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Immunochemical Studies on the Binding Specificity of the Blood Group Le^b Specific Lectin *Griffonia simplicifolia* IV[1]

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The specificity of the *Griffonia simplicifolia* IV (GS-IV) lectin was studied by quantitative precipitin and quantitative precipitin inhibition assays. The lectin precipitated most strongly with a human H,Le^b blood group substance and reacted strongly with an Le^a and an A_2 blood group substance with Le^b activity. Because of the heterogeneity of the blood group glycoproteins, the lectin reacted to different extents with substances of the same blood group activity. Specific precipitates of lectin with blood group substances which reacted strongly were less soluble than with those which reacted weakly. By inhibition of precipitation of GS-IV with H,Le^b blood group substance, the lectin is most specific for the Le^b oligosaccharide lacto-*N*-difucohexaose I,

$$\begin{array}{l} \text{LFuc} \qquad\quad \text{LFuc} \\ \quad \alpha\downarrow^{1}_{2} \qquad\quad \alpha\downarrow^{1}_{4} \\ \text{DGal}\beta 1 \rightarrow 3\text{DGlcNAc}\beta 1 \rightarrow 3\text{DGal}\beta 1 \rightarrow 4\text{DGlc.} \end{array}$$

Lacto-*N*-difucohexaose II,

$$\begin{array}{l} \qquad\qquad\quad \text{LFuc} \qquad\qquad\quad \text{LFuc} \\ \qquad\qquad\quad \alpha\downarrow^{1}_{4} \qquad\qquad\quad \alpha\downarrow^{1}_{3} \\ \text{DGal}\beta 1 \rightarrow 3\text{DGlcNAc}\beta 1 \rightarrow 3\text{DGal}\beta 1 \rightarrow 4\text{DGlc,} \end{array}$$

was about one-fourth as active on a molar basis and other difucosyl oligosaccharides were somewhat less active but were more potent than monofucosyl Le^a and H active oligosaccharides. LFuc was inhibitory only at very high concentrations. The binding site of the GS-IV lectin appears most specific for difucosyl oligosaccharides. Although the most active is an Le^b oligosaccharide, the site may prove not to be Le^b specific.

Four lectins, with different carbohydrate binding specificities, have been isolated from the seeds of *Griffonia simplicifolia* (GS)[3] previously called *Bandeiraea simplicifolia* (BS). GS-A_4 and GS-B_4 are isolectins specific for terminal nonreduc-

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[3] Abbreviations used: DGal, D-galactose; DGlc, D-

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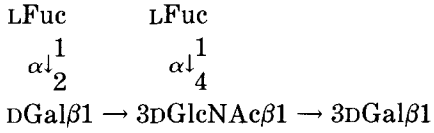
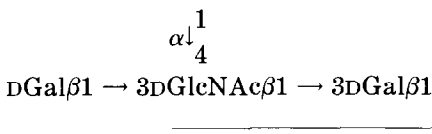
ing αDGalNAc and αDGal residues, respectively (1). The GS-II lectin was found to be specific for terminal nonreducing DGlcNAc residues (2). The BS-IV (GS-IV) lectin was isolated by affinity chromatography on a blood group Le^b oligosaccharide adsorbent (3). Inhibition of GS-IV binding to an Le^b oligosaccharide-BSA conjugate using various oligosaccharides showed the lectin to be most specific for structures containing two LFuc residues on either a type-1 or type-2 chain. However, the lectin was found to have equal agglutinating activity with ficin treated type-O red cells regardless of their Le phenotype. This is an unusual finding when compared to the specificity of human anti-Le^b sera which agglutinate Le^a and Le^- group O red cells only weakly (4).

In the present study, the specificity of the combining site of the GS-IV lectin was studied further by quantitative precipitation with A, B, H, Le^a, Le^b, and precursor I and i blood group substances and by inhibition of precipitation using various monosaccharides, glycosides, as well as milk and blood group oligosaccharides. The lectin was found to precipitate well both with an Le^a and an H,Le^b blood group substance. Although the most active inhibitor was an Le^b active hexasaccharide, lacto-*N*-difucohexaose I,



another hexasaccharide, lacto-*N*-difucohexaose II,



was also a good inhibitor. Thus, of the oligosaccharides studied, although the GS-IV reacts best with Le^b structures, it reacts more strongly with compounds in which two LFuc residues are accessible to the lectin binding site, than with lacto-*N*-fucopentaose I and II which contain only one of the two LFuc residues of the Le^b determinant.

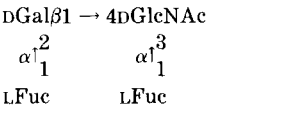
MATERIALS AND METHODS

Lectin. The GS-IV lectin was prepared as described previously (3) by affinity chromatography on an Le^b (LFucα1 → 2DGalβ1 → 3[LFucα1 → 4]DGlcNAcβ1 →) active oligosaccharide coupled to Synsorb (3), followed by elution with 0.1 N acetic acid.

Blood group substances. The purified blood group A, B, H, Le^a, Le^b, and precursor I substances were from human saliva or ovarian cyst fluid and from horse, cow, or hog gastric mucosae (5–10). Periodate-oxidized and Smith-degraded H substance cyst JS phenol insoluble, (11) and A_2 substance cyst MSS 10% 2×, (11, 12) were described previously. The P1 fractions of the B substance PM phenol insoluble (13) and A_1 substance McDon 15% (14) are the nondialyzable portions of the blood group substance after mild acid hydrolysis at pH 1.5 to 2.0 at 100°C for 2 h. This treatment removed most of the αL-fucosyl end groups as well as some of the group B and group A active side chains from PM and McDon, respectively.

Sugar inhibitors. Mono and disaccharides were from Sigma Chemical Company (St. Louis, Mo.), Eastman Organic Chemical Company (Rochester, N. Y.), and Nutritional Biochemicals (Cleveland, Ohio). DGalβ1 → 3DGlcNAc and DGalβ1 → 4DGlcNAc were from Dr. F. Zilliken (University of Bonn, West Germany). The blood group A specific oligosaccharides were described previously (14–17). DGalα1 → 3DGal, the blood group B-specific disaccharide was prepared in this laboratory from carrageenan by partial hydrolysis.

glucose; DGalNAc, *N*-acetyl-D-galactosamine; DGlcNAc, *N*-acetyl-D-glucosamine; DMan, D-mannose; GS-IV, *Griffonia simplicifolia* IV; LFuc, L-fucose; LRha, L-rhamnose; BSA, bovine serum albumin.



was a gift from Dr. P. Sinaÿ (University of Orleans, France) (18). Oligosaccharides from human milk lacto-*N*-difucohexaose I and II, lacto-*N*-fucopentaose I and II, lacto-*N*-tetraose, and 2'-fucosyllactose were gifts from the late Professor Richard Kuhn and Dr. Adeline Gauhe (Max Planck Institute for Biochemistry, Heidelberg, West Germany) and two samples of lacto-*N*-difucohexaose II, one from a nonsecretor, were from Dr. Victor Ginsburg (National Institutes of Health, Bethesda, Md.) (cf. (19)). Urine oligosaccharides were from Dr. Arne Lundblad (University of Lund, Sweden).

The A, B, Le^a, and Le^b activity of various blood group substances were determined by Dr. Richard E. Rosenfield (Mt. Sinai Medical Center, New York, N. Y.) using an agglutination inhibition assay.

Quantitative precipitation and inhibition assays with the purified lectin were carried out by a micro-precipitin technique (20). Six micrograms of lectin N was mixed with varying amounts of blood group substance in a constant volume of 200 μl, the mixtures were incubated at 37°C for 1 h, kept at 4°C for 1 week with mixing twice daily, and washed twice with chilled saline. The total nitrogen of the precipitate was determined by the ninhydrin method (21). For inhibition assays, known quantities of sugars were added to amounts of lectin and blood group substance giving maximum precipitation under the same conditions.

RESULTS

Precipitin curves of total N precipitated by varying amounts of A, B, H, Le^a, Le^b, and precursor I blood group substances, or glycoproteins, are shown in Figs. 1A–D. The lectin precipitated best with an A_1 blood group substance, cyst McDon 15% ppt, and an H,Le^b substance, cyst JS phenol insoluble, both precipitating 6.0 μg of lectin N and with 3 and 2 μg, respectively, giving 50% precipitation. Various fractions of an Le^a substance, cyst N-1, also reacted strongly. Cyst N-1 10% 2× precipitated 6.0 μg N with 4.0 μg giving 50% precipitation; N-1 phenol insoluble and N-1 20% 2× gave different curves, precipitating 5.3 and 5.5 μg of lectin N, respectively, with 4.8 μg giving 50% precipitation. The lectin failed to react with the first Smith degradation product of cyst JS phenol insoluble and with the P1 fraction of cyst McDon 15%. A_1 blood group substances, cyst MSS 10% 2× and cyst MSM 10% ppt, reacted poorly both precipitating 1.5 μg N with 10 μg giving 50% precipitation. The first Smith degradation product of cyst MSS did not react. Another A_1 substance, cyst 9 phenol insoluble (22), reacted moderately precipitating 3.9 μg N with 12 μg required for 50% precipitation. An A_2 substance, cyst 14 phenol insoluble, precipitated 4.8 μg N with 3 μg of blood group substance giving 50% precipitation. The lectin did not react appreciably with a hog A substance, Hog 75 10%, or with Hog A + H substance.

B substances tested reacted weakly with the lectin. Cyst Beach phenol insoluble precipitated 2 μg N, with 10 μg needed for 50% precipitation. PM phenol insoluble, from human saliva reacted moderately, precipitating 3.6 μg N with 13 μg giving 50% precipitation. The P1 fractions of Beach phenol insoluble and PM-phenol insoluble failed to react. Cow B substance, Cow 21 phenol insoluble, reacted very weakly. Various fractions of Cyst Tij II, with weak B activity, reacted moderately; Tij II phenol insoluble precipitated 4.7 μg lectin N and 7.5 μg gave 50% precipitation.

The lectin reacted to various extents with H blood group substances. Cyst Tighe phenol insoluble precipitated 4.8 μg lectin N with 2 μg giving 50% precipitation. Hog 59 10% ppt (H) did not react and Morgan Standard (H) reacted weakly precipitating 1.8 μg N with 8 μg needed for 50% precipitation.

The lectin precipitated poorly with two fractions of a precursor substance OG with I and i activity, OG 20% from 10% and OG 10% from 20%; both precipitated 2.7 μg and 3 μg gave 50% precipitation. The lectin did not react with fetuin and thyroglobulin before and after removal of sialic acid nor did it react with antifreeze glycoprotein or with type XIV pneumococcal polysaccharide.

The A, Le^a, and Le^b activities of several blood group substances are shown in Table I. JS phenol insoluble, cyst 14 phenol insoluble, Tighe phenol insoluble, and McDon 15% ppt which precipitated strongly with the lectin showed high Le^b activity. MSS 10% 2× which reacted poorly had low Le^b

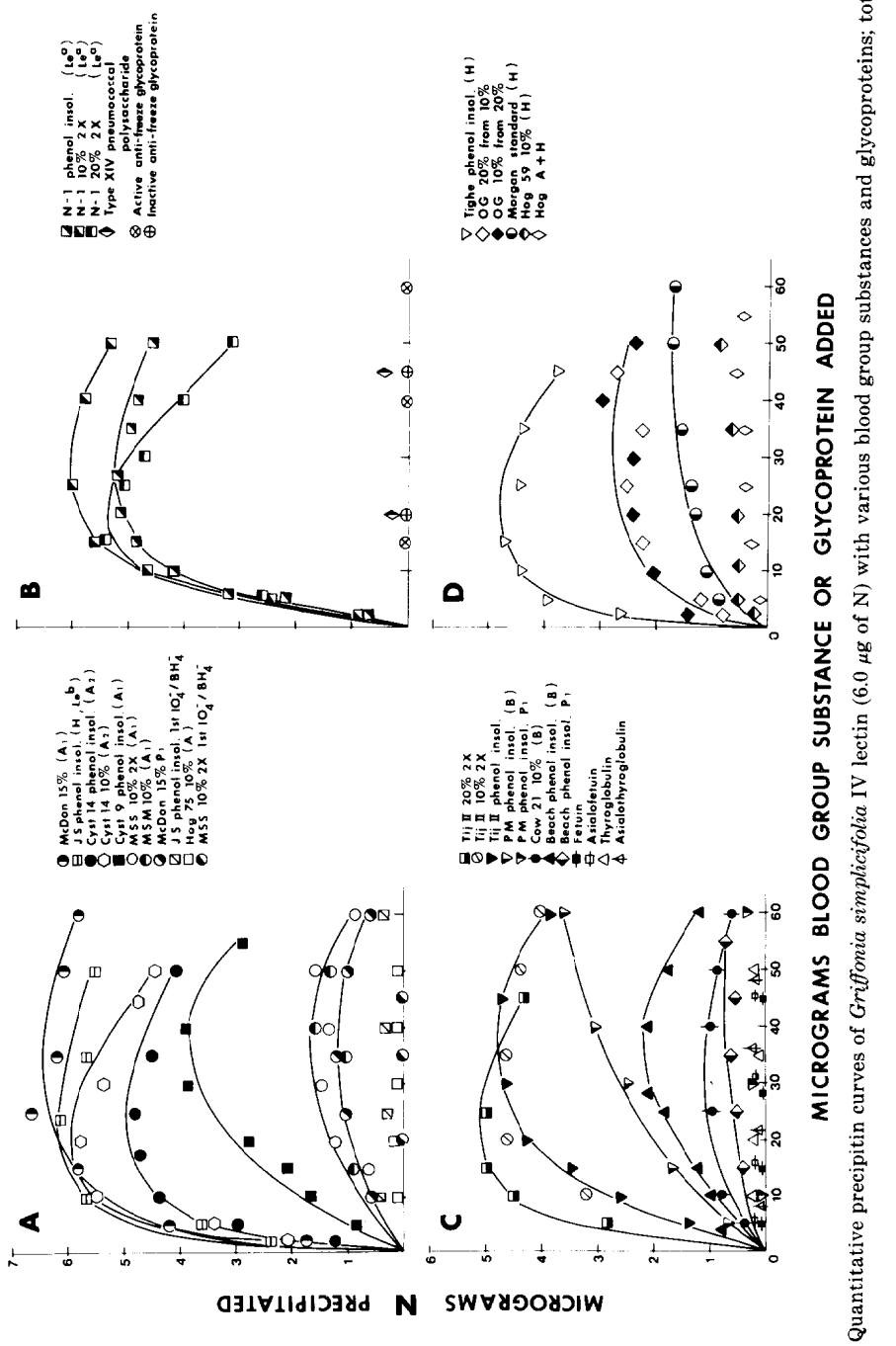


FIG. 1. Quantitative precipitin curves of *Griffonia simplicifolia* IV lectin (6.0 μg of N) with various blood group substances and glycoproteins; total volume 200 μl.

TABLE I

INHIBITORY ACTIVITIES OF BLOOD GROUP SUBSTANCES[a]

	Le^a	Le^b	A	Maximum N precipitated[b] (μg)
JS phenol insoluble, 0.96 mg/ml	1000	10,000	0	6.2
McDon 15%, 1.16 mg/ml	80	10,000	20	6.3
McDon 15% P1, 1.17 mg/ml	100	8,000	20	1.2
Cyst 14 phenol insoluble, 1.2 mg/ml	1000	10,000	<20	5.0
Cyst 14 10% ppt, 0.99 mg/ml	100	2,000	<20	5.7
Tighe phenol insoluble, 1.42 mg/ml	50	10,000	0	4.8
MSS 10% 2×, 1.0 mg/ml	100	1,000	20	1.6

[a] Highest dilution giving complete inhibition of agglutination of A, Le^a, Le^b erythrocytes by the corresponding blood group specific antiserum.

[b] From Fig. 1.

activity and was A active. Surprisingly, McDon 15% P1, which did not react appreciably with the lectin showed only slightly lowered Le^b activity and cyst 14 10% ppt, which had much less Le^b activity than cyst 14 phenol insoluble, precipitated strongly. Similarly the precipitating potency of these substances was unrelated to their Le^a activity.

Many of the blood group substances failed to precipitate all of the lectin N, as previously noted for other lectins and ascribed to differing solubilities of complexes of lectin with blood group substances (23-26). These differences were detected by determining the effect of increasing volume on the quantity of precipitate.

Figure 2 shows that precipitation of the lectin with cyst JS phenol insoluble (H,Le^b) is unaffected by increasing volume, while precipitation with cyst N-1 20% 2× (Le^a)

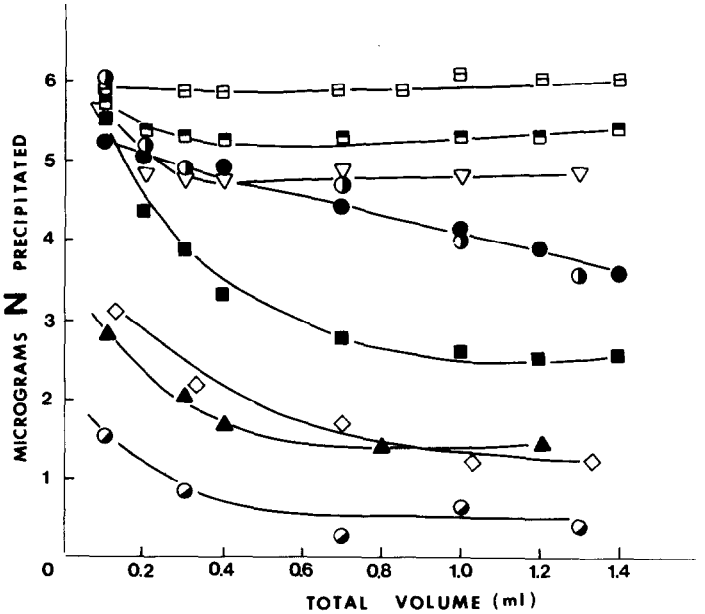


FIG. 2. Effect of volume on the precipitation of *Griffonia simplicifolia* IV lectin (6.0 μg of N) by blood group substances. Symbols are as shown on Fig. 1.

and cyst Tighe phenol insoluble (H) are affected slightly. The precipitates of the lectin with cyst McDon 15% (A_1) and cyst 14 phenol insoluble (A_2) are similar and show considerable solubility. Cyst 9 phenol insoluble (A_1) which reacts strongly with the lectin in small volumes gives a precipitate most of which is highly soluble. Cysts OG 20% from 10% (Ii), McDon 15% P1, and Beach phenol insoluble (B) react poorly with the lectin and substantial portions of their precipitates are highly soluble. In all instances except cyst 14 and McDon, complexes seem to reach a level at which more does not dissolve with increased volume.

Quantitative precipitin inhibition assays. The abilities of various monosaccharides, milk, and blood group oligosaccharides to inhibit precipitation of the GS-IV lectin by H,Le^b blood group substance cyst JS phenol insoluble are shown in Fig. 3. The structures of the oligosaccharides and amounts giving 50% inhibition are shown in Table II. The best inhibitor was an Le^b active oligosaccharide, lacto-*N*-difucohexaose I (line 1), 108 nmol giving 50% inhibition; all other compounds are compared to it. A different difucosyl oligosaccharide, lacto-*N*-difucohexaose II (line 2) inhibited 50% at 475 nmol, 4.4 times poorer than lacto-*N*-difucohexaose I. Since lacto-*N*-difucohexaose II was obtained from a nonsecreting individual (27), it cannot contain any of the most potent inhibitor lacto-*N*-difucohexaose I. Absence of the C-2 linked LFuc as in lacto-*N*-fucopentaose II (line 5) results in a 22-fold decrease in inhibitory activity. Lacto-*N*-fucopentaose I without the LFuc-linked $\alpha 1 \rightarrow 4$ to the subterminal DGlcNAc was inactive at 2000 nmol. Lactodifucotetraose (line 3) and lacto-*N*-difucotetraose (line 4) were similar, inhibiting 50% at 620 and 630 nmol, respectively, 5.2 times more than the best inhibitor being required. N-terminal DGalNAc, the immunodominant group of blood group A, linked $\alpha 1 \rightarrow 3$ to the DGal of lactodifucotetraose (urine A oligosaccharide), completely blocked the activity. The trisaccharide 2′-fucosyllactose was inactive at 600 nmol and 3 fucosyllactose gave 23% inhibition at 1600 nmol. LFuc$\alpha 1 \rightarrow$ 4DGlcNAc$\beta 1 \rightarrow O(CH_2)_8CO_2ME$ was not inhibitory at 398 nmol. The nonfucose-containing oligosaccharides lacto-*N*-tetraose, DGal$\beta 1 \rightarrow$ 4DGlcNAc, and DGal$\beta 1 \rightarrow$ 3DGlcNAc were also inactive over the range studied. Lactose (line 6) and LFuc (line 7) were inhibitory at high concentrations, 1.7×10^5 and 2×10^5 nmol, respectively, giving 42% inhibition. No significant inhibition was obtained with the following monosaccharides and glycosides:

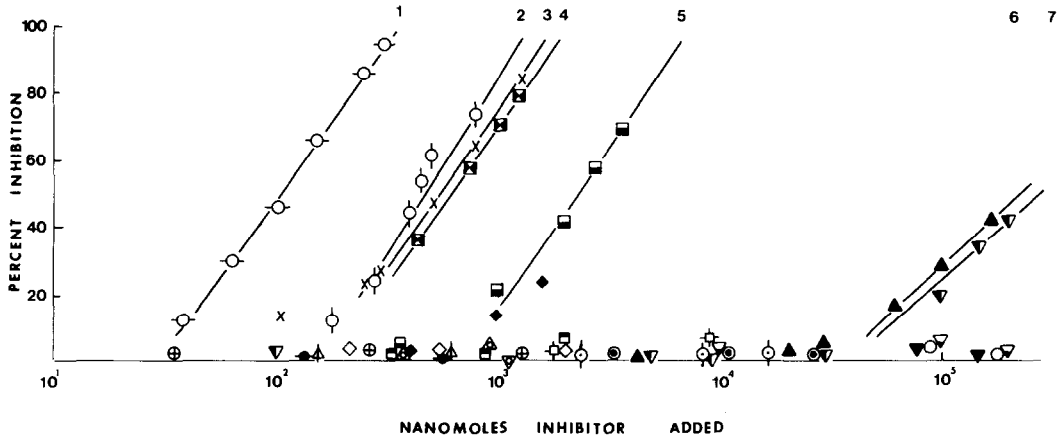


FIG. 3. Inhibition by glycosides and mono- and oligosaccharides of precipitation of *Griffonia simplicifolia* IV lectin (6.0 μg of N) with Le^b active substance JS phenol insoluble (12.5 μg). Total volume in all cases was 200 μl. Symbols are shown in Table II. The following monosaccharides and glycosides were not inhibitory: DGal, ▽; DGlc, ▼; *p*-nitrophenyl αLFuc, ⚱; methyl αLFuc, ⊙; DFuc, ○; DGalNAc, ⊹; and LRha, ⚲.

DGal (2×10^5 nmol), DGlc (1.5×10^5 nmol), *p*-nitrophenyl αLFuc (480 nmol), methyl αLFuc (2.7×10^4 nmol), DFuc (1.8×10^5 nmol), DGalNAc (9×10^3 nmol), and LRha (1.65×10^4 nmol).

DISCUSSION

In a previous quantitative precipitin study (3) of the reaction of GS-IV lectin with blood group active oligosaccharides coupled to BSA, the lectin precipitated best with conjugates of Le^b and H active blood group oligosaccharides to BSA and poorly with a conjugate of an Le^a oligosaccharide to BSA. From these findings and from inhibition data with various oligosaccharides, a difucosylated type structure was proposed as the most likely specific determinant. However, the lectin-agglutinated ficin treated group O erythrocytes equally regardless of their Le phenotype. Because of this, it seemed desirable to study the reactivity of the lectin with water soluble blood group A, B, H, Le^a, Le^b, and precursor I substances by quantitative precipitin asays and to define its combining site by inhibition of precipitation by various oligosaccharides.

The results presented show the combining site of the GS-IV lectin to be most specific for a blood group Le^b oligosaccharide, the best inhibitor of precipitation by an H,Le^b blood group substance being lacto-*N*-difucohexaose I,

$$\begin{matrix} \text{LFuc} & & \text{LFuc} \\ \alpha\downarrow^{1}_{2} & & \alpha\downarrow^{1}_{4} \end{matrix}$$
$$\text{DGal}\beta 1 \rightarrow 3\text{DGlcNAc}\beta 1 \rightarrow 3\text{DGal}\beta 1 \rightarrow 4\text{DGlc}.$$

The LFucα1 → 4 contributes relatively more to the binding than does the LFucα1 → 2 linkage in that although lacto-*N*-fucopentaose II, which lacks the LFucα1 → 2, inhibits 50% at 2,350 nmoles while this amount of lacto-*N*-fucopentaose I, in which the LFucα1 → 4 is missing, gives no inhibition. Lactodifucotetraose,

$$\begin{matrix} \text{LFuc} & & \text{LFuc} \\ \alpha\downarrow^{1}_{2} & & \alpha\downarrow^{1}_{3} \end{matrix}$$
$$\text{DGal}\beta 1 \rightarrow 4\text{DGlc},$$

and lacto-*N*-difucotetraose,

$$\begin{matrix} \text{LFuc} & & \text{LFuc} \\ \alpha\downarrow^{1}_{2} & & \alpha\downarrow^{1}_{3} \end{matrix}$$
$$\text{DGal}\beta 1 \rightarrow 4\text{DGlcNAc},$$

had identical activities 5.8 times less active than the best inhibitor, indicating that the β1 → 4 (type 2) structure reduces efficiency of binding. This is consistent with the finding that the multivalent Le^b-BSA and the

$$\begin{matrix} \text{LFuc} & & \text{LFuc} \\ \alpha\downarrow^{1}_{2} & & \alpha\downarrow^{1}_{2} \end{matrix}$$
$$\text{DGal}\beta 1 \rightarrow 4\text{DGlcNAc}\beta 1 \rightarrow \text{BSA}$$

were equally effective in precipitating the lectin (3). LFuc was more than 1800 times less active than the best inhibitor and no inhibition was obtained with methyl αLFuc and *p*-nitrophenyl αLFuc at the highest concentrations tested.

Lacto-*N*-difucohexaose II,

$$\begin{matrix} \text{LFuc} & & \text{LFuc} \\ \alpha\downarrow^{1}_{4} & & \alpha\downarrow^{1}_{3} \end{matrix}$$
$$\text{DGal}\beta 1 \rightarrow 3\text{DGlcNAc}\beta 1 \rightarrow 3\text{DGal}\beta 1 \rightarrow 4\text{DGlc},$$

was a surprisingly potent inhibitor of the lectin activity, with about one-fourth the activity of the best inhibitor, lacto-*N*-difucohexaose I, and five times better than lacto-*N*-fucopentaose I. This suggests that

TABLE II

STRUCTURES AND ACTIVITIES OF MILK AND BLOOD GROUP OLIGOSACCHARIDES REACTING WITH *Griffonia simplicifolia* IV LECTIN

Line No.	Symbol	Structure	Nanomoles for 50% inhibition	Symbol	Structure	Nanomoles for 50% inhibition
1	⊸	LFuc $\alpha1\downarrow^{1}_{2}$ LFuc $\alpha1\downarrow^{1}_{4}$ DGalβ1→ 3DGlcNAcβ1 → 3DGalβ1 → 4DGlc Lacto-*N*-difucohexaose I	108	⊕	LFuc $\alpha1\downarrow^{1}_{2}$ DGalβ1 → 4DGlcNAcβ1 → 6R JS R_L 0.75	No inhibition at 1300
2	ϕ	LFuc $\alpha1\downarrow^{1}_{4}$ LFuc $\alpha1\downarrow^{1}_{3}$ DGalβ1 → 3DGlcNAcβ1 → 3DGalβ1 → 4DGlc Lacto-*N*-difucohexaose II	475	⊸	LFuc $\alpha1\downarrow^{1}_{2}$ LFuc $\alpha1\downarrow^{1}_{3}$ DGalNAcα1 → 3DGalβ1 → 4DGal Urine A oligosaccharide	No inhibition at 585
3	X	LFuc $\alpha1\downarrow^{1}_{2}$ LFuc $\alpha1\downarrow^{1}_{3}$ DGalβ1 → 4DGlc Lactodifucotetraose	620	⍋	LFuc $\alpha1\downarrow^{1}_{2}$ DGalβ1 → 4DGlc 2′-fucosyllactose	No inhibition at 600
4	▪	LFuc $\alpha1\downarrow^{1}_{2}$ LFuc $\alpha1\downarrow^{1}_{3}$ DGalβ1 → 4DGlcNAc Lacto-*N*-difucotetraose	630	◆	LFuc $\alpha1\downarrow^{1}_{3}$ DGalβ1 → 4DGlc 3-fucosyllactose	26% inhibition at 1600
5	▫	LFuc $\alpha1\downarrow^{1}_{4}$ DGalβ1 → 3DGlcNAcβ1 → 3DGalβ1 → 4DGlc Lacto-*N*-fucopentaose II	2,350	◇	DGalβ1 → 3DGlcNAcβ1 → 3DGalβ1 → 4DGlc Lacto-*N*-tetraose	No inhibition at 2000
	▬	LFuc $\alpha1\downarrow^{1}_{2}$ DGalβ1 → 3DGlcNAcβ1 → 3DGalβ1 → 4DGlc Lacto-*N*-fucopentaose I	6% at 2,000	▲	DGalβ1 → 4DGlc Lactose	1.7×10^5 (42%)
				▽	LFuc	2.5×10^5 (43%)

the oligosaccharide may be able to assume a conformation in which the two LFuc residues are in an orientation resembling that in lacto-*N*-difucohexaose I or that the Le^b structure may ultimately prove not to be the best fit for the combining site or that the site has an as yet undetermined specificity but one in which both lacto-*N*-difucohexaose I and II can fit. Conformations of types 1 and 2 (DGalβ1 → 3DGlcNAc, DGalβ1 → 4DGlcNAc) H, Le^a, and Le^b oligosaccharides have been determined by NMR studies and by hard sphere molecular modelling based on X-ray crystallographic measurements (28–30). However, lacto-*N*-difucohexaose II has not been investigated and such studies might shed light on this question. These crystallographic and NMR studies (28–30) have shown that the most stable conformation in Le^b oligosaccharides of different sizes is influenced by the other glycosidic bonds in the molecule.

The results of inhibition of precipitation of the lectin with the H,Le^b active blood group substance, cyst JS phenol insoluble, are consistent with those obtained previously by inhibition of precipitation of the lectin with a synthetic Le^b oligosaccharide–BSA conjugate (3). In both systems, lacto-*N*-difucohexaose I was most active. Of the monofucosylated compounds, 3-fucosyllactose inhibited precipitation of the lectin with the Le^b oligosaccharide–BSA conjugate and was 17.6 times less active than the best inhibitor. In our quantitative precipitin assay, this would correspond to 1900 nmol for 50% inhibition, an amount of oligosaccharide which was not available.

Most blood group substances precipitated to some extent with the lectin. Substances of the same blood group specificity reacted quite differently, a finding ascribable to the heterogeneity of blood group glycoproteins resulting from incomplete biosynthesis (31–33). For example, blood group A substances would have different ability to precipitate with the lectin depending upon how many chains lacked the terminal nonreducing DGalNAc, the immunodominant blood group A determinant, giving H,Le^b determinants which could react with the lectin.

The quantitative precipitin and precipitin inhibition results show the lectin to react strongly with Le^b structures. However, the degree of Le^b activity of several blood group substances did not parallel this precipiting activity with the lectin. McDon 15% and McDon 15% P1 both had high Le^b activity, yet the lectin precipitated strongly with McDon 15% and weakly with McDon 15% P1. This reflects the fucose content of the substance, which comprises 20 and 3% by weight of McDon 15% and McDon 15% P1, respectively (34). Furthermore, cyst 14 10% and cyst 14 phenol insoluble, while differing significantly in their Le^b activity, both precipitated strongly. In a study of their composition (35) fucose comprised 14.7 and 10.9% by weight of cyst 14 10% and cyst 14 phenol insoluble, respectively, and hexosamine 23.1 and 26.2%. The lower hexosamine content of cyst 14 10% may allow the lectin greater accessibility to the slightly reduced number of fucose residues, allowing both substances to precipitate well. Thus reactivity with the lectin did not always reflect the Le^b activity of the carbohydrate. These data suggest the site may not have Le^b specificity.

Studies with other lectins have demonstrated that the solubility of specific precipitates is related to reactivity (23–26). The solubility profiles of the precipitates of the lectin with blood group substances reflects both the lectin binding site specificity and the heterogeneity of each blood group substance. In small volumes, the lectin is likely to precipitate with substances with weakly or strongly reactive determinants. With increasing volume interactions with weakly reactive determinants would dissociate and in the absence of other specific determinants, the precipitate would dissolve. It is thus interesting to note the differing slope and plateau levels of the solubility profiles.

The GS-IV lectin differs in specificity from the other LFuc binding lectins *Lotus tetragonolobus* and *Ulex europeus*. The *Lotus* lectin reacts best with monofucosyl type 2 (DGalβ1 → 4DGlcNAc) oligosaccharides (36) and *Ulex* lectin (37) with both type 1 (β1 → 3) and type 2 (β1 → 4) monofucosyl oligosaccharides, whereas GS-IV shows

specificity only for difucosylated compounds.

Since it was previously shown (3) that the lectin-agglutinated ficin treated type O erythrocytes independent of their Lewis activity, it was presumed to be reacting with non-Le^b difucosylated H structures on the erythrocytes. The findings presented provide additional indications that other difucosylated or even monofucosylated oligosaccharides other than those examined, not having the specific Le^b structure may be present on erythrocytes and that GS-IV may not be truly Le^b specific.

REFERENCES

1. WOOD, C., KABAT, E. A., MURPHY, L. E., AND GOLDSTEIN, I. J. (1979) *Arch. Biochem. Biophys.* **148,** 1-11.
2. WOOD, C., KABAT, E. A., EBISU, S., AND GOLDSTEIN, I. J. (1978) *Ann. Immunol. (Inst. Pasteur)* **129C,** 143-158.
3. SHIBATA, S., GOLDSTEIN, I. J., AND BAKER, D. A. (1982) *J. Biol. Chem.* **257,** 9324-9329.
4. RACE, R. R., AND SANGER, R. (1975) Blood Groups in Man, 6th ed., pp. 323-349, Blackwell, London.
5. BEISER, S. M., AND KABAT, E. A. (1952) *J. Immunol.* **68,** 19-40.
6. ALLEN, P. Z., AND KABAT, E. A. (1959) *J. Immunol.* **82,** 340-357.
7. VICARI, G., AND KABAT, E. A. (1969) *J. Immunol.* **102,** 821-825.
8. LLOYD, K. O., KABAT, E. A., AND LICERIO, E., (1968) *Biochemistry* **7,** 2976-2990.
9. MAISONROUGE-MCAULIFFE, F., AND KABAT, E. A. (1976) *Arch. Biochem. Biophys.* **175,** 1071-1080.
10. NEWMAN, W., AND KABAT, E. A. (1976) *Arch. Biochem. Biophys.* **175,** 510-523.
11. LLOYD, K. O., AND KABAT, E. A. (1968) *Proc. Nat. Acad. Sci. USA* **61,** 1470-1477.
12. FEIZI, T., KABAT, E. A., VICARI, G., ANDERSON, B., AND MARSH, W. L. (1971) *J. Exp. Med.* **133,** 39-52.
13. ALLEN, P. Z., AND KABAT, E. A. (1959) *J. Immunol.* **82,** 340-357.
14. SCHIFFMAN, G., KABAT, E. A., AND LESKOWITZ, S. (1962) *J. Amer. Chem. Soc.* **84,** 73-77.
15. CHEESE, I. A. F. L., AND MORGAN, W. T. J. (1962) *Nature (London)* **191,** 149-150.
16. LLOYD, K. O., KABAT, E. A., LAYUG, E. J., AND GRUEZO, F. G. (1966) *Biochemistry* **5,** 1489-1501.
17. ETZLER, M. E., ANDERSON, B., BEYCHOK, S., GRUEZO, F. G., LLOYD, K. O., RICHARDSON, N. G., AND KABAT, E. A. (1970) *Arch. Biochem. Biophys.* **141,** 588-601.
18. JACQUINET, J. C., AND SINAŸ, P. (1977) *J. Org. Chem.* **42,** 720-724.
19. GINSBURG, V. (1972) *Advan. Enzymol.* **36,** 131-149.
20. KABAT, E. A. (1961) Kabat and Mayer's Experimental Immunochemistry, 2nd ed., Thomas, Springfield, Ill.
21. SCHIFFMAN, G., KABAT, E. A., AND THOMPSON W. (1964) *Biochemistry* **3,** 113-120.
22. BAER, H., NAYLOR, I., GIBBEL, N., AND ROSENFIELD, R. E. (1959) *J. Immunol.* **82,** 183-189.
23. ETZLER, M. E., AND KABAT, E. A. (1970) *Biochem. J.* **9,** 869-877.
24. PEREIRA, M. E. A., SHARON, N., AND KABAT, E. A. (1974) *Carbohydr. Res.* **37,** 89-102.
25. WU, A. M., KABAT, E. A., GRUEZO, F. G., AND PORETZ, R. D. (1981) *Arch. Biochem. Biophys.* **209,** 191-203.
26. KALADAS, P. M., KABAT, E. A., KIMURA, A., AND ERSSON, B. (1981) *Mol. Immunol.* **18,** 969-977.
27. KOBATA, A., GROLLMAN, E. F., TORAIN, B. F., AND GINSBURG, V. (1970) *in* Blood and Tissue Antigens (Aminoff, D., ed.), pp. 497-504, Academic Press, New York.
28. ROSEVEAR, P. R., NUNEZ, H. A., AND BARKER, R. (1982) *Biochemistry* **21,** 1421-1431.
29. LEMIEUX, R. U., BOCK, K., DELBAERE, L. T. J., KOTO, S., AND RAO, V. S. (1980) *Canad. J. Chem.* **58,** 631-652.
30. RAO, V. S. R., AND BISWAS, M. (1981) *Biochem. Soc. Trans.* **9,** 508-510.
31. LLOYD, K. O., KABAT, E. A., AND LICERIO, E. (1968) *Biochemistry* **7,** 2976-2990.
32. VICARI, G., AND KABAT, E. A. (1969) *J. Immunol.* **102,** 821-825.
33. ROVIS, L., ANDERSON, B., KABAT, E. A., GRUEZO, F. G., AND LIAO, J. (1973) *Biochemistry* **12,** 5340-5359.
34. SCHIFFMAN, G., KABAT, E. A., AND THOMPSON, W. (1962) *J. Amer. Chem. Soc.* **84,** 463-467.
35. MORENO, C., LUNDBLAD, A., AND KABAT, E. A. (1971) *J. Exp. Med.* **134,** 439-457.
36. PEREIRA, M. E. A., AND KABAT, E. A. (1974) *Biochemistry* **13,** 3184-3192.
37. PEREIRA, M. E. A., KISAILUS, E. C., GRUEZO, F. G., AND KABAT, E. A. (1978) *Arch. Biochem. Biophys.* **185,** 108-115.