) recently showed by X-ray crystallography that hydrogen bonds are involved in the binding of *N*-acetyl-D-glucosamine and *N*-acetylneuraminic acid to wheat germ agglutinin. Also, hydrogen bonding as a main force in association between lectin and carbohydrate was postulated by Goldstein et al. (1974), Kronis and Carver (1985), Ochoa (1981), Poretz and Goldstein (1970), and So and Goldstein (1967). It appears probable that hydrophobic bonds may not be the main driving force in the interaction between carbohydrates and proteins as was postulated earlier (Lemieux, 1982).

Biswas and Rao (1982) in their conformation studies of the ABH and Lewis blood group oligosaccharides suggested that lectins and antibodies nonspecific for type 1 and type 2 chains may have small binding sites. However, results of our studies on binding properties of *Evonymus* lectin are contrary to this suggestion.

Lotus lectin was reported to precipitate with blood group Le^a (N-1) substance despite the fact that oligosaccharides bearing the Le^a determinant lacto-*N*-fucopentaose II were poor lectin inhibitors (Pereira & Kabat, 1974). From these studies the Le^a determinant can be excluded as responsible for precipitation of N-1 glycoprotein with *Lotus* lectin. However, it was found by Rovis et al. (1973) that N-1 glycoprotein also contains an Le^x determinant on R_{IM8} 0.78 oligosaccharide, and Pereira and Kabat (1974) showed that lacto-*N*-fucopentaose III, bearing Le^x, was only 3 times poorer an inhibitor than 2′-fucosyllactose. These studies show that fucosylated *N*,*N*′-diacetylchitobiose, which is also 3 times poorer an inhibitor than 2′-fucosyllactose, is still capable of precipitating with *Lotus* lectin. Therefore, it appears likely that the Le^x determinant might be responsible for the reaction of *Lotus* lectin with N-1 glycoprotein.

The reactivity of *Lotus* and *Ulex* I lectins toward oligosaccharides containing L-fucose linked α-(1→6) to a reducing *N*-acetyl-D-glucosamine unit, using an inhibition of hemagglutination assay, was reported by Allen et al. (1977) and Debray et al. (1981). However, neither group compared the inhibitory power of these oligosaccharides with blood group H active oligosaccharides, making it impossible to conclude whether oligosaccharides containing α-(1→6) fucosyl groups linked are bound with high affinity by these lectins. The present studies compare the reactivity of αLFuc(1→6)-βDGlcNAc and βDGlcNAc[αLFuc(1→6)]βDGlcNAc with H-active 2′-fucosyllactose, against *Lotus* lectin, and show that fucosylated *N*,*N*′-diacetylchitobiose, despite its lower inhibitory power when compared with αLFuc(1→6)βDGlcNAc and 2′-fucosyllactose, still provides enough binding energy to be precipitated by *Lotus* lectin. Debray et al. (1981) observed that substitution of αLFuc(1→6)βDGlcNAc with mono- or oligosaccharide decreased the inhibitory power of the resulting oligosaccharide. Our observations are in agreement with this report.

Our studies also point out the substantial difference in specificity between *Lotus* and *Ulex* I lectin with respect to oligosaccharides containing α-(1→6)-linked fucose units. *Ulex* I lectin, despite some inhibition by oligosaccharides with LFuc α-(1→6)-linked to a reducing *N*-acetyl-D-glucosamine unit as reported by Debray et al. (1981), is not able to precipitate glycoconjugates with these determinants.

The *Ulex* I lectin binding site differs from the *L. tetragonolobus* site in that it was reactive with H type 1 oligosaccharides (Pereira et al., 1978) whereas *Lotus* was completely nonreactive (Pereira & Kabat, 1974) by the hapten inhibition assay. However, H type 1 oligosaccharides were poor *Ulex* I lectin inhibitors. This study shows that the *Ulex* I lectin is not able to precipitate blood group H (type 1)–BSA conjugates, and therefore it is an essentially blood group H type 2 specific lectin. The basic difference between these lectins resides in their ability to precipitate glycoconjugates with fucose α-(1→6)-linked to reducing *N*-acetylglucosamine residues.

Comparison of the specificity of *Evonymus* lectin with those of *Lotus* (Pereira & Kabat, 1974) and *Ulex* I (Pereira et al., 1977) lectins shows that *Evonymus* is unique among these three lectins in its ability to bind with high affinity to H type 1 oligosaccharides.

Our results also indicate that the specificity of some lectins must be interpreted in terms of the conformation of the oligosaccharides with which they interact, that is, in terms of the spatial arrangement of various groupings on different sides of the oligosaccharides as is the case with *Evonymus* lectin.

Some oligosaccharides used in these inhibition studies contained a linker arm [$-O-(CH_2)_8CO_2CH_3$]. The question arises as to whether this aliphatic grouping can affect the binding of oligosaccharides to lectins. It is of course not easily possible to assess the contribution of the linker arm to the inhibition potency of each lectin; however, there are indications that the linker arm does not participate in binding. Thus, comparison of the inhibitory power of αDGal(1→3)[αLFuc(1→2)]βDGal-1-*O*-$(CH_2)_8CO_2CH_3$ (present data) with that of the horse BR_L 0.65,0.66 oligosaccharide [αDGal(1→3)[αLFuc(1→2)]βDGal-*N*-acetyl-D-galactosaminitol; Petryniak et al., 1977] shows that both oligosaccharides, despite differences between hydrophilic and hydrophobic aliphatic reducing termini, were essentially equally active. Similar observations were also made by Shibata et al. (1982) and Baker et al. (1983).

Registry No. αDGal(1→3)βDGal(2←1)αLFuc, 72002-35-8; αLFuc(1→2)βDGal(1→3)βDGlcNAc, 81243-84-7; αLFuc(1→2)-βDGal(1→4)βDGlcNAc, 47776-54-5; αLFuc(1→2)βDGal, 16741-18-7; βDGal(1→3)βDGlcNAc(4←1)αLFuc, 79951-60-3; αLFuc(1→2)-βDGal(1→3)βDGlcNAc(4←1)αLFuc, 80081-06-7; βDGlcNAc(1→4)βDGlcNAc(6←1)αLFuc, 79391-05-2; βDGal(1→4)βDGlcNAc-(1→2)βDMan(1→6)βDMan[βDGal(1→4)βDGlcNAc(1→2)βDMan-[βDGal(1→4)βDGlcNAc[αLFuc(1→3)](1→3)](1→3)](1→4)-βDGlcNAc(1→4)βDGlcNAc, 101517-23-1; βDGal(1→4)-βDGlcNAc(1→6)βDMan[βDGal(1→4)βDGlcNAc(1→2)βDMan-[βDGal(1→4)βDGlcNAc[αLFuc(1→3)](1→3)](1→3)](1→4)-βDGlcNAc(1→4)βDGlcNAc, 101517-24-2; αDGal(1→3)βDGal, 72597-57-0; αDGal(1→3)βDGal(1→4)βDGlcNAc, 101627-01-4; αDGal(1→4)βDGal(1→4)βDGlcNAc, 101492-17-5; βDGal(1→4)-βDGlcNAc, 47491-70-3; βDGal(1→4)βDGlcNAc(1→2)αDMan(1→6)βDMan[βDGal(1→4)βDGlcNAc(1→2)αDMan(1→3)](1→4)-

βDGlc(1→4)βDGlcNAc, 101492-15-3; βDGal(1→4)βDGlcNAc(1→6)αDMan[βDGal(1→4)βDGlcNAc(1→2)](1→6)βDMan[βDGal(1→4)βDGlcNAc(1→2)αDMan[βDGlc(1→4)βDGlcNAc(1→4)](1→3)](1→4)βGlcNAc, 101517-25-3; βDGal(1→4)βDGlcNAc(1→2)αDMan(1→6)βDMan[βDGal(1→4)βDGlcNAc[βDGal(1→4)βDGlcNAc(1→3)](1→2)αDMan(1→3)](1→4)βDGlcNAc(1→4)βDGlcNAc, 101492-16-4; βDGal(1→3)αDGalNAc, 5143-15-7; αLFuc(1→6)DGlcNAc, 37776-59-3; 2′-fucosyllactose, 41263-94-9.

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