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IMMUNOCHEMICAL STUDIES ON THE SPECIFICITY OF SOYBEAN AGGLUTININ*†

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ABSTRACT

The specificity of the purified soybean agglutinin has been studied immunochemically by quantitative precipitin and quantitative precipitin inhibition assays. The lectin is precipitated by human A and Le^a blood-group substance, by the products of the second, third, fourth, and fifth stages of periodate oxidation of a human H blood-group substance (JS), and by precursor blood-group substances, as well as by a pig-submaxillary mucin having blood-group A activity, by partially hydrolyzed blood-group B substances (P1 fraction), and by group C streptococcal polysaccharide. The activity is attributable to terminal α-linked 2-acetamido-2-deoxy-D-galactopyranosyl or to α- or β-D-galactopyranosyl residues. The lectin did not precipitate with human blood-group H substances, with the product of the first stage of periodate oxidation (JS), with streptococcal group A polysaccharide, or with pig-submaxillary mucin devoid of blood-group A activity, and is poorly precipitated by blood-group B substances. Inhibition of precipitation with various monosaccharides indicated that the lectin is strongly specific for 2-acetamido-2-deoxy-D-galactose and for its oligosaccharides, and to a lesser extent for D-galactose and its oligosaccharides; the α-glycosides of both sugars were slightly more reactive than the β-glycosides of 2-acetamido-2-deoxy-D-galactose, and both α- and β-glycosides were more active than the free monosaccharides. Aromatic α- and β-glycosides of 2-acetamido-2-deoxy-D-galactose and D-galactose were better inhibitors than the corresponding methyl or ethyl compounds. The blood-group A trisaccharide α-D-GalNAc*p*-(1→3)-β-D-Gal*p*-(1→3)-D-GlcNAc was more active than the disaccharide

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α-D-GalNAc*p*-(1→3)-D-Gal and methyl α-D-galactopyranoside, both of which were equally inhibitory. Blood-group A and B reduced pentasaccharides that have a subterminal D-galactose residue substituted at O-2 with an α-L-fucopyranosyl residue alone or with an additional α-L-fucopyranosyl residue at O-3 of the 2-acetamido-2-deoxy-β-D-glucopyranosyl residue substantially lowered or eliminated inhibitory activity. The implications of these findings for the elucidation of the combining site of the lectin and to the structure of blood-group substances are discussed.

INTRODUCTION

Proteins or glycoproteins having the ability to agglutinate erythrocytes are found in many plants and invertebrates[1,2]. These hemagglutinins, known as lectins, bind to various mono- and oligo-saccharides, and specifically precipitate certain polysaccharides and glycoproteins[3–9]. Several hemaglutinins have been used as reagent for elucidating structures of oligosaccharides[10] and reduced oligosaccharides isolated from blood-group substances[11].

The soybean agglutinin (SBA), isolated in highly pure form from soybean oil meal[12–14], is a glycoprotein containing 4.5% of D-mannose and 1.2% of 2-acetamido-2-deoxy-D-glucose[13] and having a molecular weight of about 120,000 daltons. Polyacrylamide-gel electrophoresis and gel filtration[2,13], both in the presence of sodium dodecyl sulfate, showed SBA to be composed of four apparently similar subunits, each of molecular weight 30,000 ±500 daltons. By equilibrium dialysis[2,13], SBA was found to contain two identical binding sites for 2-acetamido-2-deoxy-D-galactose per 120,000 daltons, with $K^a = 3.0 \times 10^4$ l/mole. The SBA agglutinates erythrocytes nonspecifically and also agglutinates mouse, rat, and human cell lines transformed by viral or chemical carcinogens; agglutination, in all cases, is specifically inhibited by 2-acetamido-2-deoxy-D-galactose[15,16]. Transformed hamster cells are not agglutinated. The present study presents experimental data on the precipitin reactions of SBA with A, B, H, Le^a, Le^b, and precursor I blood-group substances, and on inhibition of precipitation by various mono- and oligo-saccharides and glycosides.

EXPERIMENTAL

Materials. — A highly purified SBA prepared, as described previously[14], was available.

Monosaccharides were obtained from Nutritional Biochemical (Cleveland, Ohio 44128) and Schwartz/Mann (Orangeburg, N.Y. 10962). The blood-group oligosaccharides used were those prepared and described earlier[10,17]. Milk and urine oligosaccharides were gifts from the late Prof. R. Kuhn and Dr. A. Gauhe, and from Dr. A. Lundblad[18], respectively; ethyl 2-acetamido-2-deoxy-β-D-galactopyranoside from Dr. R. W. Jeanloz; β-D-Gal*p*-(1→3)-D-GlcNAc and β-D-Gal*p*-(1→4)-D-GlcNAc from Dr. F. Zilliken (*cf.* Ref. 19), β-D-Gal*p*-(1→6)-D-GlcNAc from the late Prof. Kuhn, and allolactose from the late Prof. M. L. Wolfrom.

Streptococcal group A and C polysaccharides were provided by Dr. R. M. Krause[20], pig-submaxillary mucins by Dr. D. M. Carlson[21], and the human ovarian-cyst precursor-substance F1 by Prof. W. T. J. Morgan[22]. The blood-group substances used were those purified from human ovarian-cyst fluid or saliva, and from horse- or hog-gastric linings by digestion with pepsin, precipitation with ethanol, extraction with 90% phenol, and fractionation with ethanol[23–28]. The P1 fractions tested represent the nondialyzable portion of blood-group substances after hydrolysis for 2 h at 100° at pH 1.5 to 1.8, with the resultant removal of most of the α-L-fucosyl end groups[29] and some blood-group A or B active oligosaccharide side-chains[24,30,31]. Blood-group H substance (JS phenol insoluble) and its five products of sequential stages of degradation by periodate oxidation, reduction, and hydrolysis (Smith degradation) were previously described[32,33]; from their reactions with a mouse-myeloma protein specific for terminal β-linked 2-acetamido-2-deoxy-D-glucose, stages 1 and 3 contained terminal nonreducing 2-acetamido-2-deoxy-β-D-glucosyl residues, whereas stages 2, 4, and 5 did not, in accordance with the proposed, composite structure of the carbohydrate moiety of the blood-group substances[32,34].

Quantitative precipitin analyses. — The analyses with the purified SBA were performed with a microprecipitin technique[35]; SBA (9.1 μg of N) was mixed with different amounts of blood-group substances or other polysaccharides, the mixtures were incubated for 1 h at 37° and then kept for one week at 4°, unless otherwise stated. The N content in the washed precipitates was determined by the ninhydrin procedure[25]. For inhibition assays, known quantities of sugar were added to amounts of lectin and blood-group substance giving maximum precipitation.

RESULTS

The quantitative precipitin reactions of the purified SBA with A, Le^a, and Smith degraded human blood-group A_1 substances are shown in Fig. 1A. The four blood-group A_1 substances tested (MSM 10% ppt., MSS 10% ppt., McDonough 10% ppt., and Cyst 9) reacted equally well with the purified SBA in a typical precipitin curve, reaching a maximum of 6 μg of N of the 9.1 μg of N added. The N-1 10% 2×(Le^a) and the first stage of the Smith degradation of MSS also precipitated a maximum of 6 μg of N, but were only about 70 and 60%, respectively, as active as the A_1 blood-group substances, per unit weight, in precipitating the SBA; 10 and 12.5 μg, respectively were required to precipitate 50 percent of the maximum precipitable N, as compared with 6 μg for the blood-group A_1 substances. The blood-group A_2 substances, however, precipitated only a maximum of about 4 μg of N of the 9.1 μg of SBA N added, and were considerable less active than the other materials.

Figure 1B shows the precipitation of the SBA with the precursor blood-group substances with I activity (OG 20% 2x, OG 10% from 20%, OG 10% 2x, and F1) and with cow P1 substances (Cow 45 P1 and Cow 21 P1); all fall on a single curve showing them to be of equal potency. For 50% precipitation, 5 μg of each were needed.

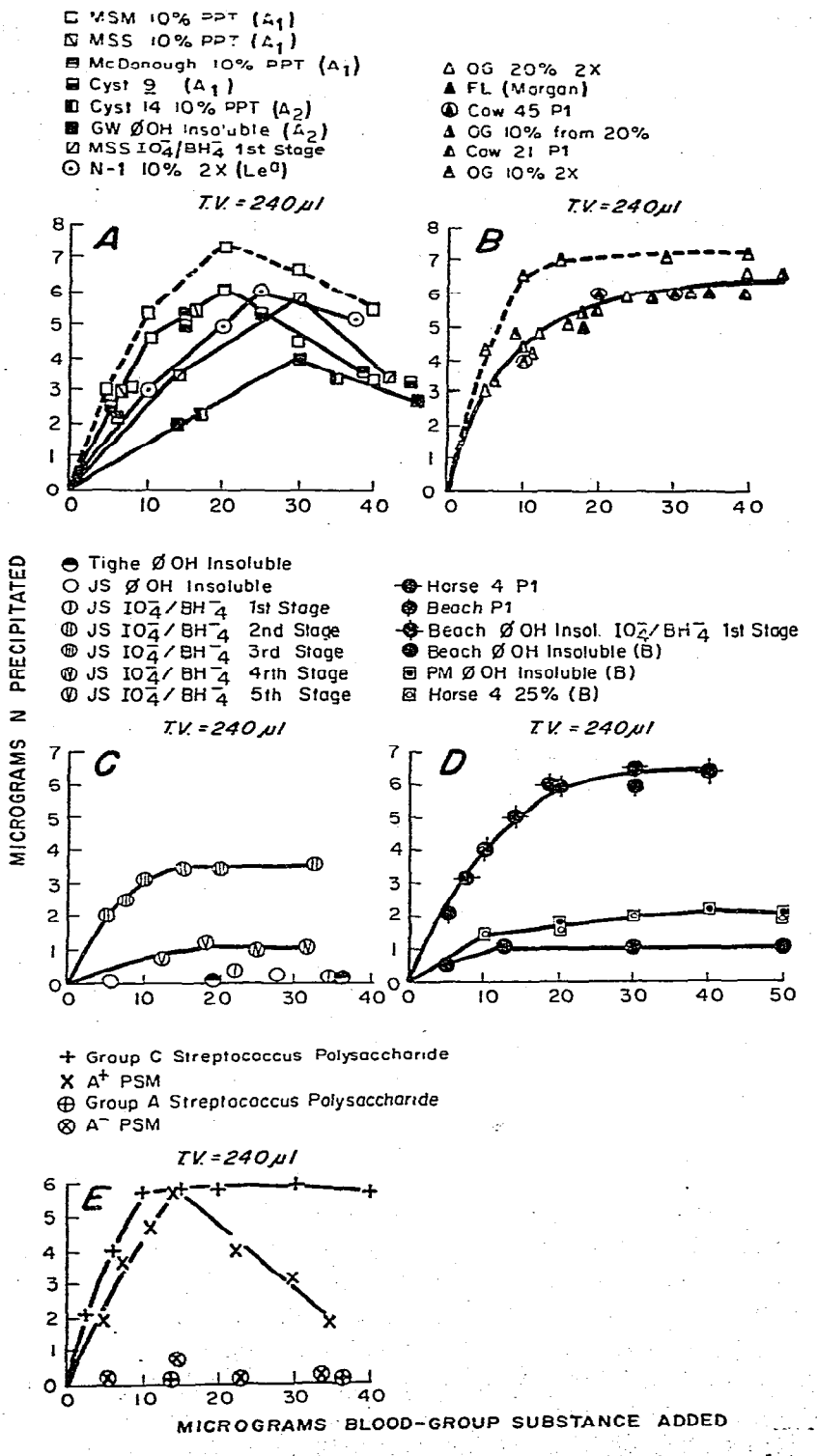

Fig. 1. Precipitation of purified SBA (9.1 µg of N) by blood-group substances and other polysaccharides. The dashed lines in A and B represent the precipitin reaction performed entirely at 0°. Total volume: 240 µl. IO_4^-/BH_A^-: Smith degradation.

The precipitation, with the SBA, of human blood-group H substances (JS phenol insoluble and Tighe phenol insoluble) and the stages of Smith degradation of blood-group H substance (JS) are shown in Fig. 1C. Precipitation of up to 3.5 μg of N occurred with the second and third stages of the Smith degradation of JS and both fell on the same curve. The H blood-group substances (JS phenol insoluble and Tighe phenol insoluble) and the first stage of the Smith degradation of JS did not precipitate, while the products of the fourth and fifth stages precipitated only 1.2 μg of N of the SBA.

The SBA reacted poorly with the blood-group B substances tested (Beach phenol insoluble, PM phenol insoluble, and horse 4 25%), as seen in Fig. 1D. However, the nondialyzable P1 fractions obtained after mild acid hydrolysis of the blood-group B substances (horse 4 P1 and Beach P1) and the Smith-degraded blood-group B substance (Beach phenol insoluble, first stage of Smith degradation) reacted equally well, giving a single precipitin curve and 6.5 μg of each precipitating 50% of the maximum.

The SBA was also precipitated by group C streptococcal polysaccharide, which has terminal nonreducing 2-acetamido-2-deoxy-α-D-galactopyranosyl residues linked to a rhamnose backbone[20], but did not precipitate group A polysaccharide, which has 2-acetamido-2-deoxy-β-D-glucopyranosyl residues[36] (Fig. 1E). In addition, it reacted with a pig submaxillary mucin containing blood-group A activity (A^+ PSM), but failed to precipitate a mucin devoid of A activity[21] (A^- PSM).

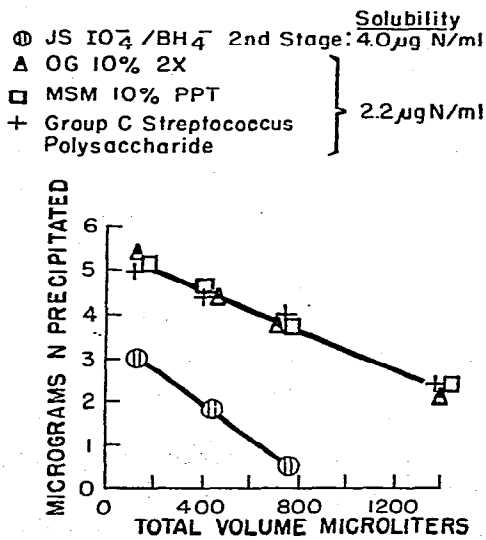

Fig. 2. Effect of volume on precipitation of the SBA (9.1 μg of N) by the product of the 2nd stage of Smith degradation of JS (20 μg), OG 10% 2x (21 μg), MSM 10% ppt. (20 μg), and group C streptococcus polysaccharide (11.6 μg).

If all of the added blood-group substance up to the point of maximum precipitation is assumed to be in the precipitate, then subtraction of the blood-group substance nitrogen added from the total nitrogen precipitated at equivalence shows that 50–60% of the purified SBA is precipitable by the blood-group A_1 and precursor substances, and by the group C streptococcus polysaccharide. However, the 2nd and 3rd stages of the Smith degradation of JS, which have a higher N content, precipitated only 27–30% of the SBA in solution. The failure to precipitate all of the SBA seems to be due to the formation of soluble complexes between the SBA and the polysaccharides. This is indicated by the high solubility of specific precipitates. Thus, the solubility (Fig. 2) of the precipitate with the 2nd stage of the Smith degradation of JS is 4.0 μg of N per ml, and that of the precipitate OG 10% 2x (precursor), MSM 10% ppt. (A_1), and blood-group C streptococcus polysaccharide is 2.2 μg of N per ml (Fig. 2). These solubilities are somewhat higher than those obtained for the precipitates of the purified *Lotus* lectin[37] with human H blood-group substance JS (1.2 μg of N per ml) or the *Dolichos* lectin[6] with human A blood-group substance MSM (1.4 μg of N per ml). Moreover 500 μl of the SBA solution (91 μg of N per ml) was absorbed at equivalence with 75 μg of the 2nd stage of the Smith degradation of JS, at 37° for 1 h and 4° for 1 week. The precipitate was centrifuged off and discarded; the absorbed supernatant failed to react with OG 10% 2x, MSM 10% ppt., group C streptococcus polysaccharide, and horse 4 P1 substance. In addition, if the quantitative precipitin reaction of the SBA with MSM 10% ppt and OG 10% 2x was performed entirely at 0° instead of 4° (see Experimental section), considerably more lectin nitrogen was precipitated (Fig. 1A and 1B, dashed lines); a maximum of 7.4 μg as compared with 6 μg of N.

The ability of several monosaccharides, glycosides, and oligosaccharides to inhibit the precipitation of the SBA with the precursor blood-group substance (OG 10% from 20%) is shown in Fig. 3. It is evident that 2-acetamido-2-deoxy-D-galactose, its glycosides, the A-active trisaccharide **1**,(A_5II, line 2) and disaccharide (**2**, R_L 1.85, line 3) (both having terminal 2-acetamido-2-deoxy-α-D-galactopyranosyl residues) were the best inhibitors. The blood-group A-active disaccharide, α-D-GalNAc*p*-(1→3)-D-Gal and methyl 2-acetamido-2-deoxy-α-D-galactopyranoside were equally active (line 3), on a molar basis, and four times more potent than 2-acetamido-2-deoxy-D-galactose. The blood-group A-active trisaccharide (**1**) (line 2) was more potent than the blood-group A-active disaccharide (**2**), 19 and 25nM concentrations, respectively, being required for 50 percent inhibition, as compared to 100nM for 2-acetamido-2-deoxy-D-galactose (line 5). The glycoside methyl 2-acetamido-2-deoxy-α-D-galactopyranoside was significantly a better inhibitor than the corresponding ethyl β-D-glycoside (line 4), 25 and 37nM concentrations, respectively, giving 50% inhibition; both were more reactive than 2-acetamido-2-deoxy-D-galactose. The most effective inhibitor was phenyl 2-acetamido-2-deoxy-α-D-galactopyranoside (line 1), which required a concentration of only 14nM for 50% inhibition and was about twice as active as the corresponding methyl α-D-glycoside (line 2) or the blood-group A-active disaccharide (**2**) (line 2). However, the mono-L-fucosyl A-penta-

α-D-GalNAc*p*-(1→3)-β-D-Gal*p*-(1→3)-D-GlcNAc

1

α-D-GalNAc*p*-(1→3)-D-Gal

2

α-L-Fuc*p*
1
↓
2
α-D-GalNAc*p*-(1→3)-β-D-Gal*p*-(1→4)-β-D-GlcNAc*p*-(1→6)-R

3

α-L-Fuc*p* α-L-Fuc*p*
1 1
↓ ↓
2 3
α-D-GalNAc*p*-(1→3)-β-D-Gal*p*-(1→4)-D-Glc

4

α-L-Fuc*p* α-L-Fuc*p*
1 1
↓ ↓
2 3
α-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→4)-β-D-GlcNAc*p*-(1→6)-R

5

α-L-Fuc*p* α-L-Fuc*p*
1 1
↓ ↓
2 3
α-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→4)-D-Glc

6

α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→4)-D-Glc

7

α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→4)-β-D-GlcNAc*p*-(1→6)-R

8

α-L-Fuc*p*
1
↓
3
α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→4)-D-Glc

9

α-L-Fuc*p* α-L-Fuc*p*
1 1
↓ ↓
2 4
α-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→3)-D-GlcNAc

10

α-L-Fuc*p* α-L-Fuc*p*
1 1
↓ ↓
2 3
α-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→4)-D-GlcNAc

11

β-D-Gal*p*-(1→3)-β-D-GlcNAc*p*-(1→3)-β-D-Gal*p*-(1→4)-D-Glc

12

R = 3-hexenetriols

saccharide, AR_L 0.52 (3) (line 7) was a much poorer inhibitor than **1** and **2** devoid of a L-fucose residue, and the urine A-pentasaccharide containing 2 L-fucosyl residues (4) (line 9) was even poorer, in concentration of 0.6 and 1.4μM, respectively, producing 50% inhibition.

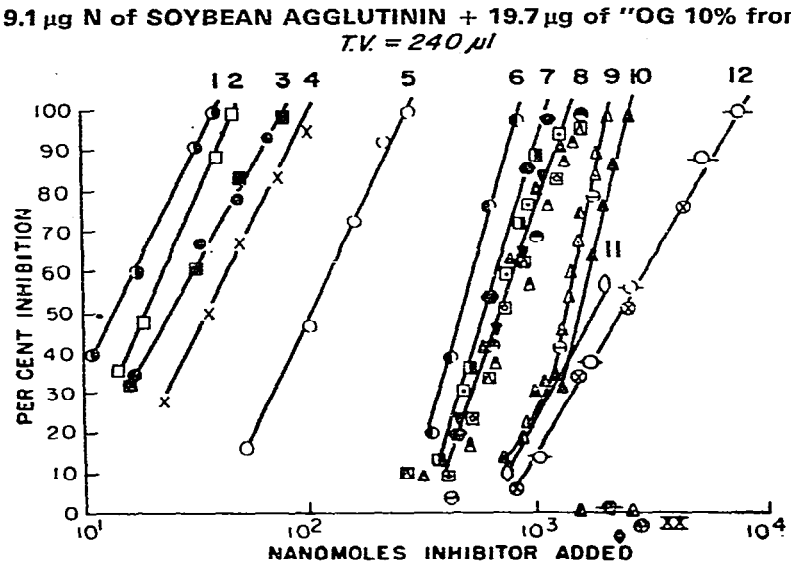

Fig. 3. Inhibition of precipitation of SBA with human blood-group precursor substance "OG 10% from 20%", by monosaccharides, glycosides, and oligosaccharides. Line 1: Phenyl 2-acetamido-2-deoxy-α-D-galactopyranoside (◑). Line 2: Trisaccharide **1** (A_5II) (□). Line 3: Disaccharide **2** (R_L 1.85) (■); methyl 2-acetamido-2-deoxy-α-D-galactopyranoside (●). Line 4: Ethyl 2-acetamido-2-deoxy-β-D-galactopyranoside (×). Line 5: 2-Acetamido-2-deoxy-D-galactose (○). Line 6: *p*-Nitrophenyl α-D-galactopyranoside (◐). Line 7: Pentasaccharide **3** (AR_L 0.52) (⬟); 6-*O*-(α-D-galactopyranosyl)-D-glucose (◨). Line 8: 6-*O*-(β-D-Galactopyranosyl)-D-glucose (▼); *p*-nitrophenyl β-D-galactopyranoside (◓); methyl α-D-galactopyranoside (◬); 3-*O*-(α-D-galactopyranosyl)-D-galactopyranosyl)-D-galactose (⊡); raffinose (⊡); stachyose (⊡); 2-acetamido-2-deoxy-6-*O*-(β-D-galactopyranosyl)-D-glucose (▲). Line 9: Methyl β-D-galactopyranoside (△); 2-acetamido-2-deoxy-4-*O*-(β-D-galactopyranosyl)-D-glucose (◭); 2-acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-D-glucose (◮); lacto-*N*-tetraose (**12**) (⊖); pentasaccharide **4** (Urine A oligosaccharide) (◬). Line 10: 4-*O*-(β-D-Galactopyranosyl)-D-glucose (◭). Line 11: Tetrasaccharide **8** (HJS R_L 0.75) (⬡). Line 12: D-Galactose (–○–); trisaccharide **7** (2-fucosyllactose) (⊗). Pentasaccharide **6** (Urine B oligosaccharide (–◓–); hexasaccharide **5** (Beach BR_{IM5} 1.2) (⊕); tetrasaccharide **9** (lactodifucotetraose) (◆); 4-*O*-(β-D-glucopyranosyl)-D-galactose (◬); 2-acetamido-2-deoxy-3-*O*-(β-D-glucopyranosyl-D-galactose (◬); 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactose (XX).

Among the glycosides of D-galactose, *p*-nitrophenyl α-D-galactopyranoside (line 6) and methyl α-D-galactopyranoside (line 8) appeared to inhibit better than the corresponding β-D-anomers (lines 8 and 9), concentrations of 0.48, 0.71, 0.71, and 1.4μM showing 50% inhibition, respectively. However, the *p*-nitrophenyl galactosides were more effective on a molar basis than the corresponding methyl galactosides; α-D-Gal*p*-(1→6)-D-Glc (line 7) was also a better inhibitor than β-D-Gal*p*-(1→6)-D-

Glc (line 8). If the β-D-galactopyranosyl residue is linked (1→6) to a D-glucose or 2-acetamido-2-deoxy-D-glucose residue, the resultant disaccharide is more reactive than the corresponding disaccharide having a (1→3) or a (1→4) linkage. Thus, β-D-Gal*p*-(1→6)-D-Glc (line 8) is more active than β-D-Gal*p*-(1→4)-D-Glc (line 10), concentrations of 0.7 and 1.6μM giving 50% inhibition, respectively. Similarly, β-D-Gal*p*-(1→6)-D-GlcNAc (line 8) gave 50% inhibition with a 0.7μM concentration, as compared with 1.4μM (line 9) for β-D-Gal*p*-(1→4)-D-GlcNAc, β-D-Gal*p*-(1→3)-D-GlcNAc, methyl β-D-galactopyranoside, and lacto-*N*-tetraose.

The blood-group B-active disaccharide, α-D-Gal*p*-(1→3)-D-Gal, was as inhibitory on a molar basis as methyl α-D-galactopyranoside, raffinose [α-D-Gal*p*-(1→6)-β-D-Glc*p*-(1→2)-β-D-Fru], and stachyose [α-D-Gal*p*-(1→6)-α-D-Gal*p*-(1→6)-β-D-Glc*p*-(1→2)-β-D-Fru]. However, the blood-group B-active oligosaccharide, Beach R_{IM5} 1.2-(5) and the urine B oligosaccharide (6), each having two L-fucosyl residues attached, were noninhibitory up to a concentration of 2.8μM. A similar blocking effect of L-fucosyl residue was also observed with lactose and its L-fucosyl derivatives, since the concentration needed for 50% inhibition of β-D-Gal*p*-(1→4)D-Glc is increased by the presence of an L-fucosyl residue at C-2 of the D-galactose residue (2′-fucosyllactose, 7) from 1.6 to 2.4μM. Indeed, the activity of 2′-fucosyllactose (7) was lowered to that of D-galactose. The blood-group H-active oligosaccharide, $HJSR_L$ 0.75 (8), was a slightly poorer inhibitor than lactose. The presence of an additional L-fucosyl residue in lactodifucotetraose (9) resulted in inactivity up to a 2.3μM concentration. For inhibitory activity of oligosaccharides, the 2-acetamido-2-deoxy-D-galactose and D-galactose residues had to be at the nonreducing end, since β-D-Glc*p*-(1→3)-D-GalNAc, β-D-Glc*p*-(1→4)-D-Gal, and β-D-GlcNAc*p*-(1→3)-D-Gal were inactive at a concentration as high as 3.6μM. Galactitol was also inactive. No significant inhibition was observed with the following sugars at the concentrations indicated: 2-acetamido-2-deoxy-D-glucose (5.6μM), D-glucose (6.8μM), D-mannose (4.5μM), 2-acetamido-2-deoxy-D-mannose (7.8μM), L-rhamnose (3.8μM), methyl α-L-fucopyranoside (4.6μM), methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (5.1μM), and methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (6.1μM).

DISCUSSION

The results of inhibition tests described in Fig. 3 clearly show that precipitation of SBA by human-cyst precursor substance (OG 10% from 20%) is best inhibited by 2-acetamido-2-deoxy-D-galactose and its glycosides, and by oligosaccharides containing this sugar as residue at the nonreducing end, and to a lesser extent by D-galactose and the corresponding derivatives (*cf.* Ref. 15). The SBA showed some slight preference for α-D over β-D linkages, as determined by inhibition with glycosides of 2-acetamido-2-deoxy-D-galactose and D-galactose, although both α- and β-glycosides of each sugar were better inhibitors, on a molar basis, than the corresponding monosaccharides.

Comparison of the specificity of SBA with other lectins is of interest. Thus,

precipitation by hog blood-group A+H substance of the two lectins isolated from *Ricinus communis*[38] was inhibited by D-galactose and 2-acetamido-2-deoxy-D-galactose, but this precipitation differed from that produced by SBA in that the amino sugar was the poorer inhibitor; one of the lectins, RCA_I, bound the β anomer of D-galactose derivatives preferentially, whereas the other lectin, RCA_{II}, bound both the α- and β-D-galactosides equally. Unlike the SBA, both RCA lectins reacted to a limited extent with L-rhamnose, L-arabinose, and D-fucose. The lectin from the snail *Helix pomatia*[5] is also highly specific for 2-acetamido-2-deoxy-α-D-galactopyranosyl residues but also differs from SBA in that ethyl 2-acetamido-2-deoxy-β-D-galactopyranoside was inactive, even at high concentration. Whereas the inhibition of precipitation of the *Helix pomatia* lectin by blood-group A substance was not inhibited by D-galactose, even at high concentrations, D-galactose and α-D-galactosides showed weak activity in inhibiting precipitation of the lectin by the core polysaccharide of *S. typhimurium*, which contains terminal nonreducing α-D-Gal*p*-(1→6)-D-Glc residues[39]. The snail agglutinin also differs from the SBA in that it is inhibited by 2-acetamido-2-deoxy-D-glucose and was precipitated by teichoic acids containing 2-acetamido-2-deoxy-α-D-glucopyranosyl residues linked to the ribitol phosphate backbone. The lectin of *Sophora japonica*[40] resembles the SBA most closely in the inhibition data reported; thus, both lectins are inhibited better by 2-acetamido-2-deoxy-D-galactose than by D-galactose, the methyl α- and β-D-galactopyranosides are both more active than free D-galactose, and neither lectin is precipitated by human H substance. The lectins, however, have very different specificities; β-D-galactosides were more active than α-D-galactosides with the *Sophora japonica* lectin and this lectin precipitated with human blood-group B substance better than with blood-group A substance. Unlike the SBA, the *Sophora japonica* lectin does not agglutinate transformed cells[41]. Of special interest is the observation that the phenyl or nitrophenyl α- and β-D-glycosides were better inhibitors, on a molar basis, than the corresponding methyl and ethyl glycosides with SBA, concanavalin A[42], and the *Sophora japonica* lectin[40]. Indeed, the best inhibitor of precipitation of SBA, phenyl 2-acetamido-2-deoxy-α-D-galactopyranoside (Fig. 3, line 1), is even more reactive than the trisaccharide **1**, and this might reflect an apolar interaction between the aglycone part of the glycoside and the corresponding region on the hemagglutinin, as was proposed for concanavalin A[43].

Inhibition of precipitation of soybean agglutinin by human precursor substance showed the blood-group A-active trisaccharide **1** to be approximately twice as active as the disaccharide **2** and methyl 2-acetamido-2-deoxy-α-D-galactopyranoside, and about five times better than 2-acetamido-2-deoxy-D-galactose. Whether the increased potency of the A trisaccharide **1**, relative to the disaccharide **2**, is ascribable to a more favorable conformation of the 2-acetamido-2-deoxy-D-galactose residue, linked (1→3) to the D-galactose residue, in the trisaccharide than in the disaccharide, or to a difference in the size of the combining region, is not clear.

Although 2-acetamido-2-deoxy-D-galactose was about 25 times more reactive than D-galactose toward the SBA, the lectin precipitated blood-group substances

having a terminal 2-acetamido-2-deoxy-α-D-galactopyranosyl residue as well as those having a terminal β-D-galactopyranosyl residue. Thus, the human blood-group A_1 substances tested, which have a terminal, immunodominant nonreducing 2-acetamido-2-deoxy-α-D-galactopyranosyl residue[44,45], are as active as the precursor blood-group substances, which possess a terminal β-D-galactopyranosyl residue[46] (Figs. 1A and 1B). The lectin is also precipitated by blood-group Lewis substance, which also contains a terminal β-D-galactopyranosyl residue[44,45].

A surprising finding is the poor precipitability of SBA with the human blood-group B substances used, since an α-D-galactopyranosyl residue is the immunodominant group of these substances[44,45]. Furthermore, the B-active disaccharide, α-D-Gal*p*-(1→3)-D-Gal, is about four times more active than D-galactose. However, the blood-group B-active oligosaccharides, Beach $R_{IM5}1.2$ (**5**) and urine B (**6**), each having two L-fucosyl residues, did not inhibit the precipitation of SBA when used at concentrations higher than those giving 100% inhibition with α-D-Gal*p*-(1→3)-D-Gal and 50% with D-galactose. The structures of the oligosaccharides isolated from blood-group substances by alkaline borohydride degradation suggested a composite structure[32] for the complex oligosaccharide moiety of blood-group A, B, H, Le^a, Le^b, and precursor substances. The blood-group B specific determinants were of two types (**10** and **11**). The α-L-fucosyl residues evidently block the reaction of the α-D-galactopyranosyl residues, which accounts for the poor reactivity of the blood-group B substances. Precipitation of the SBA by blood-group B substances was considerably increased (Fig. 1D) after mild acid hydrolysis (P1 fractions), which split off L-fucose and certain oligosaccharides[29,30], and exposed the D-galactopyranosyl residues. The activities of the mono- and di-fucosyl blood-group A oligosaccharides, AR_L 0.52 (**3**) and urine A (**4**) (lines 7 and 9, Fig. 3), are considerably lower than that of A_5II (**1**) (line 2, Fig. 3), a blood-group A-active trisaccharide devoid of L-fucose, which indicates that the steric block created by the substitution of one or two L-fucosyl residues has much less effect on the more strongly reacting 2-acetamido-2-deoxy-α-D-galactopyranosyl residue than on the more weakly binding α-D-galactopyranosyl residue.

The SBA was not precipitated by the human blood-group H substances tested, and the precipitation of the lectin with the precursor substance OG was not inhibited by lactodifucotetraose (**9**) (a blood-group H-active difucosyltetrasaccharide), and poorly inhibited by 2′-fucosyllactose (**7**) (line 12) and H JSR_L 0.75 (**8**) (line 11) (two blood-group H-active monofucosyl oligosaccharides). 2′-Fucosyllactose (**7**) was as active as D-galactose, and H JSR_L 0.75 was somewhat more active, which indicates that substitution at C-2 of a terminal D-galactopyranosyl residue does not completely prevent its access to the site.

Substitution of an L-fucosyl residue at O-2 of the subterminal D-galactopyranosyl residue of the blood-group A trisaccharide A_5II (**1**) (line 2) (giving the tetrasaccharide AR_L 0.52 (**3**) (line 7) increased the concentration for 50% inhibition from 19 to 710nM, whereas a similar substitution to give the blood-group H oligosaccharide HR_L 0.75 (**8**) (line 11) only modifies slightly the concentration needed for 50%

inhibition to 1.8μM, as compared with 1.55μM for the unsubstituted disaccharide, β-D-Gal*p*-(1→4)-D-GlcNAc and for methyl β-D-galactopyranoside (line 9). Thus, substitution of a L-fucosyl residue on the subterminal D-galactosyl residue has a much greater effect on the reactivity of a terminal 2-acetamido-2-deoxy-α-D-galactopyranosyl or α-D-galactopyranosyl residue than it does on the residue onto which it is attached. Such findings imply that the 2-fucosyl residue, by its bulk, blocks sterically the reactive surface of the terminal 2-acetamido-2-deoxy-D-galactopyranosyl or D-galactopyranosyl residues, whereas its linkage to O-2 of the subterminal β-D-galactopyranosyl residue permits access to the site. Molecular models show this interpretation to be reasonable[45], and it is evident that an α-L-(1→2) substitution would have the least effect on immunodominant, terminal nonreducing residues. The ability of a D-galactopyranosyl residue substituted at C-2 to function as an immunodominant group was noted by Heidelberger and Rebers[47] with respect to the cross-precipitin reaction of SVI and SXIV with their respective antisera. The substitution of two L-fucosyl residues blocks reactivity completely, and thus accounts for the failure of the two blood-group H substances to react with the SBA.

The reaction of the blood-group H substance and of its five sequential periodate-oxidation stages (Smith degradation) is of considerable interest. In an earlier study[33], the original JS and the second, fourth, and fifth stages did not react with a mouse myeloma antibody specific for terminal 2-acetamido-2-deoxy-β-D-galactopyranosyl residues, whereas the first and third stages did react, the latter having fewer terminal 2-acetamido-2-deoxy-β-D-glucopyranosyl residues, and larger amounts were required for precipitation; these findings agree well with the composite structure for the carbohydrate moiety of the blood-group substances[32,34,45].

Since the SBA reacts with terminal nonreducing D-galactopyranosyl and 2-acetamido-2-deoxy-D-galactopyranosyl residues, one would have expected the products of the second, fourth, and fifth stages of the Smith degradation to precipitate the lectin while the original JS, and the first and third stages would be negative. Indeed, the original JS and the product of the first stage did not precipitate, that of the second stage reacted well, and those of the fourth and fifth stages reacted, but much less strongly, all as expected. However, the product of the third stage gave a precipitin curve identical to that of the product of the second stage. This finding is understandable in view of the considerable degree of heterogeneity of the carbohydrate moiety of the blood-group substances, as shown by the recent isolation, after alkaline borohydride degradation by the method of Iyer and Carlson[48], of many oligosaccharides having different structures[49] and chain-lengths. Thus, whereas almost every oligosaccharide isolated would have exposed, terminal 2-acetamido-2-deoxy-β-D-glucopyranosyl residues after the first stage of degradation and, thus, would not be expected to react with the SBA as observed, several of the blood-group H oligosaccharides, such as JS R_{IM5} 1.04 and JS R_{IM5} 2.35a, would, after three stages of degradation, have exposed, terminal 2-acetamido-2-deoxy-α-D-galactopyranosyl residues linked to serine or threonine residues, which would be expected to react with the SBA. It is evident that a detailed exploration of the specificities of various

lectins by the use of precipitation with polysaccharides, as well as inhibition reactions, is essential to the understanding of their reactivity with cell-surface receptors.

REFERENCES

1 N. SHARON AND H. LIS, *Science*, 117 (1972) 949–959.
2 H. LIS AND N. SHARON, *Ann. Rev. Biochem.*, 42 (1973) 541–574.
3 O. MÄKELA, *Ann. Med. Exp. Biol. Fenn.*, 35 (1957) supp. 11.
4 E. E. SMITH AND I. J. GOLDSTEIN, *Arch. Biochem. Biophys.*, 121 (1967) 88–95.
5 S. HAMMARSTRÖM AND E. A. KABAT, *Biochemistry*, 8 (1969) 2696–2705.
6 M. E. ETZLER AND E. A. KABAT, *Biochemistry*, 9 (1970) 869–877.
7 V. HARISDANGKUL, E. A. KABAT, R. J. MCDONOUGH, AND M. M. SIGEL, *J. Immunol.*, 5 (1972) 1244–1258.
8 V. HARISDANGKUL, E. A. KABAT, R. J. MCDONOUGH, AND M. M. SIGEL, *J. Immunol.*, 5 (1972) 1259–1270.
9 M. E. A. PEREIRA AND E. A. KABAT, *Ann. N.Y. Acad. Sci.*, (1974) in press.
10 M. E. ETZLER, B. ANDERSON, S. BEYCHOK, F. GRUEZO, K. O. LLOYD, N. G. RICHARDSON, AND E. A. KABAT, *Arch. Biochem. Biophys.*, 141 (1970) 588–601.
11 L. ROVIS, E. A. KABAT, M. E. A. PEREIRA, AND T. FEIZI, *Biochemistry*, 12 (1973) 5355–5360.
12 S. WADA, M. J. PALLANSCH, AND I. E. LIENER, *J. Biol. Chem.*, 233 (1958) 395–405.
13 H. LIS, N. SHARON, AND E. KATCHALSKI, *J. Biol. Chem.*, 241 (1966) 684–689; R. LOTAN, H. W. SIEGELMAN, H. LIS, AND N. SHARON, *ibid.*, in press.
14 J. A. GORDON, S. BLUMBERG, H. LIS, AND N. SHARON, *FEBS Lett.*, 24 (1972) 193–196.
15 H. LIS, B. SELA, L. SACHS, AND N. SHARON, *Biochim. Biophys. Acta*, 211 (1970) 582–585.
16 B. SELA, H. LIS, N. SHARON, AND L. SACHS, *J. Membr. Biol.*, 3 (1970) 267–279.
17 K. O. LLOYD, E. A. KABAT, E. J. LAYUG, AND F. GRUEZO, *Biochemistry*, 5 (1966) 1489–1501.
18 A. LUNDBLAD, *Biochim. Biophys. Acta*, 148 (1967) 151–157.
19 E. A. KABAT, *Arch. Biochem. Biophys.*, (Supl. 1) (1962) 181–186.
20 R. M. KRAUSE AND M. MCCARTY, *J. Exp. Med.*, 115 (1962) 49–62.
21 D. M. CARLSON, *J. Biol. Chem.*, 243 (1968) 616–626.
22 W. T. J. MORGAN, *Proc. Roy. Soc., Ser. B*, 151 (1960) 308–312.
23 E. A. KABAT, *Blood Group Substances. Their Chemistry and Immunochemistry*, Academic Press, New York, 1956, pp. 135–139.
24 P. Z. ALLEN AND E. A. KABAT, *J. Immunol.*, 82 (1959) 340–357.
25 G. SCHIFFMAN, E. A. KABAT, AND W. THOMPSON, *Biochemistry*, 3 (1964) 113–120.
26 G. VICARI AND E. A. KABAT, *J. Immunol.*, 102 (1969) 821–825.
27 K. O. LLOYD, E. A. KABAT, AND E. LICERIO, *Biochemistry*, 7 (1968) 2976–2990.
28 E. A. KABAT, A. BENDICH, A. E. BEZER, AND S. M. BEISER, *J. Exp. Med.*, 85 (1947) 685–699.
29 E. A. KABAT, H. BAER, A. E. BEZER, AND V. KNAUB, *J. Exp. Med.*, 88 (1948) 43–57.
30 S. LESKOWITZ AND E. A. KABAT, *J. Amer. Chem. Soc.*, 76 (1954) 5060–5065.
31 P. Z. ALLEN AND E. A. KABAT, *J. Immunol.*, 82 (1959) 358–372.
32 K. O. LLOYD AND E. A. KABAT, *Proc. Nat. Acad. Sci. U.S.*, 61 (1968) 1470–1477.
33 G. VICARI, A. SHER, M. COHN, AND E. A. KABAT, *Immunochemistry*, 7 (1970) 829–838.
34 E. A. KABAT, in D. AMINOFF (Ed.), *Blood and Tissue Antigens*, Academic Press, New York, 1970, pp. 187–198.
35 E. A. KABAT, *Kabat and Mayer's Experimental Immunochemistry*, 2nd ed., Thomas, Springfield, III, 1961, pp. 29–96.
36 R. M. KRAUSE AND M. MCCARTY, *J. Exp. Med.*, 114 (1961) 127–140.
37 M. E. A. PEREIRA AND E. A. KABAT, unpublished results.
38 G. L. NICHOLSON, J. BLAUSTEIN, AND M. E. ETZLER, *Biochemistry*, 13 (1974) 196–204.
39 S. HAMMARSTRÖM, A. A. LINDBERG, AND E. S. ROBERTSON, *Eur. J. Biochem.*, 25 (1972) 274–282.
40 R. D. PORETZ, H. RISS, J. W. TIMBERLAKE, AND S. CHIEN, *Biochemistry*, 13 (1974) 250–256.
41 M. TOMITA, T. KUROKAWA, T. OSAWA, Y. SAKURAI, AND T. UYKITA, *Gann*, 63 (1972) 269–271.
42 R. D. PORETZ AND I. J. GOLDSTEIN, *Biochem. Pharmacol.*, 20 (1971) 2727–2739.
43 R. D. PORETZ AND I. J. GOLDSTEIN, *Biochemistry*, 9 (1970) 2890–2896.

44 W. M. WATKINS, in A. GOTTSCHALK (Ed.), *Glycoproteins*, 2nd ed., Elsevier, Amsterdam, 1972, pp. 886–891.
45 E. A. KABAT, in H. S. ISBELL (Ed.), *Carbohydrates in Solution*, American Chemical Society, Washington D. C., 1973, p. 334.
45 E. A. KABAT, *Carbohydrates in Solution*, American Chemical Society, Washington, 1973, p. 334.
46 G. VICARI AND E. A. KABAT, *Biochemistry*, 9 (1970) 3414–3421.
47 M. HEIDELBERGER AND P. A. REBERS, *J. Bacteriol.*, 80 (1960) 145–153.
48 R. H. IYER AND D. M. CARLSON, *Arch. Biochem. Biophys.*, 142 (1970) 101–105.
49 L. ROVIS, B. ANDERSON, E. A. KABAT, F. GRUEZO, AND J. LIAO, *Biochemistry*, 12 (1973) 5340–5354.