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PURIFICATION AND SACCHARIDE-BINDING CHARACTERISTICS OF A RICE LECTIN*

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ABSTRACT

A lectin was purified from rice flour by aqueous extraction followed by precipitation by ammonium sulfate and affinity chromatography on *p*-aminobenzyl 2-acetamido-2-deoxy-1-thio-β-D-glucoside–succinyl-aminohexylaminyl–Sepharose 4B. The molecular weight of the lectin is ~36,000, as determined by sedimentation-equilibrium analysis. It is a tetramer consisting of two different subunits (M_r = 12,000 ±1,000 and 9,000 ±1,000). Amino acid analysis indicated that the lectin contains very high proportions of half-cystine, glycine, and glutamic acid. All of the half-cystines are present as -S-S- bridges. The lectin agglutinates human A, B, AB, and O erythrocytes, rabbit erythrocytes, human leukocytes, and is mitogenic to human lymphocytes. The hemagglutinating activity of rice lectin is inhibited by 2-acetamido-2-deoxy-D-glucose, methyl 2-acetamido-2-deoxy-β-D-glucoside, chitobiose, and chitotriose. *N*-Acetylneuraminic acid was a noninhibitor, but *N*-acetylneuramin-(2→3)-lactose showed weak inhibition. The agglutinating activity was also inhibited by various sialoglycoproteins. The immobilized rice-lectin bound glycophorin, α_1-acid glycoprotein, and fetuin. Asialoglycophorin, asialofetuin, ovomucoid, and human chorionic gonadotropin were bound only partially to the column.

INTRODUCTION

The presence of a lectin in rice seeds was first reported by Takahashi *et al.*[1]. They purified the lectin by ion-exchange and molecular-sieve chromatography and reported that it was a glycoprotein having a molecular weight of 10,000. We (I.P. and H.S.S.) purified a lectin from rice seeds by affinity chromatography on a

*PBS, phosphate-buffered saline (0.14M Na^+, 0.1M K^+, 6mM PO_4^{3-}, pH 7.2); GlcNAc, 2-acetamido-2-deoxy-D-glucose, NeuNAc, *N*-acetylneuraminic acid; SDS, sodium dodecyl sulfate.

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column of 2-acetamido-2-deoxy-D-glucose–Sepharose 6B and established its specificity for 2-acetamido-2-deoxy-D-glucose[2]. Our lectin was distinct from that of Takahashi *et al.* in that it had a higher molecular weight and was stable to denaturants, pH changes, heat treatment, and limited proteolysis[3].

This paper describes a method for isolation of the rice lectin in high yields and presents recent information on its carbohydrate-binding characteristics.

EXPERIMENTAL

Materials. — Defatted flour from the S-701 variety of rice was prepared as described previously[2]. 2-Acetamido-2-deoxy-D-glucose, *N*-acetylneuraminic acid, 2-acetamido-2-deoxy-D-galactose, *N*-acetylneuraminic acid–lactose, trypsin, neuraminidase *(V. cholerae)*, thyroglobulin, ovalbumin, and ovomucoid were purchased from Sigma Chemical Co., St. Louis, MO. Per-*N*-acetylated chitobiose and chitotriose were gifts from Dr. I. J. Goldstein, University of Michigan, Ann Arbor, MI. Methyl 2-acetamido-2-deoxy-D-glucoside was a gift from Dr. Evelyne Walker-Nasir, Laboratory of Carbohydrate Research, Massachusetts General Hospital, Boston, MA. Toad egg-coat jelly glycoprotein[4] and the perchloric acid-soluble sheep serum glycoproteins[5] were prepared as described. Salivary glycoproteins were isolated from saliva of secretors by precipitation with ammonium sulfate. Pronase was obtained from Kaken Chemical Co., Japan. The following radioactively labelled glycoproteins were available from previous studies[6]: [NeuNAc-^{3}H]glycophorin, [NeuNAc-^{3}H]α_1-acid glycoprotein, [NeuNAc-^{3}H]fetuin, [^{14}C]dimethyl ovalbumin, [^{3}H]acetylovomucoid, [^{3}H]acetyl asialofetuin, and [^{3}H-galactose]asialoglycophorin. [^{125}I]Human chorionic gonadotropin (~60 μCi per μg) was purchased from Wampole Labs., Cranbury, NJ. Phytohemagglutinin was obtained from Burroughs–Wellcome, Kent, U.K. [^{3}H]Thymidine was obtained from BARC, Bombay, India. All other chemicals used were of the highest purity available. Human A, B, AB, and O blood-group blood was obtained from a local hospital.

Methods. — *Preparation of affinity gels.* *p*-Aminobenzyl 2-acetamido-2-deoxy-thio-β-D-glucoside–succinyl-aminohexylaminyl–Sepharose 4B was prepared as described[7]. Rice lectin was coupled to CNBr-activated Sepharose 4B according to the method of March *et al.*[8].

Hemagglutination and hemagglutination-inhibition assays. Hemagglutination assays were performed in multiwell microtitre plates by using a 2% suspension of washed erythrocytes. For agglutination-inhibition assays, the lectin required to give complete agglutination (8 μg in 25 μL) was incubated with various concentrations of the test sugar or glycoprotein (in 25 μL) for 30 min before the addition of 25μL of rabbit erythrocytes. After shaking the mixture on a rotary shaker for 1 h at room temperature, the plates were examined under a microscope and the degree of agglutination or inhibition in each well was assessed.

Enzyme-treated erythrocytes. Trypsinized erythrocytes were prepared by in-

cubating 10 parts of a 2% cell suspension with one part of 1% trypsin solution in PBS for 1 h at 37°. The cells were washed several times by resuspension in PBS and centrifugation, and were made up to a final concentration of 2%.

Pronase-treated erythrocytes were prepared as just described, but using one part of 0.5% pronase and incubating for only 15 min.

Neuraminidase-treated erythrocytes were prepared by treating 10 mL of a 2% cell suspension in PBS with 2 units of *V. cholerae* neuraminidase for 1 h at 37°. The cells were washed with PBS and suspended to a final concentration of 2%.

Assay of mitogenic activity. Mitogenicity of rice lectin was determined by measuring the [^{3}H]thymidine incorporated by human peripheral lymphocytes in the presence and absence of the lectin as described by Wybran *et al.*[9] Phytohemagglutinin (PHA) was used as a positive control.

Electrophoresis. Polyacrylamide-gel electrophoresis in 7.5% gels at pH 4.3 was carried out in 0.05M β-alanine–acetate buffer (calculated using the Henderson–Hasselbalch equation), as described by Reisfeld *et al.*[10]. SDS gel electrophoresis in 12.5% gels was conducted as described by Laemmli[11].

Molecular weight. Molecular weight of the lectin was determined by sedimentation-equilibrium analysis on a Beckman Model E analytical ultracentrifuge. The sample, dissolved in PBS, was run at 25° in a three-channel cell at three different concentrations[12].

Protein estimation and amino acid analysis. Protein was estimated by the method of Lowry *et al.*[13]. For amino acid analysis, the rice lectin (50 μg) was dialyzed against 0.1M acetic acid, lyophilized, and hydrolyzed *in vacuo* with 6M HCl, for 24 h at 110°. The dried hydrolyzates were dissolved in buffer and an aliquot analyzed on a Dionex amino acid analyzer. For the estimation of cysteine, the sample was hydrolyzed in the presence of dimethyl sulfoxide according to Spencer and Wold[14] and analyzed as before. Free SH groups were estimated by Ellman's method, using DTNB in 3M guanidine thiocyanate[15].

Affinity chromatography of glycoproteins on a rice lectin–Sepharose 4B column. The radioactively labelled sample was applied in a volume of 50–100 μL to a lectin column (2 mL of gel) equilibrated with PBS. The sample was washed twice into the column with ~0.5 mL of buffer and allowed to incubate for ~15 min. The column was then eluted with the foregoing buffer, followed by 0.1M 2-acetamido-2-deoxy-D-glucose in the same buffer, and fractions of 1 mL were collected. The flow rate of the column was controlled to ~10–15 mL per h. The radioactivity in the eluted fractions was measured after mixing with 10 mL of scintillation-counting liquid. The total radioactivity recovered from the column was 70–100%.

RESULTS

Purification of the lectin. — Defatted rice flour (50 g) was stirred in 300 mL of PBS for 2–4 h at 4° and the extract centrifuged. The crude lectin was precipitated

by the addition of solid ammonium sulfate to 50% saturation. The precipitate was collected by centrifugation, dissolved in 50 mL of PBS, and dialyzed against PBS. The dialyzed solution was applied to a column (1 × 13 cm) packed with *p*-aminobenzyl 2-acetamido-2-deoxy-1-thio-β-D-glucoside–succinyl-aminohexyl-aminyl–Sepharose 4B. The column was washed with PBS to remove all unbound material, and the bound lectin was eluted with 25 mL of 0.1M acetic acid containing 2-acetamido-2-deoxy-D-glucose (50 mg/mL). The eluate was collected in fractions and assayed for absorbance at 280 nm. The result of a typical experiment is shown in Fig. 1. The fractions containing the lectin were combined, dialyzed against water, and lyophilized. The yield of the lectin was 7–8 mg per 50 g of the rice flour. We also tested the use of chitin as an affinity ligand for the lectin. The lectin bound so tightly to the chitin column that it could not be eluted with GlcNAc or acetic acid, but only with 0.1M HCl. However, several other proteins also bound non-specifically to chitin and were eluted with HCl.

Purity and molecular weight. — The purified lectin gave a single band on polyacrylamide-gel electrophoresis at pH 4.3 under non-denaturing conditions (Fig. 2, lane A). The molecular weight of the lectin was estimated to be 36,000 by sedimentation-equilibrium analysis, using 0.746 for the partial specific volume of

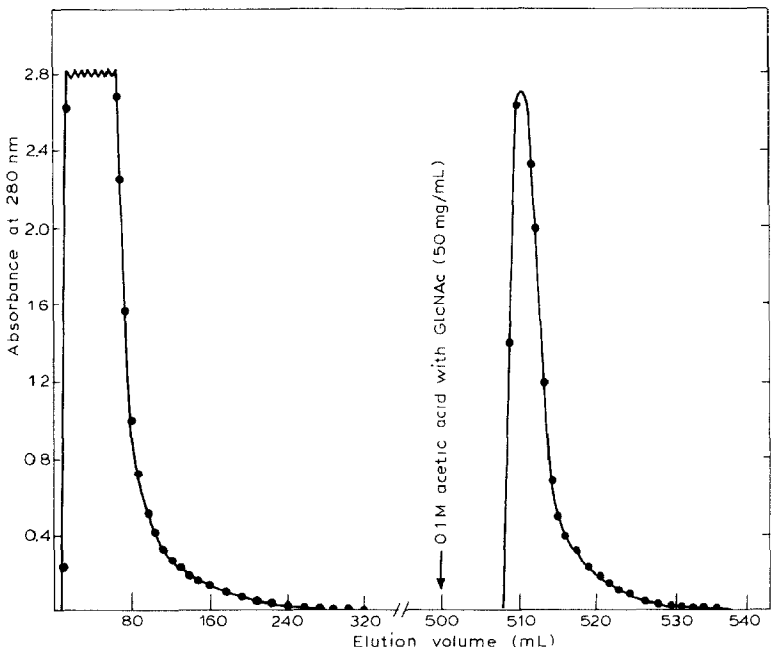

Fig. 1. Affinity purification of rice lectin on *p*-aminobenzyl 2-acetamido-2-deoxy-1-thio-β-D-glucoside–succinyl-aminohexylaminyl–Sepharose 4B column (1 × 13 cm). The ammonium sulfate fraction (0–50% saturation) was dissolved in PBS, applied to the column, and the column eluted with PBS (500 mL) to remove unbound material. The bound lectin was eluted with 25 mL of 0.1M acetic acid containing GlcNAc (50 mg/mL). Fractions of 1 mL were collected and analyzed for 280nm-absorbing material.

the lectin[12]. On polyacrylamide-gel electrophoresis in the presence of SDS and 2-mercaptoethanol the lectin, if heated in sample buffer for 5 min, showed two bands (M_r 12,000 ±1,000 and 9,000 ±1,000) (Fig. 3, lane A). When the lectin was heated in sample buffer for only 2 min, three bands having M_r 20,000 ±1,000; 12,000 ±1,000, and 9,000 ±1,000 (Fig. 3, lane B) were observed. The amino acid composition of the purified lectin is presented in Table I; the very high content of half cystine, and glycine is noteworthy. Aspartic and glutamic acids, serine and alanine are also rather high. No free SH groups were detectable by the Ellman method.

Agglutination and inhibition of agglutination. — Rice lectin agglutinated human erythrocytes of blood groups A, AB, B, and O groups equally well. It also agglutinated rabbit erythrocytes. The agglutinability of the rabbit erythrocytes increased after limited treatment with proteases (trypsin and pronase), so that the

Fig. 2. Polyacrylamide-gel electrophoresis of the purified rice lectin. About 40 μg of lectin was applied to 7.5% gels in 0.05M β-alanine–acetate buffer, pH 4.3. Lectin isolated by extraction with *A*, PBS; *B*, citrate buffer pH 4.0.

TABLE I

AMINO ACID COMPOSITION OF RICE LECTIN

Amino acid	*Residues per molecule*[a]	*Amino acid*	*Residues per molecule*[b]
Half-cystine[b]	51	Isoleucine	10
Aspartic acid	36	Leucine	22
Threonine	14	Tyrosine	10
Serine	33	Phenylalanine	8
Glutamic acid	49	Histidine	8
Glycine	68	Lysine	20
Alanine	31	Arginine	17
Valine	14		
Methionine	6	Total	397

[a]The molecular weight of the lectin was assumed to be 36,000, and the values for the nearest integer are presented. Proline was not estimated. [b]Estimated as cysteic acid after hydrolysis of the protein with Me_2SO.

TABLE II

INHIBITORY EFFECT OF VARIOUS SACCHARIDES[a] ON THE AGGLUTINATING ACTIVITY OF RICE LECTIN[b]

Sugar	*Concentration needed for 50% inhibition (mM)*
2-Acetamido-2-deoxy-D-glucose	15.0
Methyl 2-acetamido-2-deoxy-β-D-glucoside	1.4
2-Amino-2-deoxy-D-glucose	(0% at 266mM)
D-Glucose	(0% at 400mM)
2-Acetamido-2-deoxy-D-galactose	66.5
N-Acetylneuraminic acid	(0% at 66.7mM)
N,N′-Di-*N*-acetylchitobiose	0.036
N,N′,N″-Tri-*N*-acetylchitotriose	0 0027
N-Acetylneuraminyl-(2→3 and 2→6)-β-D-galactopyranosyl-(1→4)-D-glucopyranose	8.4

[a]Maltose, lactose, sucrose, melibiose, and melizitose were all noninhibitory up to 100mM. [b]The assay was performed in a total volume of 75 μL and contained 8 μg of lectin.

specific activity of the lectin with treated erythrocytes was ~3,000 times more than with untreated erythrocytes. Neuraminidase treatment of the erythrocytes did not alter their agglutinability by the lectin. Rice lectin also agglutinated untreated leukocytes from normal individuals and leukemic patients equally well.

The results of the hemagglutination inhibition experiment are summarized in Tables II and III. The agglutination of rabbit erythrocytes by rice lectin was inhibited by 2-acetamido-2-deoxy-2-glucose, its analogs, and polymers. Per *N*-acetylated chitotriose and chitobiose are the best inhibitors, followed by the methyl glycoside of 2-acetamido-2-deoxy-D-glucose. 2-Acetamido-2-deoxy-D-glucose and

TABLE III

INHIBITORY EFFECT OF GLYCOPROTEINS ON THE AGGLUTINATING ACTIVITY OF RICE LECTIN[a]

Glycoprotein	*Concentration needed for 50% inhibition (μg/mL)*
Toad egg-coat jelly glycoprotein	2.5
Salivary glycoproteins	10.3
Ovomucoid	12.0
Thyroglobulin	17.0
Sheep serum glycoproteins (0.6M perchloric acid-soluble fraction)	20.6
Ovalbumin	67.0

[a]The assay was performed in a total volume of 75 μL and contained 8 μg of lectin.

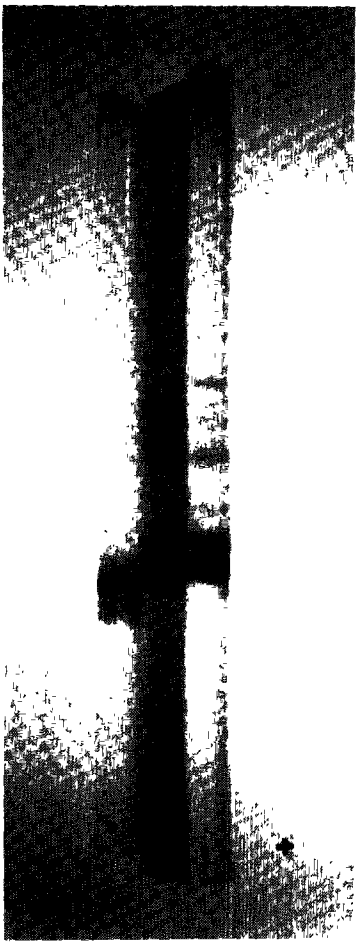

Fig. 3. Polyacrylamide-gel electrophoresis of rice lectin on 12.5% gels in the presence of SDS. About 30 μg of the lyophilized rice lectin was dissolved in the stacking-gel buffer containing 0.1% SDS, 5% 2-mercaptoethanol, and 10% glycerol, heated at 100° as indicated. Lectin was isolated by extraction with *A*: PBS and heated in SDS buffer for 5 min; *B*: PBS, but heated in SDS buffer for only 2 min; *C*: citrate buffer pH 4.0 and heated in SDS buffer for 5 min.

TABLE IV

INTERACTION OF LABELED GLYCOPROTEINS WITH RICE LECTIN–SEPHAROSE 4B

Glycoprotein	*% Radioactivity eluted with*	
	PBS	*0.1M GlcNAc*
[NeuNAc^{3}H]glycophorin	11.0	73.8
[NeuNAc^{3}H]fetuin	31.4	63.3
[NeuNAc^{3}H]α_1-acid glycoprotein	24.5	47.0
[^{3}H-Gal]asialo glycophorin	71.1	19.6
[^{3}H-Gal]asialofetuin	71.5	5.2
[^{3}H]acetyl ovomucoid	79.1	21.0
[^{14}C]dimethyl ovalbumin	79.4	0
[^{125}I]human chorionic gonadotropin	71.1	16.1

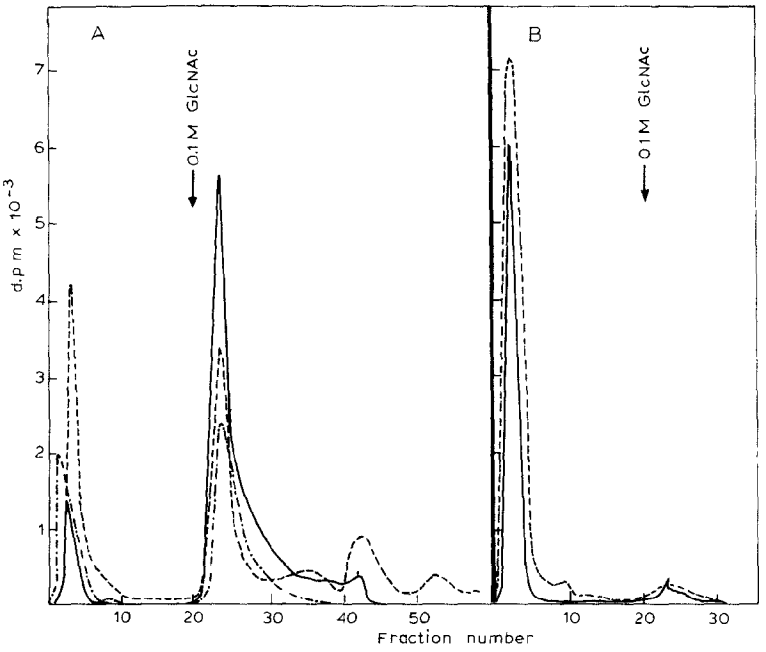

Fig. 4. A. Affinity chromatography of [NeuNAc^{3}H]glycophorin (——), [NeuNAc^{3}H]fetuin (— · — · — ·) and [NeuNAc^{3}H]α_1-acid glycoprotein (-----) on a column of rice lectin–Sepharose 4B as described in the text. B. [^{3}H Galactose]asialoglycophorin (——) and [^{3}H]acetyl asialofetuin (— · — · — ·) on a rice-lectin–Sepharose 4B column as described in the text.

N-acetylneuraminyl→lactose were respectively 5650 and 3110 times less inhibitory than tri-*N*-acetylchitotriose. D-Glucose, 2-amino-deoxy-D-glucose, and *N*-acetylneuraminic acid were noninhibitory at 30, 20, and 5μM, respectively.

Mitogenic activity of rice lectin. — Rice lectin stimulated the incorporation of [^{3}H]thymidine by human peripheral lymphocytes. [^{3}H]thymidine incorporated by lymphocytes when cultured in the presence of 0, 5, 10, 25, and 50 μg of rice lectin

per mL of medium was 50, 330, 420, 1116, and 1260 c.p.m. per 10^6 cells, respectively. In control experiments, cells cultivated in the presence of 200 μg of PHA per mL of medium incorporated 3067 c.p.m. of [^{3}H]thymidine per 10^6 cells.

Interaction of labelled glycoproteins with rice lectin immobilized on Sepharose. — The amount of lectin covalently bound to the Sepharose was calculated to be 1.5 mg/mL of the gel, by estimating the unbound protein in the supernatant solution and washes after the coupling reaction. The results of the specific interaction of different glycoproteins with immobilized rice lectin are summarized in Table IV and Fig. 4. Glycophorin, fetuin, and α_1-acid glycoprotein were specifically bound to the column and whereas the first two were readily eluted with 0.1M 2-acetamido-2-deoxy-D-glucose, α_1-acid glycoprotein was not. Asialofetuin and asialoglycophorin were only partially retained on the column. Ovomucoid, ovalbumin, and human chorionic gonadotropin were also bound to the column only partially.

DISCUSSION

We had previously extracted rice lectin with 0.05M sodium citrate buffer, pH 4.0, and purified it on a column of 2-acetamido-2-deoxy-D-glucose–epoxy-Sepharose 6B, resulting in a yield of 0.5 mg per 50 g of rice flour[2]. In the present study, we found that extraction at neutral pH with PBS resulted in higher yields. As good yields of 2-acetamido-2-deoxy-D-glucose-binding lectins have been obtained using *p*-aminobenzyl 2-acetamido-2-deoxy-1-thio-β-D-glucoside–succinyl-amino-hexylaminyl–Sepharose 4B[7,16,17], in this study we tested this affinity gel. The bound lectin could be eluted from the affinity column with 0.1M acetic acid, but slight turbidity developed in the eluate which could be circumvented by including GlcNAc (50 mg/mL) in the acetic acid. GlcNAc alone could not elute the lectin, even at 100 mg/mL. The total yields obtained (~8 mg per 50 g of rice flour) are the highest reported for rice lectin. The lectin could also be eluted from the column with 0.1M ammonium hydroxide, as reported for potato lectin[18], but the acid-eluted lectin was ten times more active in the agglutination assays than the alkali-eluted lectin.

Considerable differences have been reported for the molecular size of the rice lectin. In our previous studies, we observed two molecular species of the lectin on Sephadex G-100 (M_r 85,000 and 14,500), which on SDS-PAGE showed two bands of M_r 20,000 and 17,000 (ref. 3). Takahashi *et al.*[1] reported a single subunit of M_r 10,000 for their preparation. A value of 37,000 and two subunits of M_r 11,000 and 8,000 was reported for the rice-bran lectin[19]. Recently Tabary *et al.*[20] purified lectin from rice embryos, but no information was given regarding the molecular nature of the lectin. To determine the reason for these differences we isolated the lectin at neutral pH (extraction with PBS as described in this paper) and acidic pH (extraction with citrate–phosphate buffer, pH 4.0 as described previously), and compared its molecular properties by gel electrophoresis.

The lectin isolated at neutral pH exhibited lower mobility on electrophoresis

in non-denaturing gels at pH 4.3 as compared with the lectin isolated at acidic pH (Fig. 2, lanes A and B). On SDS-gel electrophoresis, the lectin isolated at neutral pH gave either two bands (M_r 12,000 ±1,000 and 9,000 ±1,000) or three bands (M_r 20,000 ±1,000, 12,000 ±1,000, and 9,000 ±1,000), depending on whether the lectin had been heated for 5 or 2 min with the SDS buffer (Fig. 3, lanes A and B). In contrast, the lectin isolated at acidic pH, whether heated with SDS buffer for 2 or 5 min, gave bands of M_r 25,000 and 17,000 (Fig. 3, lane C) that were distinct from those obtained from the lectin isolated at neutral pH. The reasons for the different behavior of the lectin, isolated by extraction with acidic buffer, on gel electrophoresis, and on gel filtration[3] are not clear. This lectin could be different from the one isolated by extraction with neutral buffer, or it could have undergone structural changes leading to the noted anomalous behavior. Interestingly, the rice-bran lectin, on treatment with acidic buffer, exhibited retarded elution on Sephadex columns, apparently because of interaction of the lectin with Sephadex (Tsuda[19]).

The molecular weight of 36,000 estimated by sedimentation analysis in the present study is the most reliable. This value is in agreement with those obtained by Peumans and Stinissen[21]. These workers also obtained three polypeptides of M_r 23,000, 12,000, and 10,000 on SDS gel electrophoresis and claimed that the 12,000 and 10,000 molecular-weight species were the results of a post-translational, proteolytic cleavage of the 23,000 polypeptide[21]. Our results, however, suggest that the 20,000 polypeptide seen when the lectin is heated for only 2 min with the SDS buffer could arise from incomplete dissociation.

Amino acid analysis indicated a high content of half-cystine residues. Estimation of free -SH groups in the presence of 3M guanidine thiocyanate did not give any free -SH groups, which suggests that all of the half cystines are present as disulfide bridges. The resistance of the lectin to dissociation in the presence of 1% SDS and 5% 2-mercaptoethanol indicates the presence of several inter- and intramolecular-S-S- bridges. In general, amino acid analysis indicated many differences between rice-seed and rice-bran lectins[19]. It was reported that rice-bran lectin has ~44 residues on half cystines per 19,000, and we have obtained 51 residues per 36,000. High histidine, lysine, leucine, threonine, and alanine are obtained for rice lectin, as compared with the rice-bran lectin. No valine is reported for rice-bran lectin, but we detected ~14 residues per mol of protein. The amino acid analysis clearly indicates that our rice-seed lectin is different from the rice-bran lectin purified by Tsuda.

Agglutination of rabbit erythrocytes was inhibited by various sugars. The best monosaccharide inhibitor is methyl 2-acetamido-2-deoxy-β-D-glucoside (10 times more inhibitory than 2-acetamido-2-deoxy-D-glucose). The poor inhibitory power of 2-acetamido-2-deoxy-D-galactose and the noninhibitory effect of 2-amino-2-deoxy-D-glucose demonstrates the necessity for an equatorial hydroxyl group at C-4 and an acetamido group in the D-*glycero* configuration at C-2 for binding to the rice lectin, as in the case of wheat-germ agglutinin and GS II lectins[22,23]. Of the neutral oligosaccharides examined, only those containing 2-acetamido-2-deoxy-D-glucose

residues proved effective. Maltose, lactose, sucrose, melibiose, and melizitose were all inactive. The high affinity to chitin oligomers and to chitin suggests that rice lectin has an extended binding-site which may be complementary to a sequence of β-D-(1→4)-linked 2-acetamido-2-deoxy-D-glucose units. "Neuramin–lactose", in which *N*-acetylneuraminic acid is α-linked, was inhibitory, whereas free *N*-acetylneuraminic acid, which is mainly in the β anomeric form, was non-inhibitory.

The hemagglutination-inhibition experiments with glycoproteins showed that the mucin-type glycoproteins, such as toad-jelly glycoprotein and salivary glycoproteins are potent inhibitors, which may be attributable to the presence of several terminal sialic acid residues on the *O*-glycosidically linked sialyloligosaccharides. In view of this, our finding that neuraminidase treatment of erythrocytes did not alter their agglutinability by the lectin was unexpected. It is possible that the removal of *N*-acetylneuraminic acid may lead to topographical alterations at the erythrocyte cell-surface, leading to favorable presentation of 2-acetamido-2-deoxy-D-glucose residues for interaction with the lectin. In this context, it is interesting that, even though the treatment of various cells with neuraminidase decreased their agglutinability by wheat-germ agglutinin, not all cells are affected this way, since neuraminidase digestion had no effect on the wheat-germ agglutinin-induced agglutination of Novikoff tumor cells[24]. The inhibitory effect of glycoproteins with such *N*-glycosylically linked carbohydrates as ovalbumin and ovomucoid is probably due to terminal non-reducing as well as internal 2-acetamido-2-deoxy-D-glucose residues of their saccharides.

The immobilized rice-lectin bound different glycoproteins in different proportions. Glycophorin, the major sialoglycoprotein of human erythrocyte membrane, which has 15 *O*-glycosidically linked oligosaccharides and one *N*-glycosylically linked oligosaccharide, was efficiently bound to the column. This binding arises from interaction of several terminal sialic acid residues and internal GlcNAc residues of the *N*-linked saccharide with rice lectin, as the desialylated molecules bound only partially. About 70% of the fetuin was bound to the column, whereas only 28% of asialofetuin was bound. The binding of fetuin may also be through the terminal sialic acids and the internal GlcNAc residues of three *N*-glycosylically linked oligosaccharides. The results obtained with α_1-acid glycoprotein also support the notion that sialic acids in glycoproteins are involved in interaction with rice lectin. Human chorionic gonadotropin, which has four *N*-glycosylically linked[25], and four *O*-glycosylically linked[26] oligosaccharide chains, was partially bound to the column. Recently, it has been shown that the *O*-linked oligosaccharides are present as di-, tri-, tetra-, and hexa-saccharides in different proportions[27]. The partial binding of human chorionic gonadotropin may be due to microheterogeneity arising from the uneven distribution of the terminal *N*-acetylneuraminic acid residues in the molecule. It has been previously reported that the binding of wheat-germ agglutinin to sialoglycoproteins depends on the clustering of sialyloligosaccharides[6] and that the binding of *Ricinus communis* agglutinin II to glycoproteins is influenced by the relative locations of the β-D-Gal-(1→3)-D-Gal-

NAc on the peptide backbone[28]. The poor binding and low inhibitory activity of ovalbumin could be attributable to the absence of *N*-acetylneuraminic acid residues and the low density of non-reducing terminal 2-acetamido-2-deoxy-D-glucose residues[29]. The poor binding of ovomucoid, which has several non-reducing GlcNAc terminals[30] is very surprising, in view of the good inhibition of hemagglutination by this glycoprotein. One possible explanation for this could be that, as already discussed, the spacing of the saccharide on the peptide may be unfavorable for affinity binding to the lectin–Sepharose beads. Further work is necessary to understand the exact carbohydrate structures required for binding to rice lectin.

The foregoing data clarify certain differences found in the literature about the molecular nature of the rice lectin, and also provide a method for purifying the lectin in good yields. However, the exact relationship between the lectins from rice seeds, rice bran, and rice embryos remains to be established. The immobilized rice lectin, in combination with other lectin columns of different sugar-binding specificities, could be useful tools in structural and functional characterization of membrane glycoconjugates.

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