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CARBOHYDRATE-BINDING SPECIFICITY OF THE SO-CALLED GALACTOSE-SPECIFIC PHYTOHEMAGGLUTININS*

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ABSTRACT

The carbohydrate-binding specificities of various so-called galactose-specific phytohemagglutinins were investigated by means of hemagglutination-inhibition assays. As hapten inhibitors, glycopeptides prepared by pronase-digestion of various glycoproteins (porcine submaxillary mucin, bovine submaxillary mucin, and porcine thyroglobulin), and several glycosides of D-galactose and 2-acetamido-2-deoxy-D-galactose were employed. The results indicate that these galactose-specific phytohemagglutinins may recognize the sugar residue penultimate to D-galactose or 2-acetamido-2-deoxy-D-galactose residues of the sugar chain with which they interact, and that they can be classified into three groups based on the type of sugar sequence which they primarily recognize.

INTRODUCTION

The interaction of phytohemagglutinins with cells can generally be inhibited by simple sugars. Mäkelä classified simple sugars into four groups based on the configuration of hydroxyl groups at C-3 and C-4, and indicated phytohemagglutinins which could be inhibited by each group of sugars[1]. Since these phytohemagglutinins react with different cell-surface structures, they have been used as molecular probes for the investigation of the architecture of the cell surface. However, in these investigations, the results are often correlated simply to the specificities of phytohemagglutinins disclosed by haptene inhibition assays using simple sugars as haptene inhibitors. We have previously indicated that subtle differences in specificity exist even among the same group of anti-H phytohemagglutinins[2], and also showed that the results of haptene inhibition assays using simple sugars as inhibitors cannot always be correlated to the structure of receptor sites on the cell surface[3,4].

We now present evidence suggesting that the so-called galactose-specific phytohemagglutinins can be classified into three groups based on the type of sugar chains with which they primarily interact on the cell surface.

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EXPERIMENTAL

Hemagglutinins. — *Bauhinia purpurea* hemagglutinin[5], *Ricinus communis* hemagglutinin[6], *Maackia amurensis* hemagglutinin[7], and *Maackia amurensis* mitogen[7] were purified according to the methods previously described. Purified *Sophora japonica* and *Wistaria floribunda* hemagglutinin used in this study were fractions SJ-III and A, respectively, in the methods previously described[8,9]. *Glycine max* (Soy bean) hemagglutinin was purified according to the method of Gordon *et al.*[10]. *Arachis hypogoea* (peanut) hemagglutinin was purified by affinity chromatography, according to the following method which will be described in detail elsewhere[11]. The crude extract of the seeds was first fractionated with $(NH_4)_2SO_4$. The fraction that precipitated between 0.3 and 0.75 saturation of $(NH_4)_2SO_4$ was further subjected to affinity chromatography on a column of Sephadex 6B. *Phaseolus vulgaris* hemagglutinin was a product of Difco (PHA-M).

Glycoproteins. — Porcine thyroglobulin (PT) was prepared from porcine thyroid glands as described by Ui and Tarutani[12]. Porcine submaxillary mucin (PSM) was purified from porcine submaxillary glands showing blood-group A activity, according to the method of Katzman and Eylar[13]. Purified, bovine submaxillary mucine (BSM) glycopeptide, prepared by pronase digestion of BSM followed by repeated gel-filtration, was kindly provided by Dr. Fukuda, Showa University.

Sugars. — 2-Acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-glucose (*N*-Acetyllactosamine) was a gift from Dr. M. C. Glick, University of Pennsylvania. Methyl 2-acetamido-2-deoxy-α(and β)-D-galactopyranosides [methyl *N*-acetyl-α(and β)-D-galactosaminides] were purchased from Nakarai Chemical Co. (Kyoto). Phenyl α(and β)-D-galactopyranosides and phenyl 2-acetamido-2-deoxy-α(and β)-D-galactopyranosides [phenyl *N*-acetyl-α(and β)-D-galactosaminides] were kindly provided by Dr. T. Okuyama, Seikagaku Kogyo Co. (Tokyo).

Enzymes. — *Vibrio cholerae* neuraminidase (mucopolysaccharide *N*-acetylneuraminylhydrolase, E.C. 3.2.1.18) was purchased from Calbiochem. Highly purified *N*-acetyl-α-D-galactosaminidase and β-D-galactosidase from the livers of *Charonia lampas* were purchased from Seikagaku-Kogyo Co. (Tokyo). Each purified enzyme used in this study was virtually devoid of other glycosidase activity. One unit of enzyme activity was defined as the amount of enzyme which could liberate 1.0 μmole of *p*-nitrophenol in 1 min.

Preparation of PSM glycopeptides. — PSM (1 g) showing blood-group A activity was incubated with 100 mg of pronase in 100 ml of 0.05M sodium borate buffer (pH 7.9) containing mM $CaCl_2$. Incubation was carried out for 96 h at 37° under a toluene atmosphere with further additions of 50 mg of pronase at 48 h and 72 h. The reaction mixture was then lyophilized and applied to a column (2.6 × 70 cm) of Sephadex G-50, after dissolution in a small amount of water. Elution was carried out with water (Fig. 1), and the glycopeptide material was identified by analyzing each fraction by the phenol–H_2SO_4 method[14]. The glycopeptide material thus obtained

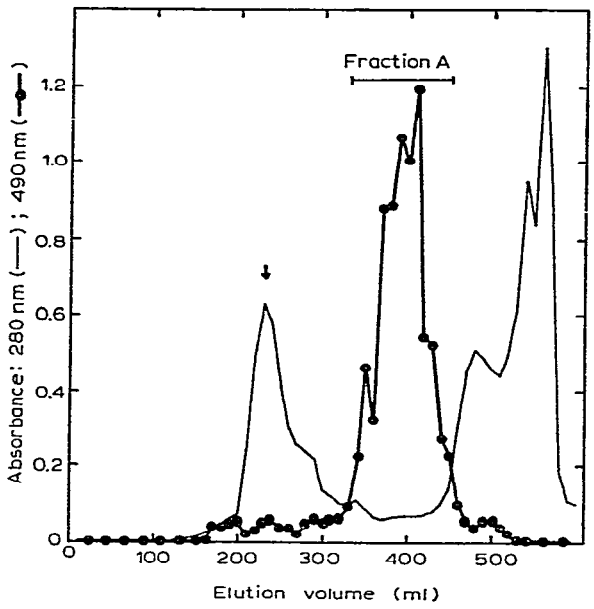

Fig. 1. Sephadex G-50 gel-filtration of pronase-digested PSM. Fractions (10 ml) were collected at a flow rate of 20 ml/h at 4°. Other experimental details are given in the text. The void volume is indicated by a vertical arrow: ———, A_{280}; —●—, phenol–H_2SO_4 reaction.

(Fraction *A*) was incubated, as described above, with 100 mg of pronase for 48 h and with a further addition of pronase at 24 h. The reaction mixture was lyophilized and then applied to a column (2.0 × 55 cm) of Sephadex G-25. Elution was carried out with water (Fig. 2), and the positive fractions (phenol–H_2SO_4 reaction) were combined (Fraction *B*) and further applied to a column (2.6 × 30 cm) of DEAE-Sephadex A-25 which had been washed successively with 0.2M sodium phosphate buffer (pH 7.0) and water. Elution was carried out with water and, after elution of the first peak, gradient elution was performed with 600 ml of water in the mixing vessel and 600 ml of 0.1M sodium phosphate buffer (pH 7.0) in the reservoir. A second gradient elution was then performed with 350 ml of 0.1M sodium phosphate buffer (pH 7.0) in the mixing vessel and 350 ml of 0.2M sodium phosphate buffer (pH 7.0) in the reservoir. Fractions of 20 ml were collected at 40 ml/h at 4° (Fig. 3). Fractions were analyzed for sialic acid by the periodate–resorcinol method[15]. The glycopeptide fractions were collected as shown in Fig. 3, desalted by passage through a column of Sephadex G-25, and lyophilized. The yield of each fraction was as follows: I, 40 mg; II, 20 mg; III, 20 mg; IV, 15 mg; V, 10 mg.

Preparation of PT glycopeptides B. — The glycopeptide B from porcine thyroglobulin (Fig. 4) was prepared according to the procedure described by Fukuda and Egami[16].

Sequential degradation of glycopeptides. — (*a*) *Desialization.* Desialization of the glycopeptides was performed by an acid hydrolysis in 25mM H_2SO_4 for 3 h at 80° in a

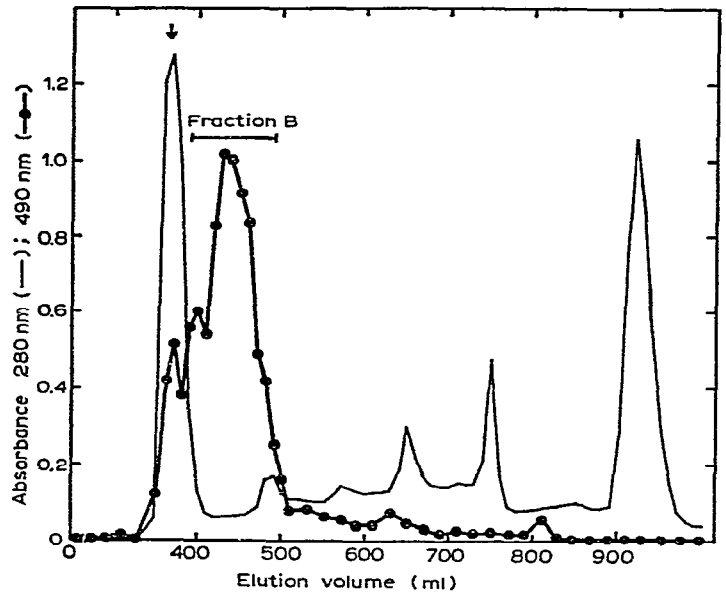

Fig. 2. Sephadex G-25 gel-filtration of pronase-digested Fraction A. Fractions (10 ml) were collected at a flow rate of 20 ml/h at 4°. Other experimental details are given in the text. The void volume is indicated by a vertical arrow: ———, A_{280}; —●—, phenol–H_2SO_4 reaction.

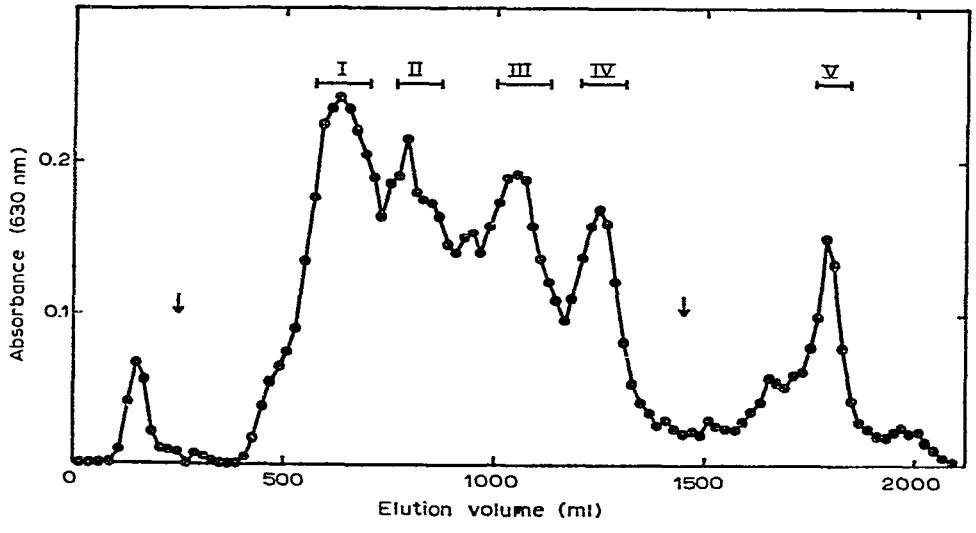

Fig. 3. DEAE-Sephadex A-25 chromatography of Fraction B. Experimental details are given in the text. The starts of the gradient elutions are indicated by vertical arrows: —●—, periodate–resorcinol reaction.

sealed tube *in vacuo*. The reaction mixture was neutralized with M NaOH and then desalted by passage through a column of Sephadex G-25.

(*b*) N-*Acetyl-α-D-galactosaminidase treatment.* Desialized PSM glycopeptide I (13 mg) was incubated with 0.2 unit of *N*-acetyl-α-D-galactosaminidase in 2 ml of 0.05M citrate buffer (pH 4.0) containing 0.1M NaCl at 37° for 48 h, with further addition of 0.1 unit of the enzyme at 24 h. The reaction mixture was then applied to a column of Sephadex G-25 to separate the residual glycopeptide from the enzyme and the released sugars.

(*c*) *Removal of fucose.* Removal of fucose residues from the desialized and *N*-acetyl-α-D-galactosaminidase-treated PSM glycopeptide I was performed by an acid hydrolysis in 0.5M H_2SO_4 for 90 min at 65°, according to the method of Carlson[17]. The residual glycopeptide was recovered after neutralization with 4M NaOH and gel filtration on a column of Sephadex G-25.

(*d*) *β-D-Galactosidase treatment.* Desialized PT glycopeptide B was treated by β-D-galactosidase, according to the method previously described[3].

Sugar analysis. — Sialic acid was measured by the periodate–resorcinol method of Jourdian *et al.*[15]. Quantitative determination of individual neutral sugars was carried out by g.l.c., after reduction to the respective alditol followed by trifluoroacetylation[18,19]. Hexosamine was determined by the method of Belcher *et al.*[20]. Hydrolysis for this assay was carried out with 4M HCl for 8 h at 100°.

Hemagglutination assays. — Titration and inhibition assays, using human erythrocytes freshly obtained from a donor, were carried out according to the methods previously described[18]. The cells used for the inhibition assays on *B. purpurea*, *G. max*, and *A. hypogoea* hemagglutinins were neuraminidase-treated cells. The neuraminidase treatment of human erythrocytes was performed as described previously[5].

RESULTS AND DISCUSSION

The hemagglutination inhibition assays against various purified galactose-specific phytohemagglutinins were carried out using glycopeptides prepared from PSM, PT, and BSM as hapten inhibitors. The sequence of sugar residues[21,22] (Fig. 4) of the major carbohydrate chain of PSM having blood-group A activity is similar to that of *O*-glycosidically linked carbohydrate chains of the major glycoprotein of human erythrocyte membrane[23]. However, in view of the fact[24] that the carbohydrate chains of PSM contain incomplete chains of various stages, the PSM having blood-group A activity was subjected to proteolytic digestion with pronase followed by repeated gel-filtration (Figs. 1 and 2) and anion-exchange chromatography on DEAE-Sephadex (Fig. 3). The carbohydrate compositions of the separated glycopeptides (Table I) indicate great heterogeneity in the carbohydrate chains of the PSM. Among the glycopeptides thus separated, glycopeptide I has the carbohydrate composition of the complete chain and was therefore used together with its sequential degradation products for the following inhibition assays. Since the molecular weight of glycopeptide I was estimated to be ~3,000 by means of gel filtration[16] on Sephadex G-50 and its amino acid content was ~14%, the presence of two complete carbohydrate

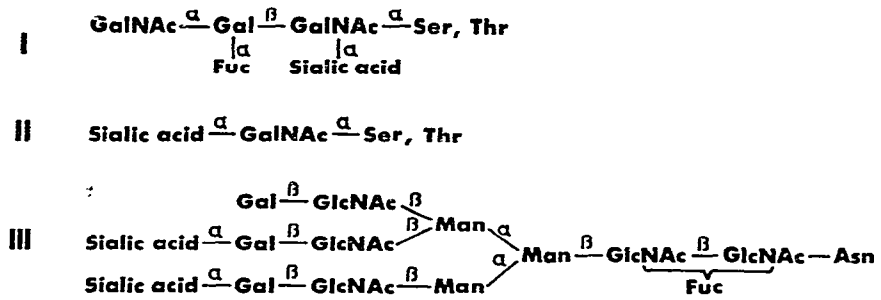

Fig. 4. Proposed structures of carbohydrate chains of glycoproteins: (I) PSM having blood-group A activity; (II) BSM; (III) glycopeptide B from PT.

TABLE I

THE CARBOHYDRATE COMPOSITION OF PSM-GLYCOPEPTIDES[a]

Fraction	*Sialic acid*	*Galactose*	*Fucose*	N-*Acetylhexosamine*
I	0.9	0.9	0.8	2.0
II	1.2	0.8	0.8	2.0
III	1.4	0.4	0.4	2.0
IV	1.5	0.5	0.3	2.0
V	2.1	0.8	0.1	2.0

[a]Values expressed in relation to *N*-acetylhexosamine taken as 2.0.

TABLE II

THE CARBOHYDRATE COMPOSITION OF VARIOUS GLYCOPEPTIDES USED AS HAPTEN INHIBITORS

	SA[a]	*Gal*	*Fuc*	*Man*	N-*Acetylhexosamine*
PSM-Glycopeptide I	0.9[b]	0.9[b]	0.8[b]	—[e]	2.0
— SA	0.1[b]	1.0[b]	0.8[b]	—[e]	2.0
— SA, GalNAc (non-reducing end)	0.1[c]	1.0[c]	0.8[c]	—[e]	1.0
— SA, GalNAc (non-reducing end), Fuc	0.0[c]	1.0[c]	0.3[c]	—[e]	1.0
BSM-Glycopeptide					
— SA	0.0	0.0	0.0	—[e]	1.0
PT-Glycopeptide B	2.1[d]	3.0[d]	1.3[d]	3.0	4.6[d]
— SA	0.0	3.1[d]	1.3[d]	3.0	4.7[d]
— SA, Gal	0.0	0.5[d]	1.3[d]	3.0	4.9[d]

[a]SA = Sialic acid. [b]Values expressed in relation to *N*-acetylhexosamine taken as 2.0. [c]Values expressed in relation to *N*-acetylhexosamine taken as 1.0. [d]Values expressed in relation to mannose taken as 3.0 [e]Trace.

TABLE III

COMPARISON OF HEMAGGLUTINATION INHIBITORY ACTIVITIES OF VARIOUS GLYCOPEPTIDES

	Minimum concentration (mM) *completely inhibiting 4 hemagglutinating doses*								
	Bauhinia purpurea	Arachis hypogoea	Maackia amurensis (*hemagglutinin*)	Sophora japonica (*SJ-III*)	Glycine max	Wistaria floribunda (*hemagglutinin*)	Maackia amurensis (*mitogen*)	Ricinus communis (*hemagglutinin*)	Phaseolus vulgaris (*PHA-M*)
PSM-Glycopeptide I	0.94	0.47	0.03	0.12	0.12	0.06	0.47	>0.94	>0.94
— SA[a]	0.57	0.04	0.57	0.07	0.04	0.02	1.1	>1.1	>1.1
— SA, GalNAc (non-reducing end)	0.32	0.04	>1.3	0.08	0.04	0.02	>1.3	>1.3	>1.3
— SA, GalNAc (non-reducing end), Fuc	0.81	0.10	>1.6	0.20	0.05	0.05	>1.6	>1.6	>1.6
BSM-Glycopeptide									
— SA	0.30[b]	>2.4[b]	>2.4[b]	2.4[b]	0.04[b]	0.04[b]	1.2[b]	>2.4[b]	>2.4[b]
PT-Glycopeptide B	>2.7	>2.7	>2.7	>2.7	>2.7	0.34	0.001	0.34	0.17
— SA	1.7	>3.4	>3.4	0.21	0.21	0.11	0.002	0.21	0.21
— SA, Gal	>3.4	>3.4	>3.4	>1.2	0.30	0.60	0.08	>1.2	>1.2

[a]SA = Sialic acid. [b]Expressed as mM of GalNAc residue of BSM-glycopeptide.

chains, as shown in Fig. 4, could be anticipated in this glycopeptide. On the other hand, the sugar sequence of the carbohydrate chain of PT glycopeptide B, which has been proposed previously[3,25,26] to be as shown in Fig. 4, is similar to that of the *N*-glycosidically linked carbohydrate chains of the major glycoprotein of human erythrocyte membrane[27]. The carbohydrate composition of the glycopeptides used in this study was confirmed and is listed in Table II.

The results of the hemagglutination inhibition assays are shown in Table III. From these results, we can classify these galactose-specific phytohemagglutinins into three groups. The members of the first group, which includes *B. purpurea*, *A. hypogoea*, and *M. amurensis* hemagglutinins, are inhibited by PSM glycopeptide I and its sequential degradation products and desialized BSM glycopeptide, but they are not significantly inhibited by PT glycopeptide B and its sequential degradation products. In contrast, the members of the second group, which includes *R. communis* and *P. vulgaris* hemagglutinins and *M. amurensis* mitogen, are effectively inhibited by the PT glycopeptide B, but they are not significantly inhibited by the glycopeptides prepared from PSM and BSM. On the other hand, the members of the third group, which includes *G. max*, *S. japonica*, and *W. floribunda* hemagglutinins, are inhibited by the glycopeptides from PSM as well as those from PT.

From the results in Table III, it might be reasonable to assume that these phytohemagglutinins can recognize the sugar residue penultimate to a 2-acetamido-2-deoxy-D-glucose (*N*-acetyl-D-galactosamine) or a D-galactose residue. Thus, each of the first group of phytohemagglutinins primarily recognizes the Gal→GalNAc or the GalNAc→Gal sugar sequence located at the terminal or internal position of the carbohydrate chain, but they cannot effectively bind to the Gal→GlcNAc sugar sequence. The *M. amurensis* hemagglutinin, however, seems to bind primarily to the Gal→(Sialic acid→)GalNAc sugar sequence, because the desialization of the PSM glycopeptide results in a remarkable diminution of the inhibitory activity, and PSM-H lacking the terminal *N*-acetyl-D-galactosamine residue also exerts a strong inhibitory activity[7]. In contrast, each of the second group of phytohemagglutinins primarily recognizes the Gal→GlcNAc sugar sequence, but they cannot effectively bind to Gal→GalNAc and GalNAc→Gal sugar sequences. Among this group, *P. vulgaris* hemagglutinin and *M. amurensis* mitogen have already been reported to recognize the Gal→GlcNAc→Man sugar sequence in the carbohydrate chain of the cell surface[3,7,27], and *R. communis* hemagglutinin has been reported to be most strongly inhibited by β-D-linked disaccharides having D-galactose at the non-reducing end, such as lactose or melibiose[28]. However, the PSM glycopeptide having a Gal→GalNAc sugar sequence at the non-reducing end is a weaker inhibitor against *R. communis* hemagglutinin than phenyl β-D-galactopyranoside, as shown in Tables III and IV. Each of the third group of phytohemagglutinins can bind to either Gal→GalNAc or Gal→GlcNAc sugar sequences. From Table III, it is also interesting to note that the inhibitory activity of the PSM glycopeptide I against *A. hypogoea* hemagglutinin was most strongly affected by the presence of a sialic acid residue. This seems to be due to the coulombic effect exerted by the sialic acid residue

TABLE IV

COMPARISON OF HEMAGGLUTINATION INHIBITORY ACTIVITIES OF VARIOUS GLYCOSIDES

	Minimum concentration (mM) *completely inhibiting 4 hemagglutinating doses*								
	Bauhinia purpurea	Arachis hypogoea	Maackia amurensis (*hemagglutinin*)	Sophora japonica (*SJ-III*)	Glycine max	Wistaria floribunda (*hemagglutinin*)	Maackia amurensis (*mitogen*)	Ricinus communis (*hemagglutinin*)	Phaseolus vulgaris (*PHA-M*)
Phenyl α-D-galactopyranoside	6.3	6.3	>50	13	1.6	3.1	>50	25	>50
Phenyl β-D-galactopyranoside	3.1	13	>50	6.3	3.1	3.1	>50	1.6	>50
Phenyl *N*-acetyl-α-D-galactosaminide	1.6	>50	>50	1.6	0.05	0.10	>50	>50	>50
Phenyl *N*-acetyl-β-D-galactosaminide	1.6	>50	>50	0.80	0.10	0.05	>50	>50	>50
Methyl *N*-acetyl-α-D-galactosaminide	0.67	>85	>85	2.7	0.01	0.04	>85	>85	>85
Methyl *N*-acetyl-β-D-galactosaminide	0.67	>85	>85	2.7	0.02	0.04	>85	>85	>85
N-Acetyllactosamine	6.5	6.5	>52	0.81	0.81	0.20	>52	1.6	>52

against the interaction of *A. hypogoea* hemagglutinin with the glycopeptide, because this hemagglutinin contains a high proportion of acidic amino acids and a low proportion of basic amino acids[11]. Furthermore, from Table III, we can see that *A. hypogoea* hemagglutinin has a much stronger affinity for D-galactose than for *N*-acetyl-D-galactosamine residues, whereas *G. max* and *W. floribunda* hemagglutinins seem to be more specific for the *N*-acetyl-D-galactosamine residue; thus, desialized BSM glycopeptide, which is practically free from neutral sugars (Table II), does not inhibit *A. hypogoea* hemagglutinin, but strongly inhibits *G. max* and *W. floribunda* hemagglutinins.

These characteristics of the hemagglutinins were also demonstrated by the hemagglutination inhibition assays using *N*-acetyllactosamine (2-acetamido-2-deoxy-4-*O*-*β*-D-galactopyranosyl-D-glucose) and methyl and phenyl glycosides of both D-galactose and *N*-acetyl-D-galactosamine as hapten inhibitors. As shown in Table IV, *N*-acetyllactosamine strongly inhibits *S. japonica*, *G. max*, *W. floribunda*, and *R. communis* hemagglutinins, while this disaccharide is a relatively weak inhibitor for *B. purpurea* and *A. hypogoea* hemagglutinins. In the experiments using methyl and phenyl glycosides as hapten inhibitors, *A. hypogoea* hemagglutinin was strongly inhibited by both *α* and *β* anomers of the D-galactopyranosides but not by the *N*-acetyl *α*(and *β*)-D-galactosaminides, whereas these glycosides of *N*-acetyl-D-galactosamine were much more potent inhibitors against *G. max*, *W. floribunda*, and *S. japonica* hemagglutinins than the corresponding glycosides of D-galactose. The strong affinity of *G. max*[29] and *S. japonica*[30,31] hemagglutinins for *N*-acetyl-D-galactosamine has already been reported. Another interesting finding from Table IV is that *R. communis* hemagglutinin is not inhibited by the glycosides of *N*-acetyl-D-galactosamine and favored the *β*-glycoside of D-galactose over the *α*-glycoside (*cf.* Ref. 32). These results are consistent with those of the hemagglutination inhibition assays using various glycopeptides as hapten inhibitors.

Thus, we conclude that members of the first group of phytohemagglutinins bind primarily to *O*-glycosidically linked carbohydrate chains of the human erythrocyte membrane glycoprotein, whereas those of the second group bind preferentially to *N*-glycosidically linked carbohydrate chains of the cell-membrane glycoprotein. Members of the third group of phytohemagglutinins can probably bind to either *O*-glycosidically linked carbohydrate chains or *N*-glycosidically linked carbohydrate chains on the cell surface. Our recent studies on the competitive binding of various ^{131}I-labelled phytohemagglutinins to human erythrocytes confirmed that *B. purpurea* and *M. amurensis* hemagglutinins share a common, possibly *O*-glycosidically linked, oligosaccharide chain, and *R. communis* hemagglutinin and *M. amurensis* mitogen share the other, possibly *N*-glycosidically linked oligosaccharide chain, on the erythrocyte surface[33].

From the results of this investigation, it is interesting to note that the phytohemagglutinins that principally interact with the Gal → GlcNAc sugar sequence, *i.e.*, *P. vulgaris* hemagglutinin[34], *M. amurensis* mitogen[7], and *R. communis* hemagglutinin[35,36], show strong mitogenic activity against human peripheral lymphocytes,

and that the phytohemagglutinins that can interact with the Gal→GlcNAc sugar sequence as well as the Gal→GalNAc sugar sequence, *i.e.*, *S. japonica* hemagglutinin (SJ-III)[8], *G. max* hemagglutinin[37], and *W. floribunda* hemagglutinin[38], show weak but definite mitogenic activity against neuraminidase-treated, human peripheral lymphocytes. On the other hand, the phytohemagglutinins that principally interact only with the Gal→GalNAc sugar sequence do not show mitogenic acitvity. Continued study on the relationship between the specificity of mitogens and the structure of their receptors on the lymphocyte cell-surface is needed for the analysis of the mitogenic mechanism.

REFERENCES

1 O. MÄKELÄ, *Ann. Med. Exp. Biol. Fenn.*, 35 (1957) Suppl. 11, 133.
2 I. MATSUMOTO AND T. OSAWA, *Vox Sang.*, 21 (1971) 548.
3 S. TOYOSHIMA, M. FUKUDA, AND T. OSAWA, *Biochemistry*, 11 (1972) 4000.
4 I. MATSUMOTO AND T. OSAWA, *Biochemistry*, 13 (1974) 582.
5 T. IRIMURA AND T. OSAWA, *Arch. Biochem. Biophys.*, 151 (1972) 475.
6 M. TOMITA, T. KUROKAWA, K. ONOZAKI, N. ICHIKI, T. OSAWA, AND T. UKITA, *Experientia*, 28 (1972) 84.
7 T. KAWAGUCHI, I. MATSUMOTO, AND T. OSAWA, *J. Biol. Chem.*, 249 (1974) 2786.
8 T. TERAO AND T. OSAWA, *J. Biochem. (Tokyo)*, 74 (1973) 199.
9 S. TOYOSHIMA, Y. AKIYAMA, K. NAKANO, A. TONOMURA, AND T. OSAWA, *Biochemistry*, 10 (1971) 4457.
10 J. A. GORDON, S. BLUMBERG, H. LIS, AND N. SHARON, *FEBS Lett.*, 24 (1972) 193.
11 T. TERAO AND T. OSAWA, unpublished results.
12 N. UI AND O. TARUTANI, *J. Biochem. (Tokyo)*, 50 (1961) 508.
13 R. L. KATZMAN AND E. H. EYLAR, *Arch. Biochem. Biophys.*, 117 (1966) 623.
14 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350.
15 G. W. JOURDIAN, L. DEAN, AND S. ROSEMAN, *J. Biol. Chem.*, 246 (1971) 430.
16 M. FUKUDA AND F. EGAMI, *Biochem. J.*, 123 (1971) 407.
17 D. M. CARLSON, *J. Biol. Chem.*, 243 (1968) 616.
18 I. MATSUMOTO AND T. OSAWA, *Arch. Biochem. Biophys.*, 140 (1970) 484.
19 Y. AKIYAMA AND T. OSAWA, *Z. Physiol. Chem.*, 353 (1972) 323.
20 R. BELCHER, A. J. NUTTEN, AND C. M. SAMBROOK, *Analyst*, 79 (1954) 201.
21 D. M. CARLSON, *J. Biol. Chem.*, 241 (1966) 2984.
22 E. H. EYLAR AND R. L. KATZMAN, *Fed. Proc.*, 26 (1967) 832.
23 D. B. THOMAS AND R. J. WINZLER, *J. Biol. Chem.*, 244 (1969) 5943.
24 A. GOTTSCHALK, A. S. BHARGAVA, AND V. L. N. MURTY, in A. GOTTSCHALK (Ed.), *Glycoproteins*, Elsevier, Amsterdam, 1972, p. 810.
25 S. TOYOSHIMA, M. FUKUDA, AND T. OSAWA, *Biochem. Biophys. Res. Commun.*, 51 (1973) 945.
26 M. FUKUDA AND E. EGAMI, *Biochem. J.*, 123 (1971) 415.
27 S. KORNFELD AND R. KORNFELD, in G. A. JAMIESON AND T. J. GREENWALT (Eds.), *Glycoproteins of Blood Cells & Plasma*, Lippincott, Philadelphia, 1971, p. 50.
28 G. L. NICOLSON AND J. BLAUSTEIN, *Biochim. Biophys. Acta*, 266 (1972) 543.
29 H. LIS, B. SELA, L. SACHS, AND N. SHARON, *Biochim. Biophys. Acta*, 211 (1970) 582.
30 T. OSAWA AND S. AKIYA, *Bull. Tokyo Med. Dent. Univ.*, 8 (1961) 299.
31 R. D. PORETZ, H. RISS, J. W. TIMBERLAKE, AND S. CHIEN, *Biochemistry*, 13 (1974) 250.
32 G. L. NICOLSON, J. BLAUSTEIN, AND M. E. ETZLER, *Biochemistry*, 13 (1974) 196.
33 T. KAWAGUCHI, I. MATSUMOTO, AND T. OSAWA, *Biochemistry*, 13 (1974) 3169.
34 P. C. NOWELL, *Cancer Res.*, 20 (1960) 462.
35 S. OLSNES, E. SALTVEDT, AND A. PIHL, *J. Biol. Chem.*, 249 (1974) 803.
36 T. KISHIYE, S. TOYOSHIMA, AND T. OSAWA, *Biochem. Biophys. Res. Commun.*, 60 (1974) 681.
37 A. NOVOGRODSKY AND E. KATCHALSKI, *Proc. Nat. Acad. Sci. U. S.*, 70 (1973) 2515.
38 S. TOYOSHIMA AND T. OSAWA, *J. Biol. Chem.*, in press.