Purification and Properties of a Novel Fucose-Specific Hemagglutinin of *Aleuria aurantia*†

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ABSTRACT: A fucose-binding lectin from fruiting bodies of *Aleuria aurantia* was purified by affinity chromatography by using the H-active glycopeptide of desialyzed porcine submaxillary mucine coupled to Sepharose 4B and eluting with L-fucose. Homogeneity of the active protein was confirmed by polyacrylamide gel electrophoresis, isoelectric focusing, column chromatography using Sephadex G-100, and ultracentrifugal analyses. It has a molecular weight of 72 000 and is proposed to be a dimer of identical subunits, each of which has one combining site of uniform affinity. Chemical analyses revealed the absence of the sulfur-containing amino acid and carbohydrate, neutral and amino sugar, in the lectin molecule. It agglutinated human erythrocytes of all ABO and Lewis types, Bombay phenotype, and group O cells treated with α(1→2)-fucosidase. In double-diffusion experiments, the lectin formed a single precipitin line which fused with all of the fucose-containing blood-group substances tested, including the α-fucosidase-treated materials. These findings together with the results of hemagglutination and precipitation studies indicate that the lectin combines the terminal fucose in the carbohydrate chain but does not require a particular linkage to the penultimate sugar moiety.

A number of lectins have been reported in higher plants, invertebrates, and lower vertebrates [for a review see Sharon & Lis (1972)], and they provide useful tools for the structural analyses of soluble and membrane glycoproteins [e.g., see Nicolson (1974)]. Fucose[1]-binding lectins have been reported from *Lotus tetragonolobus* seed (Morgan & Watkins, 1953; Watkins & Morgan, 1962), *Ulex europaeus* seed (Boyd, 1963; Flory, 1966), and normal eel (*Anguilla anguilla*) serum (Watkins & Morgan, 1952, 1962). They are primarily specific to terminal fucose linked 1→2 to the penultimate galactose, which is the H-determinant structure of human and heterologous blood-group antigens.

On the other hand, little information is available concerning a lectin which could bind fucose linked other than 1→2 in a carbohydrate chain. In the course of our preliminary survey of hemagglutinins in lower plants, we found a novel fucose-binding lectin in the fruiting body of *Aleuria aurantia* (orange peel fungus). In this paper we describe the purification of the active protein by using specific immunoadsorbent and molecular aspects of the purified lectin. Immunological experiments revealed the binding specificity of the lectin is for fucosyl residue irrespective of the linkage to the proximal sugar moiety. The usefulness of this novel lectin for studies of fucose-containing carbohydrates will be suggested.

Experimental Procedures

Materials. Blood-group substances of ovarian cyst fluids, gastric linings, and meconiums were prepared as described [see Kabat (1956), Howe & Kabat (1956), and Côté & Valet (1976), respectively]. The desialyzed and then trypsinized glycopeptide of PSM was prepared as described earlier (Kochibe, 1973). 2′-FL, LNT, LNF-I, LNF-II, and LND-I were isolated from secretor colostrum (Kobata, 1972). 3-FL was isolated from nonsecretor colostrum in the same manner, and this preparation was further purified by α(1→2)-fucosidase (see below) treatment and rechromatography for removal of contaminating 2′-FL. Methyl α-fucoside was prepared by refluxing a methanol solution of fucose in the presence of Dowex 50W (H^+ form) and crystallizing from hot methanol. The product gave $[\alpha]^{25}_D$ −185° (*c* 1.78, water) and mp 152 °C.

Fruiting bodies of *A. aurantia* were collected locally and were extracted immediately or stored at −20 °C until use. Fucose-specific lectins of *U. europaeus* and eel serum were prepared according to published methods (Matsumoto & Osawa, 1969, 1974). *Bacillus fulminans* α(1→2)-fucosidase (EC 3.2.1.63) was purified as described (Kochibe, 1973).

Preparation of Specific Adsorbent. The H-active glycopeptide of desialyzed PSM was coupled to CNBr-activated Sepharose 4B (Pharmacia) (March et al., 1974). Briefly, to 60 mL of a 50% (v/v) aqueous suspension of the gel was added 120 mL of 2 M Na_2CO_3 under cooling. The gel was activated by adding 3.8 g of CNBr in 7 mL of acetonitrile and stirring gently for 2 min. The slurry was poured onto a sintered-glass funnel, washed with cold water and then 0.25 M $NaHCO_3$ (pH 9.0), and transferred into a beaker containing 200 mg of the glycopeptide in 30 mL of 0.25 M $NaHCO_3$. The coupling was allowed to continue for 4 h at room temperature under slow stirring. The gel was then collected by filtration and washed with water, 0.05 M ethanolamine, and PBS, successively. In a typical experiment, 23.5% of the fucose-containing glycopeptide added was coupled to the gel. The calculation was based on the amount of fucose in the filtrate and the washings compared to that initially added. The fucose concentration of the settled gel was approximately 1 μmol/mL.

Isolation and Purification of Lectin. All operations were carried out at 4 °C unless otherwise noted. Fresh or frozen fruiting bodies (100 g) were homogenized with 250 mL of PBS, and the homogenate was centrifuged at 10000*g* for 20

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[1] Abbreviations and trivial names used: PSM, porcine submaxillary mucine; CNBr, cyanogen bromide; $NaDodSO_4$, sodium dodecyl sulfate; PBS, phosphate-buffered saline; CM, carboxymethyl; 2′-FL, 2′-fucosyllactose Fucα1→2Galβ1→4Glc; 3-FL, 3-fucosyllactose Galβ1→4(Fucα1→3)Glc; LNT, lacto-*N*-tetraose Galβ1→3GlcNAcβ1→3Galβ1→4Glc; LNF-I, lacto-*N*-fucopentaose I Fucα1→2Galβ1→3GlcNAcβ1→3Galβ1→4Glc; LNF-II, lacto-*N*-fucopentaose II Galβ1→3(Fucα1→4)GlcNAcβ1→3Galβ1→4Glc; LND-I, lacto-*N*-difucohexaose I Fucα1→2Galβ1→3(Fucα1→4)-GlcNAcβ1→3Galβ1→4Glc. "Fucose" indicates the L form, and other sugars are of the D form in this paper.

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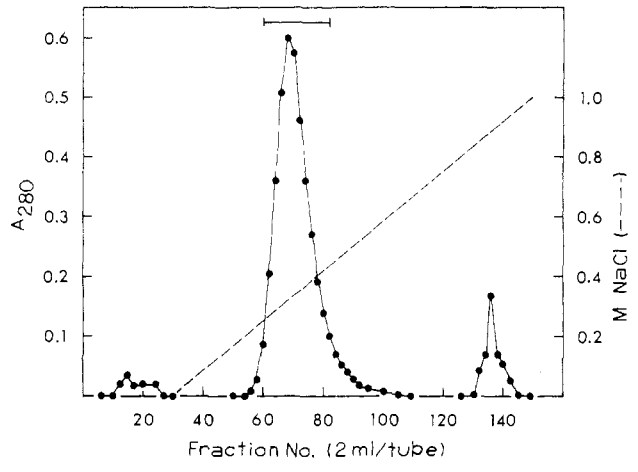

FIGURE 1: Purification of *A. aurantia* lectin by CM-cellulose column chromatography. The bar represents the active fractions which were pooled and used for further investigations. Experimental details were as described in the text.

min. The pellet was homogenized again with 150 mL of PBS, stirred for 2 h, and centrifuged as above. To the combined, pale orange colored extract was added solid $(NH_4)_2SO_4$ under continuous stirring to give 80% saturation. After the solution was allowed to stand overnight, the precipitate was collected by centrifugation and dialyzed against 4 × 1 L of PBS for 2 days (fraction F-1). This fraction, F-1 (10 mL), was applied to a column of Sephadex G-100 (Pharmacia, 4.5 × 60 cm), which had been equilibrated, and was eluted with PBS. The eluate from the column was monitored by the absorbance at 280 nm and the agglutinating activity. The protein in the active fraction was concentrated by $(NH_4)_2SO_4$ precipitation and dialyzed (fraction F-2). This was then applied to a column (1.5 × 26 cm) of specific immunoadsorbent, and the column was eluted with PBS until the absorbance decreased to as low as 0.05 at 280 nm. The bound lectin was eluted with 20 mM fucose and dialyzed until free of the sugar (fraction F-3).

Finally, CM-cellulose chromatography was conducted to purify the lectin. For this, 5 mL of fraction F-3 which had previously been dialyzed against 0.05 M citrate buffer (pH 3.8) was applied to a column (1.5 × 24 cm) of CM-cellulose equilibrated with the same buffer. A linear gradient of NaCl from 0 to 1.0 M in the buffer was employed for the elution (Figure 1). The purified lectin thus obtained (fraction F-4) was used for further investigations.

Molecular-Weight Determination. Analytical gel filtration was performed by the method of Andrews (1965) by using a Sephadex G-100 column (2 × 50 cm) equilibrated with PBS. Polyacrylamide gel electrophoresis in the presence of Na-$DodSO_4$ was done as described (Weber & Osborn, 1969). The following served as standards in the gel filtration and the electrophoresis experiments: aldolase, 160 000; human γ-globulin, 150 000; bovine serum albumin, 67 000; egg albumin, 43 000; chymotrypsinogen, 25 700; myoglobulin, 15 500; cytochrome *c*, 11 700. Sedimentation velocity was determined by employing a Beckman Spinco Model E analytical ultracentrifuge equipped with Schlieren optics. The sedimentation coefficient was calculated at a protein concentration of 1.25 mg/mL PBS from the measurement at 52 800 rpm. Sedimentation equilibrium analyses were carried out by Dr. N. Ui of the Institute of Endocrinology, Gunma University, on a Beckman Spinco Model E analytical ultracentrifuge equipped with an ultraviolet spectrophotometer. Protein concentrations varied from 0.18 to 0.27 mg/mL of either PBS or PBS made with a H_2O and D_2O mixture (1:9 v/v). Data deduction for molecular weight and partial specific volume was performed with a computer; the program was devised by Dr. Ui (unpublished procedure).

Electrophoresis and Isoelectric Focusing. Disc polyacrylamide gel electrophoresis was performed as described (Reisfeld et al., 1962) at pH 4.3 with β-alanine–acetic acid buffer. Isoelectric focusing on a thin-layer polyacrylamide gel was conducted by either horizontal runs employing an LKB Multiphor apparatus (LKB Producter) and the method recommended by the manufacturer or vertical runs using a slab gel electrophoresis apparatus (Atto Co. Ltd.). For pH-gradient measurement, the gel was cut longitudinally into 1 cm wide strips and sectioned into 0.5-cm pieces; then each piece was minced and eluted with 0.5 mL of water. The gel was stained with 7% acetic acid containing 20% (v/v) methanol and 0.02% (w/v) Coomassie Brilliant Blue R (Sigma) and destained with the same solution devoid of the dye. In the case of isoelectric focusing, the gel was treated with 6% (w/v) trichloroacetic acid for 2 h prior to staining.

Chemical Analyses. Purified, lyophilized lectin was hydrolyzed with 3 N mercaptoethanesulfonic acid (Pierce) in a sealed tube at 110 °C for 22 h (Penka et al., 1974). Amino acid analysis was carried out by Drs. Hamana and Hayashi using a Hitachi Model KLA-5 amino acid analyzer equipped with a data processor. The phenol–sulfuric acid method (Dubois et al., 1956) and the anthrone–sulfuric acid method (Spiro, 1966) were used for neutral sugar analysis. Protein was determined by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

Equilibrium Dialysis. This was conducted by use of a microchamber (polyacrylate plastic resin) that accommodated as much as 200 μL. The chamber was designed and machined according to description (Eisen, 1971) with minor modification. Each half-chamber contained 100 μL of the lectin (84.3 μg of protein) and the other half 100 μL of tritiated fucose (New England Nuclear, 12 Ci/mmol) of increasing concentration (from 0.08 to 900 μM). All solutions were made with PBS containing 0.02% sodium azide. After dialysis for 24 h at room temperature, 50 μL of the solution was withdrawn from each compartment, and the radioactivity was counted in a Packard Model 3390 liquid scintillation spectrophotometer.

Immunological Methods. (1) Hemagglutination. The titration of lectin was conducted by serial dilution of lectin with PBS. The activity was expressed as the reciprocal of the greatest dilution where the agglutination of type O cells (2% suspension in PBS) occurred on a hollowed glass plate. For inhibition studies, the substance to be tested was serially diluted with PBS, and equal volumes of four doses of agglutinin were added. After incubation for 2 h at room temperature, 1 drop of the reaction mixture was mixed with 1 drop of the cell suspension, and the hemagglutination was visually examined. Inhibiting activity was expressed as the lowest concentration of the substance in the incubation mixture with which agglutination was completely inhibited.

(2) Precipitation. Quantitative precipitation analyses were carried out by using 3-mL centrifuge tubes. For the standard assay, 52 μg of the lectin was added to an increasing amount of a meconium substance, H Le(a±b+), to a total volume of 250 μL. The tubes were incubated at 37 °C for 2 h and then at 4 °C for 2 days, centrifuged, and washed with cold PBS 3 times. The protein in the precipitate was determined as described above. For precipitation inhibition studies, a lectin solution (40 μg of protein) was added to tubes containing varying amounts of inhibitor and meconium substance (12.5 μg) to a total volume of 250 μL. The extent of inhibition was

Table I: Characteristics of Fractions of *A. aurantia* Lectin Purification[a]

fraction	vol (mL)	protein (mg/mL)	titer[b]	sp act.[c]
crude extract	370	2.8	32	11.3
F-1	55	10.6	320	30.2
F-2	23	11.7	1024	87.5
F-3	31	3.06	1024	334.6
F-4	42	2.1	1024	487.6

[a] 100 g of fresh fruiting bodies was processed. [b] Hemagglutinating activity with group O cells. [c] Titer/mg of protein.

expressed as the percentage of protein precipitated vs. that of the control including no inhibitor. Immunodiffusion was performed by using 1% agar or 1.5% agarose gel in PBS.

Results

Purification. The characteristics of the fractions obtained in various purification steps are presented in Table I. Upon Sephadex G-100 chromatography, active substance was eluted later than the large part of inactive protein which was excluded from the column. The coupling of H-active glycopeptide of desialyzed PSM to CNBr-activated Sepharose 4B yielded a good adsorbent for the lectin. Neither native PSM nor desialyzed PSM was used as a ligand because of their low solubilities in aqueous medium. When fraction F-2 was applied on the affinity column, inactive protein passed through, and subsequent elution with 20 mM fucose displaced bound lectin in a symmetrical peak. If a lower concentration of the sugar (e.g., 10 mM) was used for the elution, a diffused peak accompanying a shoulder resulted. After this purification step, the lectin solution became almost colorless and was shown to contain two components by polyacrylamide gel electrophoresis (see Figure 2B). Finally, the lectin was applied to a column of CM-cellulose equilibrated with 5 mM citrate buffer, pH 3.8, and eluted with a linear gradient of NaCl in the buffer. As much as 90% of the applied protein was eluted at 0.3 M in a symmetrical peak (Figure 1), and a minor component was eluted at 0.8–0.9 M, which was not subjected to further analysis. A 43-fold purification based on the specific hemagglutination activity was achieved, and the final product represented 8.5% of the protein in the crude extract.

It should be noted that the protein in the active fractions which arose from each purification step was concentrated by salting out with $(NH_4)_2SO_4$ (80% saturation). We found that both lyophilization and ultrafiltration through a membrane, such as collodion or cellulose acetate, caused no loss of activity but did cause aggregation of the protein molecules. This was apparent when examined by polyacrylamide gel electrophoresis at pH 4.3. Thus, the purified lectin obtained by $(NH_4)_2SO_4$ precipitation gave a single band indicating homogeneity of the product, whereas the same fraction treated further by lyophilization or ultrafiltration gave additional multiple bands of slow mobilities (see Figure 2C,D).

Purity and Molecular Weight. Purified *A. aurantia* lectin was shown to be homogeneous by the following criteria: disc polyacrylamide gel electrophoresis, polyacrylamide gel electrophoresis in the presence of $NaDodSO_4$, isoelectric focusing, chromatography on Sephadex G-100, and analytical ultracentrifuge patterns. The lectin gave a single protein band on disc polyacrylamide gel electrophoresis at pH 4.3 (Figure 2C), whereas it failed to migrate into the gel when the buffer system for pH 9.5 (Davie, 1964) was used. Therefore, the protein was assumed to charge positively in the sample buffer (pH 8.3), and this was apparent in the isoelectric-focusing experiment. As shown in Figure 2E, the lectin migrated as a single band to the area of pH 9.0–9.2. Both vertical and horizontal runs gave identical results.

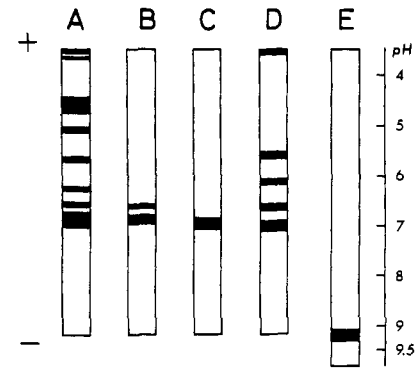

FIGURE 2: Disc polyacrylamide gel electrophoresis patterns (A–D) in 7.5% gel: (A) fraction F-1; (B) fraction F-3; (C) fraction F-4; (D) lyophilized material of fraction F-4. Isoelectric-focusing pattern of fraction F-4 (E). Amount of applied protein was ~150 μg for A and D and 50 μg for B, C, and E. See the text for experimental details.

Table II: Amino Acid Composition of *A. aurantia* Lectin

residue	mol/72000 g	residue	mol/72000 g
Try	33.5	Gly	96.7
Lys	30.6	Ala	67.2
His	3.62	Cys	0
Arg	24.6	Val	36.4
Asp	53.6	Met	0
Thr	41.2	Ile	45.8
Ser	82.3	Leu	27.1
Glu	62.4	Tyr	28.3
Pro	37.5	Phe	19.7

A gel-filtration study of the lectin using a Sephadex G-100 column revealed a molecular weight of ~70 000 where the extent of absorbance at 280 nm and that of hemagglutinating activity showed good coincidence. Upon ultracentrifugation, the lectin moved as a single peak indicating a homogeneity of the molecular species, and the sedimentation constant ($s_{20,w}$) was calculated to be 4.90 from duplicate experiments. Sedimentation-equilibrium analyses at 9000 rpm and 17.7 °C gave a molecular weight of 72 000 and a partial specific volume of 0.784; these were the averages of the data deduced from the measurements at 27- and 44-h intervals of centrifugation.

The lectin was subjected to polyacrylamide gel electrophoresis in the presence of $NaDodSO_4$ and gave a single polypeptide band of apparent molecular weight 31 000 ± 1000. This value did not vary when the lectin was treated with either 0.1 M 2-mercaptoethanol or 0.1 M dithiothreitol and then dialyzed against 0.2 M iodoacetamide. Thus, the native lectin exists probably as a dimer of identical subunits (see also Equilibrium Dialysis), and disulfide bonding is not involved for the association.

Chemical Compositions. The amino acid composition of the lectin shown in Table II indicates a high content of hydroxylic and acidic amino acids. The absence of sulfur-containing amino acid was remarkable; an appreciable recorder response was not observed in the regions corresponding to cysteine or cystine, methionine, methionine sulfoxide, or methionine sulfone. This is in support of the findings that subunit molecular weight and binding activity were unaffected by reducing reagents. It should be noted also that neither the phenol–sulfuric acid reaction nor the anthrone reaction gave appreciable color characteristic of neutral sugars when up to 2 mg of the lectin was employed. Moreover, hexosamine was not detected in either amino acid analysis or an Elson–Morgan reaction (Spiro, 1966) of the acid hydrolysate. Therefore, *A.*

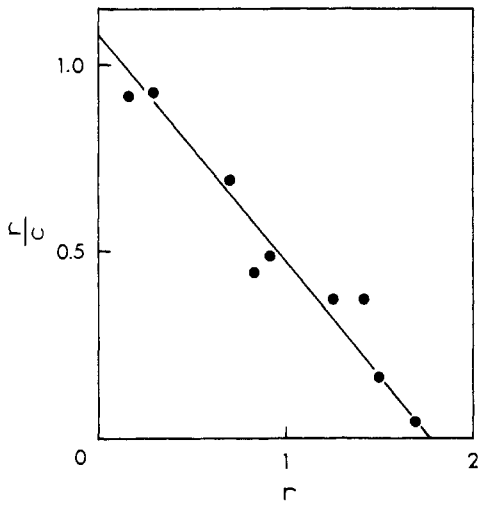

FIGURE 3: Equilibrium dialysis of *A. aurantia* lectin. See the text for details.

Table III: Inhibiting Activity of Mono- and Oligosaccharides

substance	min concn (mM) for complete inhibn	substance	min concn (mM) for complete inhibn
D-fucose	>50	3-FL	3.65
L-fucose	0.78	LNF-I	5.0
methyl α-fucoside	0.1	LNF-II	2.5
2′-FL	1.6	LND-I	5.0

aurantia lectin is not a glycoprotein.

Equilibrium Dialysis. The data are plotted in Figure 3 according to $r/c = nK - rK$, where r = moles of fucose bound per mole of protein, c = the concentration of free fucose, n = the number of combining sites per mole of protein, and K = the intrinsic association constant. The plots are notably linear, showing the binding site is homogeneous with respect to affinity for the hapten. The abscissa intercept, which corresponds to n, is 1.8, and K is calculated to be 6.1×10^4 M^{-1} from the intercept of the ordinate. These data support the fact that the lectin molecule is a dimer of univalent, identical subunits.

Hemagglutination and Hemagglutination Inhibition. A. aurantia lectin agglutinated human erythrocytes of all ABO and Lewis types tested, and the reaction was unaffected in the presence of 2-mercaptoethanol (0.1 M). Treatment of the cells with protease, such as trypsin or papain, caused a great enhancement of the reaction. Table III shows the results of hemagglutination inhibition studies and that the lectin was highly specific to fucose. Thus, methyl α-fucoside was the most potent inhibitor, and fucose came after. Of human milk oligosaccharides, 2′-FL was more active than 3-FL, and penta- and hexasaccharides were moderately active. These inhibiting patterns seem to be similar to those observed with eel serum and *Lotus* lectin (Watkins & Morgan, 1962), both of which are specific for fucose linked 1→2 to galactose (i.e., H-determinant structure). The following sugars failed to give significant inhibition when tested up to the concentrations (mM) indicated: Glc, 100; Gal, 100; Man, 50; rhamnose, 50; GlcNAc, 50; GalNAc, 50; lactose, 50; LNT, 25.

It should be emphasized that *A. aurantia* lectin was able to agglutinate Bombay phenotype cells, O_h Le(a+b−) (Iseki et al., 1970), although the reaction was weaker than with group O cells. The O_h cell lacks the H-determinant structure and fails to react with anti-H reagents. This status could be produced in vitro by treatment of appropriate group O cells

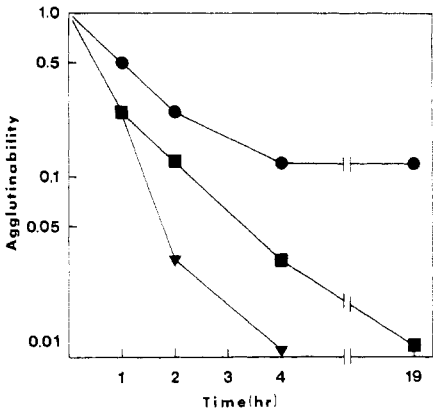

FIGURE 4: Changes of agglutinability of human erythrocytes by α(1→2)-fucosidase treatment. To a 10% (v/v) cell suspension, O Le(a−b−), in PBS was added an equal volume of 0.1% (w/v) purified enzyme, and the sample was incubated at 37 °C with gentle shaking. At the interval indicated, an aliquot of the mixture was withdrawn. The cells were washed with PBS and used for the titration of eel serum (▼), *Ulex* (■), and *A. aurantia* lectin (●) by the method described under Experimental Procedures. Agglutinability is defined here as the maximum dilution factor of the individual agglutinin with the enzyme-treated cells relative to that with nontreated cells.

with the α(1→2)-fucosidase of *B. fulminans*. Indeed, as shown in Figure 4, O Le(a−b−) cells were no longer agglutinated by either eel serum or *Ulex* lectin after a 19-h incubation with the enzyme. In contrast, the enzyme-treated cells still reacted with the *A. aurantia* lectin. Agglutinability of the cells decreased to one-eighth of that of the original during early 4-h treatment and then remained unchanged up to 19 h. When O Le(a+b−) cells were treated and titrated in the same manner, quite similar results were observed. In a parallel experiment, additional enzyme was supplied to the cell suspension at 12-h intervals of incubation and then allowed to react for a further 7 h. The agglutinability of the cells thus treated was found to be comparable to that described above. This observation should exclude the possibility that *A. aurantia* lectin may have a higher avidity to the 1→2-fucosyl group on the cell surface than the anti-H reagents employed and combines residual H determinant left after the enzyme treatment.

To obtain further information on the binding specificity of the lectin, we treated blood-group substances of various phenotypes with the fucosidase and tested the reactivities with respective fucose-binding lectins by hemagglutination inhibition assays. Table IV illustrates that H activities of all blood-group substances were degraded or greatly reduced by the enzyme treatment, whereas these substances still inhibited the reaction with *A. aurantia* lectin, although the activities decreased to one-half or one-fourth of the originals. The only exception was desialyzed PSM in which terminal fucose linked solely in 1→2 and was susceptible to the enzyme. These observations, together with the fact that the meconium substance of Lewis-negative nonsecretor exhibited significant activity for the lectin, indicate that the lectin binds specifically to fucose but does not seem to require a particular linkage to the penultimate sugar moiety. The relationship between the fucosyl linkage and the blood-group activity is shown in Table V.

Precipitation Reactions. Blood-group substances of all ABO and Lewis types precipitated with *A. aurantia* lectin. The inhibiting effect of mono- and oligosaccharides on the precipitation was measured in a system containing the optimal proportion of the lectin and the meconium substance of H Le(a±b+), as described under Experimental Procedures. Figure 5 shows that methyl α-fucoside was the most potent inhibitor among the five substances tested and caused 50%

Table IV: Changes of Inhibiting Activities of Blood-Group Substances by α(1→2)-Fucosidase Treatment[a]

substance		enzyme	inhibn act. (μg/mL) against		
source	blood-group act.	treatment	*A. aurantia*	*Ulex*	eel
human gastric lining	H Le(a−b+)	before[b]	19.5[c]	4.9	4.9
		after	78.1	625	313
	H Le(a−b−)	before	9.7	4.9	4.9
		after	39.1	1250	2500
	−Le(a+b−)	before	19.5	313	2500
		after	39.1	>5000	>5000
meconium	H Le(a±b+)	before	39.1	9.8	4.9
		after	78.1	313	1250
	H Le(a−b−)	before	39.1	4.9	4.9
		after	78.1	625	1250
	−Le(a−b−)	before	39.1	1250	1250
		after	78.1	>1250	>1250
PSM	H	before	19.5	>1250	>1250
		after	>1250	>1250	>1250

[a] Blood-group substances in PBS (0.5%) were treated with equal volumes of purified α-fucosidase (0.1%) at 37 °C for 24 h and then heated at 100 °C for 5 min. Inhibiting activity was assayed by the method described under Experimental Procedures. [b] An equal volume of PBS was added in place of the enzyme. [c] Minimum amount required to inhibit completely the agglutination reaction.

Table V: Fucose-Involved Structures of Blood-Group Antigens and Their Occurrences

structure	antigenic act.	presence in secretions of
Fucα1→2Galβ1→3GlcNAcβ1→3Gal− type 1 chain	H	secretor
Fucα1→2Galβ1→4GlcNAcβ1→3Gal− type 2 chain	H	secretor
Galβ1→3GlcNAcβ1→3Gal− (↑4 Fucα1)	Le[a]	Lewis-positive nonsecretor
Galβ1→3GlcNAcβ1→3Gal− (↑2 Fucα1, ↑4 Fucα1)	Le[b]	Lewis-positive secretor
Galα1→4GlcNAcβ1→3Gal− (↑3 Fucα1)	X[a]	secretor and nonsecretor

[a] Antigenicity of this structure has not been known.

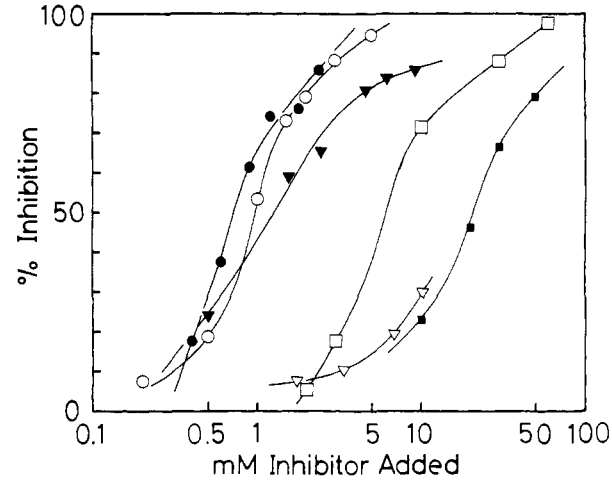

FIGURE 5: Inhibition of precipitation of a meconium substance, H Le(a±b+), with *A. aurantia* lectin. The inhibitor concentration in the reaction mixture is indicated. Methyl α-fucoside (●); fucose (○); 2′-FL (▼); 3-FL (▽); LNF-II (□); LNF-I (■). See the text for further details.

inhibition at 0.72 mM. The concentrations (mM) of the inhibitors giving half-maximal inhibition were as follows: fucose, 1.0; 2′-FL, 1.3; 3-FL, 10 (30%); LNF-II, 6.0; LNF-I, 24. The order of potency of these carbohydrates showed good agreement with that observed in hemagglutination inhibition studies as described above.

In double-diffusion experiments, the lectin formed a single and fused precipitin line with the blood-group substances from all sources tested, including the α-fucosidase-treated materials. These findings indicate, again, that the substituting position of the fucose is less important for the binding. In order to see the effect of detergents on the activity, we performed double diffusions in the presence of either Triton X-100, Tween 80, Tergitol NP-40, $NaDodSO_4$, or sodium deoxycholate (0.5% each). The lectin formed a precipitin line in all cases and seemed to be insensitive to the detergents for the binding activity.

Discussion

The lectin of *A. aurantia* was purified by affinity chromatography employing the specific immunoadsorbent, H-active glycopeptide of desialyzed PSM coupled to Sepharose 4B, and elution with fucose. On polyacrylamide gel electrophoresis at pH 4.3, the eluate gave one major band of active protein and a faint, slow-moving band that was separated from the former by subsequent CM-cellulose chromatography. The purified lectin was shown to have a molecular weight of 70000 by gel filtration, and this was further determined to be 72000 from the ultracentrifugal analyses. Polyacrylamide gel electrophoresis in the presence of $NaDodSO_4$ indicated that the lectin is composed of apparently identical subunits and that the sulfhydryl group is not involved for the association since the mobility of the subunit peptide was unaffected after reduction and alkylation. The lectin seems to be a dimer of the univalent subunits since two binding sites per molecule were evident from the equilibrium dialysis. The subunit molecular weight calculated from the electrophoretic mobility was somewhat smaller than expected from the value of the native protein; the reason for the unusual mobility of the subunit was not known.

All of the phytoagglutinins reported, except concanavalin A, contain carbohydrate in the molecule and accordingly are glycoproteins (Lis & Sharon, 1973). Peanut lectin was recently reported to have no sugar (Lotan et al., 1975). *A. aurantia* lectin is the third exception in this respect, since neither neutral nor amino sugar was detected by various analytical methods. The absence of cysteine and methionine is also notable, and this supports the findings that the immunological activity and subunit molecular weight were unaffected by reducing reagents. A number of lectins have been reported to have low sulfur-containing amino acids [see Lis & Sharon (1973) and references cited therein]. Recently, *Vicia faba* lectin was shown to lack these amino acids (Allen et al., 1976; Allen & Johnson, 1976).

In the carbohydrate chains of the mammalian glycoproteins so far examined, fucose is found always as an α anomer and binds to the nonreducing or branching terminal. Various blood-group activities are determined by this sugar, depending on its attachment position to the proximal sugar residue (see Table V). Fucose-binding lectins have been reported from three sources, and they are specific to 1→2 linkage at a nonreducing terminal of the carbohydrate chain. Of these, both *Lotus* lectin (Periera & Kabat, 1974) and *Ulex* lectin (Periera et al., 1978) were shown to have restricted specificity for the fucose linked to the type-2 chain. In contrast, *A. aurantia* lectin exhibited a unique binding property as presented. Comparison of the ability of monosaccharides and human milk oligosaccharides to inhibit hemagglutination and precipitation implies that the lectin may resemble the H-specific lectin described, since 2′-FL was more potent than 3-FL and fucosyl penta- and hexasaccharides were moderately active (see Table III and Figure 5). Whereas, as suggested by the agglutination of Bombay-type cells, the data of the inhibitory activities of macromolecular blood-group substances are consistent with the fact that the lectin combines not only H-determinant structure but also the fucose linked other than 1→2. Binding to the Fucα1→3 structure is obvious from the finding that the substances of Lewis-negative nonsecretor, –Le(a–b–), and of the secretor after the enzyme treatment were potent toward the lectin but not anti-H lectins (see Table IV). Since the substance which has fucose solely linked 1→4 to GlcNAc, i.e., Lewis a determinant, was unavailable, binding to this structure was uncertain. However, the inhibitory activity of LNF-II was stronger than the inhibitory activities of 3-FL and LNF-I for the lectin, showing a high possibility of lectin binding to the Fucα1→4 structure. The binding to fucose irrespective of the substituting position is further confirmed by the fact that, in the double-diffusion experiment, the lectin gave a homogeneous precipitin reaction with all of the fucose-containing substances tested.

There is a decrease in the inhibitory activity of the nonsecretor substance or the enzyme-treated material when compared with that of secretor or the untreated material. This may be due to the difference of binding affinity for individual fucosyl linkages, the decrease in the fucosyl content of H-negative substance, or both.

The presence of branched fucose linked 1→6 to GlcNAc was demonstrated in the glycoproteins having N-glycosidic chains (Kornfeld & Kornfeld, 1976) but not in the blood-group substances, which have O-glycosidic chains. Therefore, whether the lectin can bind to the fucose linked in such a manner is not known. Although the lectin failed to form a precipitin line with either human serum or purified human immunoglobulin, it was suggested from our preliminary experiment that the immobilized lectin could bind fucose-containing glycoproteins in the serum and release them in the presence of free fucose. The finding that the lectin activity was maintained in the presence of detergents will favor the development of a specific method for the separation of fucose-containing glycoproteins from the detergent-solubilized membrane components.

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