Purification and Characterization of a Lectin from *Phaseolus vulgaris* Seed

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Received July 19, 1979

A method is presented for the isolation of a lectin from a Japanese cultivar of *Phaseolus vulgaris* seed by an affinity chromatography on the heat-denaturated porcine thyroglobulin-Sepharose. The lectin is a glycoprotein whose molecular weight is 120,000; it consists of four apparently identical subunits, held together by non-covalent forces. The isoelectric point is 5.5 and the sedimentation constant is 6.66 S at pH 5.3. The lectin is nonspecific in agglutination for any types of human erythrocytes. The lectin induces mitosis in human lymphocytes at concentration between 10 and 100 μg per 3×10^6 lymphocytes. The hemagglutinating and mitogenic activities are inhibited by *N*-acetyl-D-galactosamine and some simple sugars.

Plant lectins isolated from wide varieties of plants have recently attracted great interest because of their remarkable effects in biological actions such as hemagglutination, lymphocyte transformation, inactivation of certain types of tumor cells, precipitation reaction with some species of polysaccharides and glycoproteins, contribution to the poor nutritive value of unheated legumes, etc. More recently, the lectins as the carbohydrate binding proteins have been investigated and utilized in various biochemical fields. Some authors have described the purification and chemical and biological properties of lectins from many kinds of plant seeds, mainly from the different cultivars of *Phaseolus vulgaris* L.^{1~6)} The lectins from the species have

also investigated with an interest possibly because of the importance of these beans for human nutrition.

This paper describes the purification and characterization of a lectin of the *P. vulgaris* seed (Tora-mame).

MATERIALS AND METHODS

PHA-P, a crude lectin which was isolated from red kidney bean, was purchased from Difco. L-PHA, agglutinated only leucocytes, and H-PHA, with both leuco- and hemagglutinating activities, were obtained from PHA-P according to the method of Allen *et al.*⁸⁾ Standard proteins were obtained from Seikagaku-kogyo Co. Ltd. (Tokyo). Other reagents were purchased from Wako Pure Chemicals Co. Ltd. (Osaka).

Purification of a lectin from Phaseolus vulgaris seeds. A preliminary survey of 10 varieties of *P. vulgaris* seeds cultivated in Japan revealed that the saline (0.9% NaCl) extract of the variety, Tora-mame, one of the Japanese cultivars of the common beans (*P. vulgaris*), with the highest hemagglutinating activity was chosen as the starting material for purification of the lectin.

The ground beans (100 g) was suspended in 1 liter deionized water. After continuous stirring for 1 hr, the suspension was adjusted to pH 2.0 with conc. HCl and allowed to stand overnight at 4°C. The

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Abbreviations: PTG, porcine thyroglobulin; BSA, bovine serum albumin; PHA(s), phytohemagglutinin(s); SDS, sodium dodecyl sulfate; EDTA, ethylenediamine tetraacetate.

clear supernatant was siphoned off, and sufficient solid ammonium sulfate was added to 0.5 saturation. The precipitate formed was removed by centrifugation, and an additional quantity of solid ammonium sulfate added to the supernatant to give a 0.75 saturated solution. The precipitate was centrifuged off. The supernatant and the precipitate as well as the one precipitated at 0.5 saturation with ammonium sulfate were dialyzed against deionized water until free of ammonium ions (negative to Nessler's reagent) and then lyophilized. Over 80% of the hemagglutinating activity present in the original aqueous extract was recovered in the fraction precipitated between 0.5 and 0.75 saturation with ammonium sulfate. Further purification of this fraction was achieved by the affinity chromatography on the heat-denaturated PTG-Sepharose 4B as described in the legend accompanying Fig. 1. The various fractions obtained during the course of this purification procedure were examined for hemagglutinating activity by a photometric technique,⁷⁾ or a 2-fold serial dilution method and for protein concentration by the measurements of absorbance at 280 nm or the Folin phenol reagent according to Lowry *et al.*⁸⁾ using BSA as standard.

Preparation of affinity adsorbent. Affinity adsorbent was prepared by coupling the heat-denaturated PTG to cyanogen bromide activated Sepharose 4B.⁹⁾ PTG was prepared from porcine thyroid glands by the method of Ui and Tarutani.¹⁰⁾ The heat-denaturation of PTG was carried out by heating at 100°C for 5 min.

Disc electrophoresis. Disc electrophoresis on polyacrylamide gel was carried out at pH 8.3 and 4.5 according to Ornstein¹¹⁾ and Reisfeld *et al.*,¹²⁾ respectively, to evaluate the purity of the purified lectin. Staining was performed with amido black 10B for proteins and periodic acid-Schiff¹³⁾ (PAS) reagent for carbohydrates.

Ultracentrifugation. Ultracentrifugation of the purified lectin was performed in Hitachi Ultracentrifuge model UCA-1A equipped with a schlieren optical system at a speed of 51,200 rpm. The observed sedimentation coefficients were converted into standard condition values, $s_{20,w}$, by correcting for density and viscosity of the solvent. The $s_{20,w}^0$ at pH 5.3 was obtained from the $s_{20,w}$ values at four protein concentrations by graphical extrapolation to zero concentration.

Amino acid analyses. The purified lectin was hydrolyzed with 6 N HCl in sealed and evacuated tubes for 24, 48 and 72 hr at 110°C. The amino acid content of the hydrolysates was determined on a amino acid analyzer according to Spackman *et al.*¹⁴⁾ Values for serine and threonine were obtained by

extrapolation to zero time and maximum values were taken for isoleucine and agrinine. The average of the three periods of hydrolysis was used for the remainder of amino acids. Tryptophan was determined by the method of Edelhoch¹⁵⁾ and cystine was determined as cysteic acid.

Sugar analyses. The carbohydrate content was determined by the phenol-sulfuric acid method.¹⁶⁾ Quantitative analysis of the carbohydrate constituents was performed by gas chromatography of acetylated alditols for neutral sugars using 30 mg of the purified lectin and methyl- β -D-glucose as an inner standard substance as described in a previous paper.⁹⁾

Glucosamine was determined separately on the 70 cm column of the amino acid analyzer. In this case, the purified lectin (34 mg) was hydrolyzed with 1 N sulfuric acid for 6 hr at 100°C. The condition was found to give maximum release of glucosamine from this glycoprotein.

Metal analysis. The quantity of metallic cations in the purified lectin was determined by atomic absorption using a Parkin-Elmer model 370 spectrophotometer. An aliquot of 0.2% of the purified lectin dialyzed against 0.05 M Tris-HCl buffer, pH 8.3, was used for metal analysis.

Isoelectric focusing. Isoelectric focusing¹⁷⁾ was carried out in a 110 ml-column using a carrier ampholyte (the pH range of 3~10) at final concentration of 0.5% and a stepwise sucrose gradient (0~50%). Electrophoresis was carried out for 84 hr (at 200 V for the first 4 hr and at 900 V for the remainder hours) and kept around 10°C by cooling with tap water. The column was drained at a flow rate of 1 ml/min and the eluate collected in 2 ml fraction. Each fraction was assayed for pH, optical density at 280 nm and hemagglutinating activity.

Molecular weight estimation. The molecular weight of the purified lectin was estimated from the data of the gel filtration on Sephadex G-200 (1.5 $\times$ 90 cm) with glutamate dehydrogenase, catalase, γ -globulin, aldolase, BSA, α -chymotrypsinogen A, and cytochrome c as the reference proteins.

SDS-polyacrylamide gel electrophoresis. SDS-polyacrylamide gel electrophoresis was carried out according to the method of Weber and Osborn.¹⁸⁾ The molecular weight of the subunit of the lectin was estimated from the relative mobility. BSA, ovalbumin, α -chymotrypsinogen A and cytochrome c served as calibration proteins for the molecular weight estimation of the subunit.

Lymphocyte mitogenic activity. The studies utilized human lymphocytes from tonsils separated by

standard Ficoll-paque density gradient centrifugation. Separated cells were washed in Hank's solution at three times and suspended in TC-medium 199 (Difco) supplemented with 20% calf serum, 100 units/ml of penicillin and 100 μ g/ml of streptomycin sulfate. Three ml of the cell suspension (1×10^6 lymphocytes/ml) were placed in sterile culture tubes that were loosely capped with aluminum foils and incubated at 37°C in an atmosphere of air containing 5% CO₂ (v/v) and, after 60 hr, 3 μ Ci of ³H-thymidine was added. The lectins used were added into the culture tubes before the 60 hr-incubation. The tubes were shaken, incubated for a further 3 hr and then centrifuged after the addition of 2 ml of 0.9% NaCl. The pellets were washed by centrifugation twice with 7% trichloroacetic acid (5 ml; w/v) and once with methanol and were then dissolved in 0.3 ml of formic acid. The solutions were transferred to the scintillation vials with 10 ml of scintillation fluid and radioactivity was counted. In morphological observation, human tonsillar lymphocytes (3×10^6 cells) were incubated with the purified lectin or PHA-P in the conditions as mentioned above. After 63 hr-incubation, the cells were collected by centrifugation and stained with Giemsa's solution. Blasts were counted by observing under a microscope and recorded as a percent of the total cells.

Inhibition studies. a) Hemagglutinating activity: Prior to the hemagglutination assay, EDTA or certain sugar solutions (0.5 ml) were mixed with the lectin solutions (0.5 ml) which were prepared by a serial 2-fold dilutions and these mixtures (1 ml) were incubated at room temperature for 1 hr. After this treatment, the mixtures were incubated with the equal volume of 2.5% rabbit erythrocyte suspension at room temperature for 2.5 hr. Hemagglutinating activity was determined by a photometric technique.⁷⁾ Thus, hemagglutination assay was carried out in the presence of EDTA or sugars. b) Lymphocyte mitogenic activity:

The lectin solutions incubated with certain sugars at 37°C for 30 min were used in the assay system mentioned above.

RESULTS

Purification of Phaseolus vulgaris lectin

Table I summarizes the data pertaining to the purification of the *P. vulgaris* lectin. The majority of the hemagglutinating activity in the pH 2.0 extract was obtained in the precipitate between 0.5 and 0.75 saturation

FIG. 1. Affinity Chromatography of the TM-lectin on Heat-Denatured PTG-Sepharose.

Lyophilized fraction (100 mg) precipitated between 0.5 and 0.75 saturation with ammonium sulfate was applied to a column of PTG-Sepharose (9 ml gel) which was equilibrated with 10 mM phosphate buffer (pH 7.2) containing 0.15 M NaCl. The column was washed with the same buffer and the protein adsorbed was eluted with 0.1 M glycine-HCl buffer (pH 3.0) containing 0.5 M NaCl (at vertical arrow).

TABLE I. DETAILS ON PURIFICATION OF TM-LECTIN

Fraction	Total protein ^a (mg)	Hemagglutinating activity ^b HU ^c	Specific activity HU/mg of protein	Recovery of activity (%)	Purification factor
Crude extract	1684.0	184,832	109.8		1.0
(NH ₄) ₂ SO ₄ fractionation					
0~0.5 satd. ppt.	807.3	7,655	9.5	4.1	
0.5~0.75 satd. ppt.	327.9	154,794	472.1	83.8	4.3
0.75 satd. sup.	90.0	1,201	13.3	0.6	
PTG-Sepharose chromatography					
Peak 1	201.6	trace			
Peak 2	94.1	134,040	1424.4	72.5	13.0

^a determined according to Lowry *et al*⁸⁾ using BSA as standard.

^b performed according to Liener⁷⁾ using trypsinized rabbit erythrocytes.

^c HU: hemagglutinating unit.⁷⁾

FIG. 2. Polyacrylamide Gel Electrophoresis of TM-lectin.

Electrophoresis was carried out on 7.5% gels in acidic (pH 4.5; gels a and b) and basic (pH 8.3; gels c and d) buffer systems. After electrophoresis, the gels were stained with PAS-reagent (gels a and c) and amido black 10B (gels b and d). Electrophoresis conditions: gels a and b, 5 mA per gel for 1 hr; gels c and d, 5 mA per gel for 40 min.

FIG. 3. Sedimentation Patterns of TM-lectin.

Concentration of protein: 10.9 mg/ml in 50 mM acetate buffer containing 0.1 M NaCl (pH 5.3). Photographs, from right to left, were taken at intervals of 6 min.

of ammonium sulfate. This fraction was fractionated by an affinity chromatography on Sepharose 4B coupled with heat-denatured PTG as shown in Fig. 1. The bulk of the activity was recovered in peak 2. The homogeneity of this fraction was shown by the disc electrophoresis and sedimentation patterns in Figs. 2 and 3, respectively. Since the

lectin was stained with PAS-reagent as well as amido black 10B, it was considered as a glycoprotein as shown in Fig. 2. Peak 2 represents a 13-fold increase in hemagglutinating activity over the crude extract. All further studies were done with peak 2 which will hereafter simply be referred to as Tora-mame lectin (TM-lectin).

Chemical composition

Presented in Table II are data pertaining to amino acid composition of the TM-lectin. About 92% of the dry weight of the lectin could be accounted for amino acid residues. The molecular weight of the polypeptide moiety of the lectin, as calculated from the number of amino acid residues per mole, is 110,000.

The percent carbohydrate, expressed as mannose, was found to be 7.5% for the lectin by phenol-sulfuric acid method. Quantitative analysis of carbohydrate constituents was

TABLE II. AMINO ACID COMPOSITION OF TM-LECTIN

Amino acid	Amino acid residues ^a (mol/120,000 g of protein)
Aspartic acid	150.1 (150)
Threonine	103.2 (103)
Serine	124.0 (124)
Glutamic acid	65.1 (65)
Proline	40.8 (41)
Glycine	84.1 (84)
Alanine	79.3 (79)
Cystine	0 (0)
Valine	84.7 (85)
Methionine	0 (0)
Isoleucine	42.4 (42)
Leucine	98.6 (99)
Tyrosine	15.4 (15)
Phenylalanine	44.0 (44)
Lysine	47.7 (48)
Histidine	12.2 (12)
Arginine	20.0 (20)
Tryptophan	26.4 (26)
Total	(1037)
Mol.wt. of protein moiety: 110,340 ^b	

^a Numbers in brackets are number of residues to the nearest whole integer.

^b Calculated from the sum of the individual amino acids from which the water of bond formation was subtracted.

TABLE III. CARBOHYDRATE COMPOSITION OF TM-LECTIN

sugar	Sugar residues	
	g/100 g	mol/120,000 g ^a
Neutral sugars		
L-fucose	0.35	2.89 (3)
L-arabinose	trace	
D-xylose	0.36	2.90 (3)
D-mannose	4.48	33.16 (33)
D-glucose	0.42	3.09 (3)
Amino sugar		
D-glucosamine	2.28	15.26 (15)
Total	7.89	(57)
Mol. wt. of carbohydrate moiety ^b : 9100		

^a Numbers in brackets are number of residues to the nearest whole integer.

^b Calculated from the sum of the individual sugars from which the water of bond formation was subtracted.

TABLE IV. METAL COMPOSITION OF TM-LECTIN

Metal	mg/100 g	g atom/120,000 g ^a
Calcium	32.6	0.98 (1)
Magnesium	12.6	0.63 (1)
Manganese	0	0
Zinc	trace	(0)

^a Numbers in brackets are number of ions to nearest whole integer.

performed by gas chromatography of acetylated alditols for neutral sugars and ion exchange chromatography for the amino sugars. The carbohydrate composition of the lectin is represented in Table III. The molecular weight of the carbohydrate moiety of the lectin, as calculated from the number of sugar residues per mole, is 9100.

Data relating to the metal content of the TM-lectin is included in Table IV. The lectin contained 1 g atom each of calcium and magnesium per mole.

Physicochemical properties

The sedimentation coefficient ($s_{20,w}^0$) calculated from the sedimentation velocity data was 6.66 S at pH 5.3. The TM-lectin has an absorption coefficient of $A_{1\text{cm}}^{1\%} = 12.7$ at 280 nm. The isoelectric point of the lectin was found to be 5.5 from the result of the isoelectric focusing as shown in Fig. 4. The mole-

FIG. 4. Isoelectric Focusing of TM-lectin on a 110 ml Carrier Ampholyte Column in a Gradient from pH 3 to 10.

The figure shows protein concentration (○—○) as measured at 280 nm, pH (●—●) and hemagglutinating activity (△—△).

FIG. 5. Molecular Weight Estimation of TM-lectin on Sephadex G-200.

Arrows: The V_e/V_0 value and the estimated molecular weight of TM-lectin. Reference proteins: a, glutamate dehydrogenase; b, catalase; c, γ -globulin; d, aldolase; e, BSA; f, ovalbumin; g, α -chymotrypsinogen A; h, cytochrome c.

cular weight was calculated to be 120,000 by the gel filtration on Sephadex G-200 (Fig. 5). The subunit structure of the lectin was identified by SDS-polyacrylamide gel electrophoresis. A single band was found on either

FIG. 6. SDS-polyacrylamide Gel Electrophoresis of TM-lectin.

Gel (a) was stained with PAS-reagent and gel (b) with Coomassie brilliant blue. Condition: 8 mA/gel for 2 hr.

FIG. 7. Estimation of the Molecular Weight of the Subunit of TM-lectin by SDS-polyacrylamide Gel Electrophoresis.

Arrows: The relative mobility and the estimated molecular weight of the subunit of TM-lectin. Reference proteins: a, BSA; b, ovalbumin; c, α -chymotrypsinogen A; d, cytochrome c.

stained with Coomassie brilliant blue or PAS-reagent (Fig. 6). The molecular weight of the subunit was calculated to be 30,000 from the relative mobilities of the reference proteins (Fig. 7).

Biological properties

The TM-lectin demonstrated identical hem-

FIG. 8. Hemagglutinating Activity of TM-lectin.

The hemagglutinating activity was determined by a photometric method⁷⁾ using human red blood cells (○: type A, ●: type B, □: type AB and ■: type O), rabbit red blood cells (△) and trypsinized rabbit red blood cells (▲). The extent of agglutination was determined after a 2.5 hr-sedimentation by absorbance at 620 nm and plotted against the logarithm of protein concentration.

agglutinating activity when reacted with human erythrocytes from A, B, O, and AB individuals as shown in Fig. 8. From these results, the specific activity for human erythrocytes was obtained as 20 HU/mg and as 130 HU/mg for rabbit erythrocytes. On the other hand, the specific activities for both human and rabbit erythrocytes treated with trypsin were increased to 1500 HU/mg in either of them. Assay of the mitogenic activity of the lectins was carried out by quantitation of the ^3H -thymidine into the cells at various doses, as explained under METHODS. Results in such an assay are represented in Fig. 9 and indicate the high mitogenic potency of the TM-lectin. The mitogenic responses of PHA-P, L-PHA, and H-PHA are examined as the reference lectins. An important observation made in these studies was that the mitogenic response of the lymphocytes first increased as the concentration of the TM-lectin until an optimum concentration of 100 μg per 3 ml cell suspension was reached, and then decreased. Morphological observation in this optimum concentration showed that over 25% of lymphocytes transform to the blasts. The maximum transformation to blasts was achieved at a concentration of 20 μg of TM-lectin or PHA-P per 3 ml cell suspension.

Hemagglutinating activity of the TM-lectin was inhibited by EDTA as shown in Table V. After EDTA was removed by dialysis from the lectin treated with EDTA, its hemagglu-

FIG. 9. Dose-responses for TM-lectin and the Reference Lectins Induced Blast Formation and Incorporation of ^3H -thymidine.

Solid lines are represented the incorporation of ^3H -thymidine and the blast formation is denoted by dashed lines. TM-lectin (●), PHA-P (○), L-PHA (Δ) and H-PHA (▲).

tinating activity corresponded to 10% of the untreated lectin and was restored to 92% by the addition of an aliquot of CaCl_2 and MgCl_2 to the dialyzed lectin solution. Hemagglutinating activity was inhibited by the addition of such sugars as *N*-acetyl-D-galactosamine or L-arabinose as shown in Table VI. The mitogenic activity was slightly inhibited by all of sugars tested at high concentration as shown in Table VI.

DISCUSSION

The lectin isolated from the Tora-mame (*P. vulgaris*) was shown to be homogeneous by polyacrylamide gel electrophoresis and ultracentrifugation. The isolation procedure was very simple, involving only two steps: fractionation by ammonium sulfate and an affinity chromatography on the heat-denatured PTG-Sepharose 4 B. In a preliminary experiment, only 10% of the activity was adsorbed on the native PTG-Sepharose 4 B prepared according to the method of Matsumoto and Osawa,¹⁹ whereas on the former

TABLE V. EFFECTS OF EDTA AND DIVALENT CATIONS ON HEMAGGLUTININATING ACTIVITY OF TM-LECTIN

Material added	Concentration (mM)	Relative hemagglutinating activity ^a (%)
None		100
EDTA	1.0	0
	0.1	0
	0.05	0
	0.01	6.6
	0.005	26.5
Ca^{2+}	0.5	155
Mg^{2+}	0.5	140
Mn^{2+}	0.5	150
Zn^{2+}	0.5	83

^a performed according to Liener⁷⁾ using rabbit erythrocytes treated with trypsin.

adsorbent, over 85% of the activity and 28.7% of proteins applied were adsorbed at neutral pH and were easily eluted with an acidic buffer. It is important to compare some properties of the TM-lectin with those of the other *P. vulgaris* lectins^{1-3,5,20} (Table VII). It is evident that the physical and chemical properties of these lectins are quite different. There is likewise a notable difference in carbohydrate contents of these lectins. The amino acid composition of the TM-lectin is similar to that of the some other lectins isolated from *P. vulgaris* seeds notably in that cyst(e)ine and methionine are absent. In the analysis of carbohydrate composition, mannose was a predominant sugar found in the TM-lectin. This sugar was also found in the largest amounts in some lectins or PHAs of Brazilian common bean,³⁾ wax bean,⁵⁾ soybean,²¹⁾ lima bean,²²⁾ castor bean,^{23,24)} *Ulex europeus* seed,²⁵⁾ *Bandeiraea simplicifolia* seed,²⁶⁾ etc. Glucosamine has also been reported to be present in smaller amounts in various lectins^{3,5,21,27)} as well as the TM-lectin. Most of glucosamine residues in the TM-lectin may actually be present as the *N*-acetyl derivative since, under the conditions employed in this experiment for hydrolysis (1 N H_2SO_4 at 100°C for 6 hr), quantitative deacetylation of *N*-acetyl-glucosamine would be expected.²⁸⁾ In addition to

TABLE VI. EFFECTS OF VARIOUS MONOSACCHARIDES ON TM-LECTIN INDUCED BIOLOGICAL ACTIVITIES

Sugar	Concentration ^a (mm)	Relative activity	
		Hemagglutinating activity (%)	Mitogenic activity (%)
None		100	100
D-Glucose	250	98	
	50		80
N-Acetyl-D-glucosamine	50		66
	20	105	
	10		79
D-Galactose	50		60
	20	101	
	10		96
N-Acetyl-D-galactosamine	50		60
	10	49	64
D-Mannose	125	99	
	50		69
	10		83
α -Methyl-mannoside	50	90	79
	10		90
D-Xylose	50		80
	10	70	94
L-Rhamnose	50		82
	10	70	100
L-Arabinose	50		69
	10	62	98

^a represented as the final concentration in the assay.

TABLE VII. COMPARISON OF THE PROPERTIES OF TM-LECTIN WITH SOME LECTINS FROM *Phaseolus vulgaris* SEEDS

Property	Red kidney bean ²⁾	Wax bean ⁵⁾	Rico 23 bean ³⁾	Blue Lake ¹⁾ a'	a''	Tora-mame (this paper)
Sedimentation constant	6.5 S	5.37 S	nd	6.4~6.7 S	6.2 S	6.66 S
Molecular weight	128,000	120,000~132,000	100,250	109,900	91,000	120,000
Isoelectric point	6.5	5.5	5.1	nd	nd	5.5
$A_{280}^{1\%}$ (cm ⁻¹)	nd	12.5	7.85	nd	10.5	12.7
Neutral sugar (%)	8.0	9.5	9.91	10.5	10	5.61
Amino sugar (%)	2.4	0.9	1.44	2.91	2.91	2.28

nd: not determined.

these sugars, L-fucose, D-xylose, and D-glucose are also present in the TM-lectin. These sugars were found in lectins isolated from the seeds of *P. vulgaris*,⁵⁾ *B. simplicifolia*,²⁶⁾ *U. europeus*,²⁵⁾ etc.

It has been reported that many lectins are the metalloproteins²⁷⁾ and a part of the metals is necessary for such activities as hemagglutination,^{20,29~31)} polysaccharide precipitation reaction,²⁹⁾ and lymphocyte transforma-

tion.^{20,31)} In the TM-lectin, calcium and magnesium are contained. The hemagglutinating activity of the TM-lectin are strongly inhibited by treatment with EDTA (Table V). The metal contents of the TM-lectin are smaller in amounts than the other lectins isolated from jack bean,²¹⁾ pea,²⁹⁾ and wax bean.²⁰⁾ This might be due to the fact that the TM-lectin was extracted in acidic condition (pH 2.0) and a part of metals was removed

from the lectin molecule in this step. This possibility may be supported by the fact that the hemagglutinating activity increases to 40 to 55% by the addition of such metals as Ca^{2+} , Mn^{2+} and Mg^{2+} to the purified TM-lectin (Table V). The TM-lectin has a mitogenic activity for human lymphocytes as well as some other lectins.²¹⁾ In this experiment, the red kidney bean lectins, PHA-P, L-PHA and H-PHA, were examined as the references. The dose-response curves of L-PHA and H-PHA exhibited a broad plateau of the mitogenic activity by the ^3H -thymidine uptake, in contrast to the relative sharp peak by the TM-lectin. The maximal mitogenic activity by the ^3H -thymidine uptake was obtained with the TM-lectin at a concentration of 100 $\mu\text{g}/3\text{ ml-culture}$, whereas it was achieved by whichever 10 μg of PHA-P, or 10~20 μg of L-PHA, or 20~50 μg of H-PHA per 3 ml-culture. From the results, a higher concentration of the TM-lectin was required to obtain a maximum incorporation of ^3H -thymidine than the reference lectins. However, in morphological observation, the TM-lectin induces more effectively blast formation than PHA-P.

The molecular weight of the TM-lectin was found to be 120,000 and that of the subunit of the lectin was 30,000. Thus, TM-lectin may consist of four identical subunits whose molecular weights are 30,000 and which are a glycoprotein.

Acknowledgments. This work was supported by the research grant from the Ministry of Education of Japan. We wish to acknowledge the help of Dr. Hiroshi Taguchi of our Department for the ultracentrifuge experiments.

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