Carbohydrate Research, 51 (1976) 107–118

IMMUNOCHEMICAL STUDIES ON THE SPECIFICITY OF THE PEANUT (*Arachis hypogaea*) AGGLUTININ*

MIERCIO E. A. PEREIRA, ELVIN A. KABAT, REUBEN LOTAN**, AND NATHAN SHARON

Departments of Microbiology, Human Genetics and Development and Neurology, Columbia University, the Neurological Institute, Presbyterian Hospital, New York 10032 (U. S. A.); and the Department of Biophysics, Weizmann Institute of Science, Rehovoth (Israel)

(Received May 7th, 1976; accepted for publication, June 14th, 1976)

ABSTRACT

The specificity of purified, peanut agglutinin has been studied immunochemically by quantitative precipitin and inhibition assays. The lectin showed substantial differences in precipitating with blood-group substances of the same specificity. Of the B substances tested, horse 4 25% completely precipitated the lectin, Beach phenol insoluble failed to interact, and PM phenol insoluble gave an intermediate reaction. The lectin did not precipitate with A_1 substances, with hog gastric mucin A+H substance, or with A_2 substance WG phenol insoluble. Another A_2 substance, cyst 14 phenol insoluble, precipitated ~2/3 of the lectin. Of the H substances, Tighe phenol insoluble was inactive, JS phenol insoluble precipitated poorly, and Morgan standard H precipitated about 80% of the lectin. However, first stage of Smith degradation, as well as PI fractions obtained by mild acid hydrolysis of blood-group substances, gave products which precipitated strongly. The lectin was also completely precipitated by all precursor blood-group substances, as well as by cows 21 and 26, all having strong I-Ma, I-Ort, I-Step, and I-Da activities. Cow 18, which does not possess significant blood-group I activity, precipitated very slightly. Fractions of blood-group substances N-1 (Le^a) and Tij (B) obtained by precipitation from 90 percent phenol at higher concentrations of ethanol interacted better with peanut agglutinin. These differences in activity are ascribable to a heterogeneity resulting from incomplete biosynthesis of carbohydrate side-chains of blood-group substances, particularly resulting in variations in the numbers of DGalβ1→3DGalNAc or DGalβ1→4DGlcNAc determinants. The agglutinin reacted with the hydatid cyst P_1 glycoprotein, as well as with the previously studied antifreeze and sialic acid-free α1 acid glycoproteins, but not with pneumococcus type XIV polysaccharide. Inhibition of precipitation showed the lectin to be most specific for the disaccharide DGalβ1→3DGalNAc, which is 14, 55, and 90 times as active as DGalβ1→4DGlcNAc, DGal, and DGalβ1→3DGlcNAc, respectively. DGalβ1→3*N*-

*Aided by grants from the National Science Foundation BMS-72-02219A02 and A03, a General Research Support Grant from the United States Public Health Service, and a grant from the United States–Israel Binational Science Foundation, Jerusalem, Israel.

**Department of Cancer Biology, the Salk Institute for Biological Studies, San Diego, Ca. 92122, U. S. A.

acetyl-D-galactosaminitol has ~1/25 th the activity of DGalβ1→3DGalNAc. Substitutions of DGlcNAc or LFuc on the DGal of active inhibitors completely blocked the activity, in line with the assumption that the combining site of the peanut lectin is a partial cavity. The oligosaccharides DGalβ1→4DGlcNAcβ1→6-hexane-1,2,4,5,6-pentol(s) and DGalβ1→3[DGalβ1→4DGlcNAcβ1→6]*N*-acetyl-D-galacto-saminitol showed the same inhibitory activity as DGalβ1→4DGlcNAc, suggesting that the combining site of the peanut agglutinin may not be complementary to more than a disaccharide. Whereas precipitin reactions of peanut agglutinin resemble those of anti-I and anti-i, DGalβ1→3DGalNAc did not inhibit anti-I Ma, anti-I Step, or anti-i Den, nor were these antibodies precipitated by the antifreeze glycoprotein.

INTRODUCTION

The presence in crude extracts of peanut (*Arachis hypogaea*) of an activity that agglutinates neuraminidase-treated cells was first observed by Bird[1]. The active material has been purified by affinity chromatography on Sepharose-*N*-(6-amino-1-oxohexyl-β-D-galactopyranosylamine, and is a noncarbohydrate-containing protein of molecular weight 110,000, consisting[2] of four identical, noncovalently linked subunits of mol. wt. 27,500. As with some other lectins (*cf.*, ref. 3), the peanut agglutinin (PNA) is devoid of cysteine and low in lysine and histidine, but rich in acidic and hydroxy amino acids[2]. The purified lectin agglutinates neuraminidase-treated human erythrocytes, irrespective of their ABO blood-groups, and binds to neuraminidase-treated lymphocytes of human, rat, mouse, and guinea-pig, although it will stimulate DNA synthesis only in human and rat lymphocytes[4]. Because its activity resembles that of the naturally occurring "T antibody", which detects a heterophile antigen on neuraminidase-treated erythrocytes[5], the peanut agglutinin has been called[1,6] T-agglutinin. It is of clinical use in diagnosis of the so-called, acquired T-polyagglutination[7] accompanying certain bacterial and viral infections that produce neuraminidase[8,9]. In the present study, results are given on the precipitin reactions of PNA with A, B, HLe^b, Le^a, and I precursor blood-group substances, and with other glycoproteins and bacterial polysaccharides. Inhibition of precipitation by various glycosides and mono- and oligo-saccharides was assayed, to provide insight into the specificity of the binding site of the lectin.

MATERIALS AND METHODS*

Peanut agglutinin was purified as described previously[2]. Monosaccharides were obtained from Nutritional Biochemicals (Cleveland, Ohio 44128) and Schwartz/Mann

*Abbreviations used: DGal, D-galactopyranose; DGlc, D-glucopyranose; DMan, D-mannopyranose; Fru, fructofuranose; LFuc, L-fucopyranose; L-Rha, L-rhamnopyranose; DGlcNAc, 2-acetamido-2-deoxy-D-glucopyranose; DGalNAc, 2-acetamido-2-deoxy-D-galactopyranose; D-ManNAc, 2-acetamido-2-deoxy-D-mannopyranose; $GalNH_2$, 2-amino-2-deoxy-D-galactopyranose; methyl β-DGlcNAc, methyl 2-acetamido-2-deoxy-β-D-glucopyranoside; methyl α-DGalNAc, methyl 2-acetamido-2-deoxy-α-D-galactopyranoside; Ala, alanine; Thr, threonine; R, 3-hexenetetrol(s); and PNA, peanut agglutinin.

(Orangeburg. N.Y. 10962). The blood-group oligosaccharides used were those described earlier[10–12]. Milk oligosaccharides were gifts from the late Prof. R. Kuhn and Dr. A. Gauhe, DGalβ1 → 3DGlcNAc and DGalβ1 → 4DGlcNAc from Dr. F. Zilliken (*cf.*, ref. 13), and DGalβ1 → 3DGalNAc from Prof. W. Gielen[14].

The purified blood-group substances used were from human ovarian-cyst fluid or saliva, and from horse, cow, or hog gastric mucosae[15–20]. The PI fractions represent the nondialyzable portion of the blood-group substances after mild hydrolysis at pH 1.5 to 2.0 for 2 h, which removed most of the α-L-fucosyl end-groups as well as some blood-group A or B active oligosaccharide side-chains[17,21,22]. Blood-group H substance (JS phenol insoluble) and its products of five sequential stages of degradation by periodate oxidation, reduction, and hydrolysis (Smith degradation) were previously described[23,24]. From their reactions with a mouse-myeloma protein (S117) specific[24] for terminal, β-linked DGlcNAc, stages 1 and 3 contained terminal, nonreducing, β-linked 2-acetamido-2-deoxy-D-glucosyl groups, whereas stages 2, 4, and 5 did not, in accordance with the proposed, composite structure of the carbohydrate moiety of the blood-group substances[23]. The human, ovarian-cyst, blood-group substance FI was provided by Prof. W. T. J. Morgan[25], α1-acid glycoprotein by Dr. K. Schmid[26], native and sialic acid-free fetuin by Dr. R. G. Spiro[27], blood-group P1 glycoprotein from hydatid-cyst fluid by Dr. W. M. Watkins[28], and the active and inactive, anti-freeze glycoprotein by Dr. R. E. Feeney[29] through Prof. M. Heidelberger. Pneumococcus type XIV polysaccharide (Squibb No. 227, lot 80320) was previously described[30].

Quantitative precipitin and inhibition assays were performed by a microtechnique[31], and the total nitrogen in the washed, specific precipitates was measured by the ninhydrin method[32]; 6.0 μg of nitrogen of peanut agglutinin was used in all the experiments.

RESULTS

Quantitative, precipitin assays

Figure 1a gives the results of the precipitation of human and horse blood-group B substances and their PI fractions with purified peanut agglutinin. There is considerable variation in the ability of these fractions to interact with the lectin. Thus, horse 4 25% (B-active) is a good reagent for precipitating PNA, as 90% of the lectin N is precipitated at equivalence, and 6 μg of the blood-group substance is required for 50% precipitation of the agglutinin. However, human ovarian-cyst B substance (Beach phenol insoluble) did not precipitate, whereas human saliva B substance PM gave an intermediate reaction, 40 μg precipitating less than 3 μg of lectin N. Mild acid hydrolysis of the nonreactive Beach substance gave a product (Beach P1) of striking reactivity that precipitates the agglutinin twice as effectively per unit weight as horse 4 25%; similarly, when Beach substance was degraded by periodate oxidation, the product reduced with borohydride, and the product treated by mild acid hydrolysis (Smith degradation), the final product was as reactive as Beach P1 in

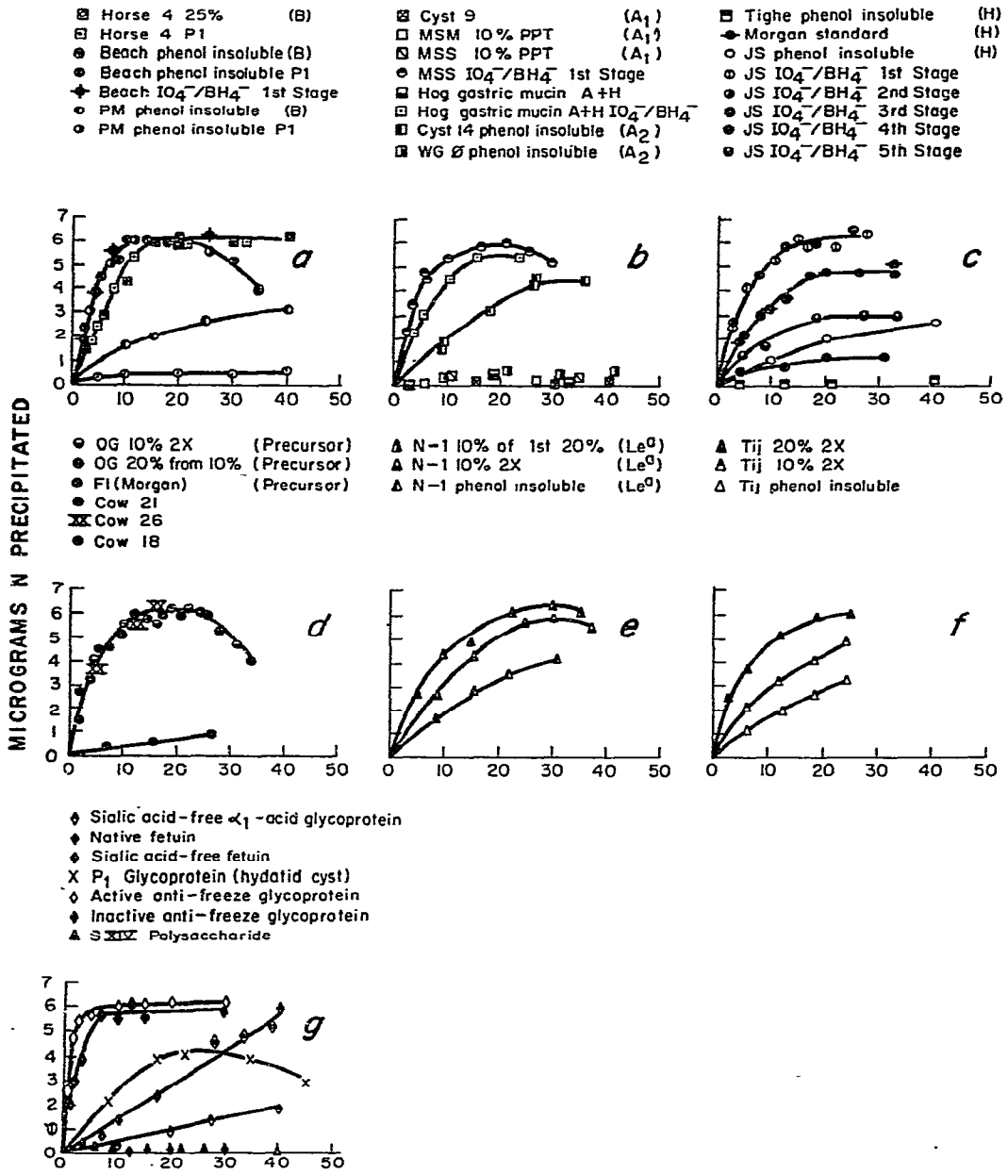


Fig. 1. Precipitation of 6.0 μg of N of purified peanut agglutinin by blood-group substances and other glycoproteins. (Total volume, 210 μl.)

precipitating PNA. The P1 fraction of PM also showed substantially increased precipitating power; with horse 4P1, PNA reacted as well as with the original material. Thus, it appears that the peanut agglutinin detects a determinant different from those involved in B specificity, and that concealed, reactive groupings can be exposed by mild acid hydrolysis or by one stage of Smith degradation (IO_4^-–BH_4^-) of B substances.

Studies with human, ovarian-cyst, A_1 substances (MSM 10% 2x, MSS 10% 2x, and cyst 9) showed them to be completely inactive in precipitating the agglutinin (see Fig. 1b), but Smith-degraded MSS 10% 2x substance reacted to about the same extent as Smith-degraded, Beach phenol insoluble B substance or its P1 fraction. With A_2 substances, heterogeneity in precipitating ability was also seen, as WG phenol insoluble was inactive, and cyst 14 phenol insoluble gave about 20% as much specific precipitate as MSS lstIO_4^-–BH_4^-. Hog gastric-mucin A+H did not give any detectable reaction with the lectin, but, after a first cycle of Smith degradation, it became highly reactive.

The interaction of peanut agglutinin with human blood-group H substances (JS phenol insoluble, Tighe phenol insoluble, and Morgan Standard H) and the stages of Smith degradation of blood-group H substance (JS) are presented in Fig. 1c. Heterogeneity was again observed, because cyst Tighe did not react, whereas cyst JS and Morgan standard H respectively precipitated 3.0 and 4.5 μg of N of the 6.0 μg of lectin N added. The first and second stages of Smith degradation of JS precipitated equally well with peanut agglutinin, the curve being almost identical to that of Smith-degraded blood-group B (Beach phenol insoluble) or A_1 (MSS 10% 2x) substances, but the third stage of Smith degradation of JS showed about 40% of these activities, and the fourth and fifth stages precipitated only 1.0 and 3.0 μg of PNA nitrogen.

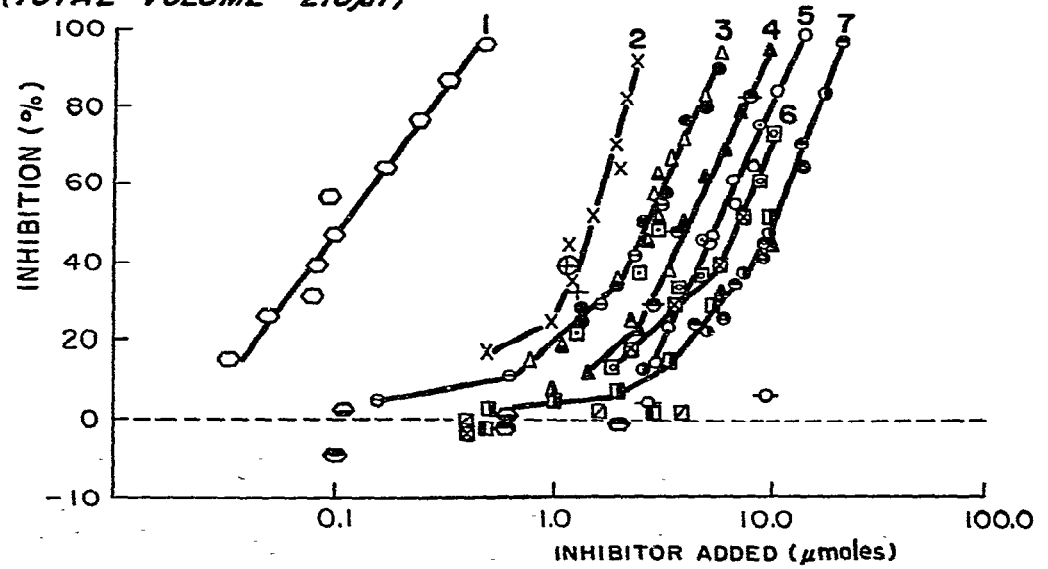
Figure 1d shows the precipitation of the PNA with precursor blood-group glycoproteins with I activity (OG 10% 2x, OG 20% from 10% and Fl) and with cow substances. It is evident that all precursor substances and cow substances 21 and 26 fall on the same curve, showing them to be of equal potency, 3.0 μg of each being required for 50% precipitation. Thus, these substances are as reactive per unit weight as the P1 fractions or the first stage of Smith degradation of B, A_1, or H blood-group substances. Cow 18 precipitated only very poorly with the agglutinin.

The reaction of Le^a blood-group substances with purified peanut agglutinin is shown in Fig. 1e. The lectin precipitated best with N-1 10% from first 20%, which was about 50% as good as Beach P1, whereas N-1 10% 2x and N-1 phenol insoluble were poorer, respectively showing, ~30 and 17% of the activity of Beach P1; 6.0, 10.0, and 17.5 μg, respectively, were needed to precipitate 50% of the maximum.

The activity of various fractions from human ovarian-cyst fluid Tij is shown in Fig. 1f. Tij 20% 2x, which is low in galactose and fucose, and has weak B activity but high reactivity toward anti-I Step, anti-i Den, and Con A (20), was the most active in precipitating PNA, and Tij phenol insoluble, having high galactose and fucose content and very weak activities toward anti-I Step, anti-i Den, and Con A (20),

	COMPOUND	CURVE NO.
○	D-Galactose	5
⊙	Methyl 6-O-methyl-α-D-galactoside	5
⊕	2-Amino-2-deoxy-D-galactose	4
⊖	D-Fucose	7
△	Methyl α-D-galactoside	3
▲	p-Nitrophenyl α-D-galactoside	3
▲	Methyl β-D-galactoside	4
△	p-Nitrophenyl β-D-galactoside	4
●	Lactose	3
⊡	Allolactose	3
+	D Gal β1→4 D GlcNAc β1→6 hexane-1,2,4,5,6-pentol(s) (N-1R_L 0.71 b)	2
⊕	D Gal β1→3 [D Gal β1→4 D GlcNAc β1→6] 1-2-acetamido-2-deoxy-D-galactitol (N-1R_L 0.44)	2
X	D Gal β1→4 D GlcNAc	2
◧	D Gal β1→3 D GlcNAc	7
⬭	D Gal β1→3 D GalNAc	1
⊖	D Gal β(1→3) 2-acetamido-2-deoxy-D-galactitol (N-1R_L 1.36)	3
◓	D GlcNAc α1→4 D Gal β1→3 D GalNAc (R_L 0.97a)	—
◓	D GlcNAc α1→4 D Gal β1→4 D GlcNAc (R_L 0.97b)	—
⊖	Melibiose	7
◐	Raffinose	7
⊡	Stachyose	6
⊠	D Gal α1→3 D Gal	6
⊠	D GalNAc α1→3 D Gal (R_L 1.85)	—
◭	Lacto-N-tetraose	7
⊸	2'-O-Fucosyllactose	—
◧	L Fuc α1→2 D Gal β1→4 D GlcNAc β1→6R	—

6.0μg of N OF PEANUT AGGLUTININ + 11.3μg OF OG 20% FROM 10% (TOTAL VOLUME 210μl)



reacted only ~1/6th as well with the peanut agglutinin. Tij 10% 2x showed intermediate reactivity; quantities of blood-group glycoproteins needed to precipitate 3.0 μg of lectin N were 3.5, 11.0, and 22.0 μg for Tij 20% 2x, Tij 10% 2x, and Tij phenol insoluble, respectively, as compared with 3.0 μg for Beach P1.

The best reagent tested for precipitating the peanut agglutinin was, however, the active anti-freeze glycoprotein from the Antarctic fish *Trematomus borchgrevinki*, a glycoprotein known to be composed[33] of repeating units of a diglycosyltripeptide, namely, DGalβ1 → 3DGalNAcα1-O-Ala-Ala-Thr; on a weight basis, this glycoprotein i sabout three times as active as Beach P1, 1 μg precipitating about 50% of the lectin (see Fig. 1g) as compared with 3 μg of Beach phenol insoluble P1. Indeed, it has recently been shown[34] to be a potent inhibitor of hemagglutination by peanut extract. The inactive, antifreeze glycoprotein, whose composition is similar to that of the active preparation, except that proline is present[29], was also very potent in precipitating the peanut agglutinin, 2 μg precipitating about 50% of the added lectin nitrogen.

As expected, sialic acid-free fetuin, which contains[27] both DGalβ1 → 3DGalNAc and DGalβ1 → 4DGlcNAc also reacted with the agglutinin, 40 μg giving a total of 6.0 μg of specific precipitate N; however, computation of lectin N precipitated is complex, as the desialyzed fetuin N that is added almost equals the total N that is precipitated (desialyzed fetuin contained 13.5 percent of N). This is probably attributable to soluble complexes, or to variations in the numbers of sugar chains on the desialyzed fetuin, so that all the fetuin molecules may not react. The nitrogen present in the amounts of blood-group substances added did not contribute appreciably to the total N. Sialic acid-free α_1-acid glycoprotein (orosomucoid), which has[35] DGalβ1 → 4DGlcNAc but not DGalβ1 → 3DGalNAc, was apparently even poorer than desialyzed fetuin in interacting with the peanut agglutinin, as 40 μg gave only 2.0 μg of precipitate N. A strict comparison between these two glycoproteins is not possible, owing to the uncertainties as to lectin N precipitated. Native fetuin, in which sialic acid is substituted on O-3 of DGal, did not precipitate. Precipitation of up to 3.5 μg of N occurred with blood-group, P_1 glycoprotein from sheep hydatid-cyst fluid. The agglutinin was not precipitated by pneumococcus type XIV polysaccharide.

Quantitative precipitin inhibition assays

The ability of various sugars to inhibit the precipitation of peanut agglutinin with precursor substance OG 20% from 10% is shown in Fig. 2 and in Table I. $DGalNH_2$ (curve 4) was the best monosaccharide inhibitor, and ~2.2 times as active as DGal (curve 5), which, in turn, was better than DFuc (curve 7), 2.7, 6.0, and 10.0 μmoles respectively being required for 50% inhibition. No inhibition was observed with other monosaccharides or glycosides: D-Man, 40, D-Glc, 20, L-Rha, 21, L-Fuc, 35, D-GalNAc, 60, D-GlcNAc, 50, D-ManNAc, 28, methyl αDGlcNAc, 25, methyl αDGalNAc, 9.0, and methyl βDGal, 8.5 μmoles. Both α- and β-glycosides

← Fig. 2. Inhibition, by monosaccharides, glycosides, and oligosaccharides, of precipitation of peanut agglutinin by human, blood-group precursor substance OG 10% from 20%.

TABLE I

ACTIVITIES OF MONOSACCHARIDES AND OLIGOSACCHARIDES IN INHIBITING THE PRECIPITATION OF PEANUT AGGLUTININ

Inhibitor	*μmoles to give 50% inhibition*	*Relative potency*
D-Gal	6.0	1.0
Methyl 6-*O*-methyl-α-D-Gal	6.0	1.0
D-GalNH_2	2.7	2.2
D-Fuc	10.0	0.6
Methyl α-D-Gal	2.7	2.2
p-Nitrophenyl α-D-Gal	2.7	2.2
Methyl β-D-Gal	4.0	1.5
p-Nitrophenyl β-D-Gal	4.0	1.5
DGalβ1→4DGlc	2.7	2.2
DGalβ1→6DGlc	2.7	2.2
DGalβ1→4DGlcNAc	1.5	4.0
DGalβ1→4DGlcNAcβ1→6hexane-1,2,4,5,6-pentol(s)	1.5	4.0
DGalβ1→3[DGalβ1→4DGlcNAcβ1→6]-2-acetamido-2-deoxy-D-galactitol	1.5	4.0
DGalβ1→3DGlcNAc	10.0	0.6
DGalβ1→3DGalNAc	0.11	54.5
DGalβ(1→3)-2-acetamido-2-deoxy-D-galactitol	2.7	2.2
DGlcNAcα1→4DGalβ1→3DGalNAc	>2.0	<3.0
DGlcNAcα1→4DGalβ1→3DGlcNAc	>1.9	<3.1
DGalα1→6DGlc	10.0	0.6
DGalα1→6DGlcβ1→2Fru	10.0	0.6
DGalα1→6DGalα1→6DGlcβ1→2Fru	7.0	0.9
DGalα1→3DGal	7.0	0.9
DGalNAcα1→3DGal	>4.0	<1.5
DGalβ1→3DGlcNAcβ1→3DGalβ1→4DGlc	10.0	0.6
LFucα1→2DGalβ1→4DGlc	>10.0	<0.6
LFucα1→2DGalβ1→4DGlcNAc1-6R	>3.0	<2.0

were somewhat better inhibitors than DGal, methyl αDGal and *p*-nitrophenyl αDGal both being ~2.2, and the methyl β-glycosides 1.5, times as active. The 6-hydroxyl group of galactose is apparently important for the binding as methyl 6-*O*-methyl-αDGal is about 45% as active as methyl αDGal, and as active as DGal (which is twice as active as DFuc).

Of the disaccharides tested, DGalβ1→3DGalNAc (curve 1), as previously noted[2,6] by hemagglutination inhibition, was the most active; ~110 nmoles was required for 50% inhibition of precipitation, as compared with 6.0 μmoles for DGal. The specificity of the lectin site for this disaccharide is evident as 1.5 μmoles of DGalβ1→4DGlcNAc (curve 2) and 10.0 μmoles of DGalβ1→3DGlcNAc (curve 7) were needed for 50% inhibition, and they are thus only 7 and 1% as active as DGalβ1→3DGalNAc. The blood-group oligosaccharides N-1 R_L 0.71 b, DGalβ1→4DGlcNAcβ1→6-hexane-1,2,4,5,6-pentol(s) and N-1 R_L 0.44, DGalβ1→3[DGalβ1→4DGlcNAcβ1→6]2-acetamido-2-deoxy-D-galactitol showed the same inhibitory power per micromole as DGalβ1→4DGlcNAc, indicating that addition of the

β-(1→6) linkage to the DGalβ1→4DGlcNAc residue did not result in an increase in the inhibitory activity, unlike the findings[36] with anti-I Ma. The reduced disaccharide, DGalβ(1→3)-2-acetamido-2-deoxy-D-galactitol (N-1 R_L 1.36) (curve 3) is ~1/25th as active as the corresponding, unreduced disaccharide, indicating a contribution of the ring structure of DGalNAc. The oligosaccharide R_L 0.97a, DGlcNAcα1→4DGalβ1→3DGalNAc, was inactive when tested up to 2.0 μmoles, as was compound R_L 0.97b, DGlcNAcα1→4DGalβ1→4DGlcNAc, up to 1.9 μmoles. Substitution of fucose on active compounds sharply lessened the activity. Thus, 2′-*O*-fucosyllactose and the blood-group, H-active oligosaccharide HR_L 0.75, L-Fucα1→2DGalβ1→4DGlcNAcβ1→6-R, were inactive at 10.0 and 3.0 μmoles, as compared with lactose and *N*-acetyllactosamine, which gave 50% inhibition at 2.7 and 1.5 μmoles, respectively. DGalβ1→3DGlcNAc and lacto-*N*-tetraose, DGalβ1→3DGlcNAcβ1→3DGalβ1→4DGlc, are only about 15% as active as DGalβ1→4DGlcNAc, suggesting that the lectin may have a preference for the β-(1→4)-linkage. Unfortunately, the compound DGalβ1→4DGalNAc was not available.

Although α-glycosides of DGal were slightly better inhibitors than the corresponding β anomers, the oligosaccharides containing terminal, α-linked DGal were all weak inhibitors. Thus, 2.7 μmoles of allolactose, DGalβ1→6DGlc, were required for 50% inhibition, whereas melibiose (DGalα1→6DGlc), raffinose (DGalα1→6DGlcβ1→2DFru), and stachyose (DGalα1→6DGalα1→6DGlcβ1→2DFru), were all poor inhibitors; 10.0, 10.0, and 7.0 μmoles were needed, and they were thus much less active than DGal. The blood-group, B-active disaccharide DGalα1→3DGal was 85% as active as DGal, and the A-active disaccharide DGalNAcα1→3DGal did not give detectable inhibition at 4.0 μmoles.

DISCUSSION

Hemagglutination inhibition studies with crude extracts[6] and purified lectin[2] showed the peanut agglutinin to be most specific for the disaccharide DGalβ1→3DGalNAc. The results presented in Fig. 2 are in general agreement with earlier studies[2], and also demonstrate specificity for terminal, nonreducing, β-linked D-galactosyl-containing oligosaccharides, particularly for the structure DGalβ1→3DGalNAc, which is 14, 55, and 90 times as active as DGalβ1→4DGlcNAc, DGal, and DGalβ1→3DGlcNAc, respectively, 110 nmoles giving 50% inhibition of precipitation of the agglutinin by a precursor, blood-group I glycoprotein (OG 20% from 10%). Oligosaccharides in which DGlcNAc and LFuc were substituted on O-4 and O-2 of the terminal, D-galactopyranosyl group of DGalβ1→3DGalNAc and DGalβ1→4DGlcNAc, respectively (as in DGlcNAcα1→4DGalβ1→3DGalNAc and LFucα1→2DGalβ1→4DGlcNAcβ1→6R), were inactive up to 2.0 and 3.0 μmoles, respectively, indicating that the lectin does not recognize internal D-galactopyranosyl residues. These findings differ from those of Irimura *et al.*[37] with glycopeptides obtained by pronase digestion of a pig submaxillary mucin having blood-group A activity; these workers found that L-fucose linked α-(1→2) to the D-galactosyl residue

of DGalβ1 → DGalNAcα-Ser/Thr increased the affinity of the glycopeptide toward the peanut agglutinin ~2.5 times; addition of a DGalNAc linked α-(1→3) to the DGal of this structure did not alter[37] the inhibitory activity. Moreover, their PNA preparation did not stimulate DNA synthesis in neuraminidase-treated, human lymphocytes[38], whereas ours was mitogenic for neuraminidase-treated, human and rat lymphocytes[4]. The two preparations could differ in specificity.

The oligosaccharides containing terminal, α-linked DGal residues were all much poorer inhibitors of precipitation than the corresponding β-linked compounds, and the methyl and *p*-nitrophenyl α-D-glycosides were slightly more active than the β anomers. However, the *p*-nitrophenyl α- and β-D-galactosides were not better inhibitors than their corresponding methyl α- and β-glycosides, unlike findings with such lectins as the soybean agglutinin[39] and the sponge agglutinin[40] of *Aaptos papillata.*

The precipitin findings with the peanut agglutinin are of interest, in that differences were observed in the interaction of the lectin with blood-group glycoproteins of the same specificity, thus providing additional evidence for heterogeneity of the carbohydrate chains of blood-group substances. Thus, the agglutinin was completely precipitated by a horse B substance (horse 4 25%), did not react with the human ovarian-cyst B substance Beach, and was poorly precipitated by human saliva B substance PM phenol insoluble (see Fig. 1a). Of the A_2 substances used, cyst 14 phenol insoluble interacted with the lectin, and WG phenol insoluble failed to precipitate (see Fig. 1b). Variation was observed among blood-group H substances, as cyst Tighe did not precipitate, whereas 4.6 and 2.0 μg of N of the 6.0 μg of lectin N added were precipitated at equivalence by Morgan standard H and by JS phenol insoluble, respectively (see Fig. 1c). None of the A_1 substances tested precipitated. However, one stage of Smith degradation, or mild, acid hydrolysis of the blood-group substances, strikingly increased the reactivity toward the agglutinin, as seen with substances PM (Fig. 1a) and JS (Fig. 1c); with Beach phenol insoluble (Fig. 1a), MSS 10% 2x, and hog gastric mucin A+H substance (Fig. 1b), the increase was even greater, as the original, blood-group glycoproteins did not precipitate with PNA. These findings strongly indicate that the peanut agglutinin does not recognize the A-, B-, or H-determinants, but, rather, interacts with residues in the interior of the blood-group substances after their exposure by mild acid hydrolysis or by Smith degradation. Reactivity is most probably ascribable to DGalβ1 → 3DGalNAc (and, to a lesser degree, to DGalβ1 → 4DGlcNAc), as both are present in blood-group substances and are the best inhibitors of precipitation by peanut agglutinin (see Fig. 2). Incomplete biosynthesis of the oligosaccharide chains by failure to add fucose and DGlcNAc could account[10,18] for such terminal, nonreducing ends. Moreover, the antarctic-fish antifreeze glycoprotein, which is composed of repeating units of the diglycosyltripeptide DGalβ1 → 3DGalNAcα1 → O-Thr-Ala-Ala was the best reagent in precipitating the peanut agglutinin, whereas sialic acid-free, α_1-acid glycoprotein (orosomucoid), which contains DGalβ1 → 4DGlcNAcβ1 → 3(or 4) DMan residues, precipitated poorly.

It is interesting that the fractions of N-1 and of Tij blood-group substances that require higher concentrations of ethanol to precipitate them from phenol reacted best with the peanut agglutinin (Fig. 1e–f); a similar parallelism was observed with N-1 fractions in their cross-reactions with type XIV and with anti-I sera[36], as well as with Tij fractions and anti-I Step, anti-i Den, and Con A[20]. It had been noted[20] that Tij phenol insoluble has a higher proportion of larger carbohydrate chains than Tij 20% 2x, accounting for its higher galactose and fucose content and its higher B activity. Thus, the poorer reactivity of the phenol-insoluble fractions toward the peanut agglutinin can be ascribed to these larger carbohydrate chains which, by themselves, are inactive, as all are substituted on the DGal of the DGalβ1 → 3DGalNAc or DGalβ1 → 4DGlcNAc determinants[20]; in addition, larger chains could hinder access of the lectin combining-sites to the remaining, unsubstituted DGalβ1 → 3DGalNAc residues linked α to serine or threonine. A similar inference could account for the heterogeneity in precipitation of peanut agglutinin with blood-group substances of the same specificity.

The pattern of precipitation of peanut agglutinin with various blood-group substances resembles that of anti-I cold autoagglutinins, in that the lectin and anti-I[36] react strongly with P1 fractions obtained by mild acid hydrolysis, as well as with the Smith-degraded materials, with precursor I antigens, OG and Fl, and with cows 21 and 26. Moreover, the 20% 2x fractions of N-1 and of Tij, which are more reactive with anti-I-Step and anti i-Den than the corresponding phenol-insoluble fractions, are also more reactive with the peanut agglutinin. Thus, it appears that the peanut agglutinin reacts with some I or i determinants, although, when tested up to 0.51 μmoles, DGalβ1 → 3DGalNAc failed to inhibit precipitation by 10 μg of OG 20% from 10% of anti-I Ma (30 μl of 1:2) or precipitation by 38 μg of Tij 20% 2x, of anti-I Step (20 μl of 1:4) and anti-i Den (20 μl of 1:16). Moreover, these antisera, in the amounts indicated, were not precipitated by 5 to 40 μg of antarctic-fish glycoproteins. Anti-I and anti-i sera of other groups[41] should be studied. It is obvious that, besides being useful in diagnosing T-polyagglutination[7], the peanut agglutinin will prove a very important reagent in structural studies of glycoproteins and of cell-surface receptors (*cf.*, ref. 42).

REFERENCES

1 G. W. G. Bird, *Vox Sang.*, 9 (1964) 748–749.
2 R. Lotan, E. Skutelsky, D. Danon, and N. Sharon, *J. Biol. Chem.*, 250 (1975) 8487–8495.
3 N. Sharon and H. Lis, *Science*, 117 (1972) 949–959.
4 A. Novogrodsky, R. Lotan, A. Ravid, and N. Sharon, *J. Immunol.*, 115 (1975) 1243–1248.
5 V. Friedenreich, *The Thomsen Hemagglutination Phenomenon. Production of a Specific Receptor Quality in Red Cell Corpuscles by Bacterial Activity*, Levin and Munksgaard, Copenhagen, 1930.
6 G. Uhlenbruck, G. I. Pardoe, and G. W. G. Bird, *Z. Immunitaetsforsch.*, 138 (1969) 423–433.
7 G. W. G. Bird and J. Wingham, *Scand. J. Haematol.*, 8 (1971) 307–308.
8 K. E. Boorman, J. F. Loutit, and D. B. Steabben, *Nature (London)*, 158 (1946) 446–447.
9 F. W. Chorpenning and J. C. Hayes, *Vox Sang.*, 4 (1959) 210–224.
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 K. O. Lloyd, E. A. Kabat, E. J. Layug, and F. Gruezo, *Biochemistry*, 5 (1966) 1489–1501.

12 L. ROVIS, B. ANDERSON, E. A. KABAT, F. GRUEZO, AND J. LIAO, *Biochemistry*, 12 (1973) 5340–5354.
13 E. A. KABAT, *Arch. Biochem. Biophys. Suppl.*, 1 (1962) 181–186.
14 E. KLENK, V. W. HENDRIKS, AND W. GIELEN, *Hoppe-Seyler's Z. Physiol. Chem.*, 330 (1962) 140–144.
15 E. A. KABAT, *Blood Group Substances. Their Chemistry and Immunochemistry*, Academic Press, New York, 1956, pp. 135–139.
16 S. BEISER AND E. A. KABAT, *J. Immunol.*, 68 (1952) 19–40.
17 P. Z. ALLEN AND E. A. KABAT, *J. Immunol.*, 82 (1959) 340–357.
18 G. VICARI AND E. A. KABAT, *J. Immunol.*, 102 (1969) 821–825.
19 K. O. LLOYD, E. A. KABAT, AND E. LICERIO, *Biochemistry*, 7 (1968) 2976–2990.
20 F. MAISONROUGE-MCAULIFFE AND E. A. KABAT, *Arch. Biochem. Biophys.*, 175 (1976) 1090–1113.
21 E. A. KABAT, H. BAER, A. E. BEZER, AND V. KNAUB, *J. Exp. Med.*, 88 (1948) 43–57.
22 S. LESKOWITZ AND E. A. KABAT, *J. Am. Chem. Soc.*, 76 (1954) 5060–5065.
23 K. O. LLOYD AND E. A. KABAT, *Proc. Natl. Acad. Sci. U. S. A.*, 61 (1968) 1470–1477.
24 G. VICARI, A. SHER, M. COHN, AND E. A. KABAT, *Immunochemistry*, 7 (1970) 829–838.
25 W. T. J. MORGAN, *Proc. R. Soc. London Ser. B*, 151 (1960) 308–312.
26 K. SCHMID, *Biochim. Biophys. Acta*, 14 (1954) 437.
27 R. J. SPIRO AND V. D. BOHYROO, *J. Biol. Chem.*, 249 (1974) 5704–5717.
28 H. T. CORY, A. D. YATES, A. S. R. DONALD, W. M. WATKINS, AND W. T. J. MORGAN, *Biochem. Biophys. Res. Commun.*, 61 (1974) 1289–1296.
29 A. L. DE VRIES, S. KOMATSU, AND R. E. FEENEY, *J. Biol. Chem.*, 245 (1970) 2901–2908.
30 C. HOWE, G. SCHIFFMAN, A. E. BEZER, AND E. A. KABAT, *J. Am. Chem. Soc.*, 80 (1958) 6656–6661.
31 E. A. KABAT, *Kabat and Mayer's Experimental Immunochemistry*, 2nd edn., Thomas, Springfield, Ill., 1961, pp. 29–96.
32 G. SCHIFFMAN, E. A. KABAT, AND W. THOMPSON, *Biochemistry*, 3 (1964) 113–120.
33 W. T. SHIER, Y. LIN, AND A. L. DE VRIES, *FEBS Lett.*, 54 (1975) 135–138.
34 W. M. GLOCKNER, R. A. NEWMAN, AND G. UHLENBRUCK, *Biochem. Biophys. Res. Commun.*, 66 (1975) 701–705.
35 R. W. JEANLOZ, in A. GOTTSCHALK (Ed.), *Glycoproteins*, 2nd edn., Elsevier, Amsterdam, 1972, pp. 565–611.
36 T. FEIZI, E. A. KABAT, G. VICARI, B. ANDERSON, AND W. L. MARSH, *J. Immunol.*, 106 (1971) 1578–1592.
37 T. IRIMURA, T. KAWAGUCHI, T. TERAO, AND T. OSAWA, *Carbohydr. Res.*, 39 (1975) 317–327.
38 T. TERAO, T. IRIMURA, AND T. OSAWA, *Hoppe-Seyler's Z. Physiol. Chem.*, 356 (1975) 1685–1692.
39 M. E. A. PEREIRA, E. A. KABAT, AND N. SHARON, *Carbohydr. Res.*, 37 (1974) 89–102.
40 H. BRETTING, E. A. KABAT, J. LIAO, AND M. E. A. PEREIRA, *Biochemistry*, (1976) in press.
41 T. FEIZI AND E. A. KABAT, *J. Exp. Med.*, 135 (1972) 1242–1258.
42 E. A. KABAT, *Structural Concepts in Immunology and Immunochemistry*, 2nd edn., Holt, Rinehart, and Winston, New York, 1976.