Role of Lectins and Lipopolysaccharides in the Recognition Process of Specific Legume-*Rhizobium* Symbiosis

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Rhizobium japonicum and *R. leguminosarum* combined specifically with lectins of host plants, and lipopolysaccharides with the strongest inhibitory activity towards the hemagglutinating reaction of host plants lectins were extracted and purified from the two *Rhizobial* species. Moreover, they inhibited the binding reaction between lectins and bacterial cells. These results support the lectin theory that the interaction between lectins of host plants and *Rhizobium* is the key to the mechanism of the host specificity in *Rhizobium*-legume symbiosis, and that the lectin-receptor of *Rhizobial* cells exists in the cell surface lipopolysaccharides.

It is well known that there is a rather strict specificity between *Rhizobial* species and the host plants in the symbiotic nitrogen fixation by leguminous plants.^{1,2)} This specificity may be determined with *Rhizobial* cells to recognize the root hairs of the corresponding leguminous hosts.

Carbohydrates on the cell surface can function in the intercellular recognition process^{3~5)} and the case between *Rhizobium* and root hair cells may not be an exception. Therefore, it is assumed that the bacterial recognition of its host is related to the carbohydrates on its cell surface and the *Rhizobial* species with different host specificity have different cell surface carbohydrates respectively. Exopolysaccharide or lipopolysaccharide, both located on the cell surface of *Rhizobial* cells, may function in the host recognition process.

Many of the leguminous plants are known to contain lectins, which bind specifically with suitable sugar or sugar chains. Since Schmidt and Bohlool⁶⁾ showed that soybean lectin binds specifically to *R. japonicum*, but

not to other *Rhizobial* species, there have appeared several papers^{7~13)} supporting the lectin hypothesis. However, there is only a few researches^{8,9,14,15)} on the nature of the lectin-receptor of *Rhizobial* cells. This paper deals with this problem and it is shown that the interaction between the lectin and lipopolysaccharide may be one of the determinative steps in the establishment of root nodule formation in the symbiotic nitrogen fixation of leguminous plants.

MATERIALS AND METHODS

Materials. *Rhizobial* strains. *Rhizobium japonicum* strains 509, 5033 and 501 and *R. leguminosarum* strains J357 and J2080 were obtained through the courtesy of Dr. Tsuru, the Institute of Agricultural Technology, the Ministry of Agriculture and Forestry.

Leguminous seeds. Soybean (*Glycine max*, var. Shiratori) and pea (*Pisum sativum*, var. Sanjūnichi-kinuzaya and var. Waisei-akabana-kinuzaya) were purchased from Takii Seeds Co., Tokyo.

Maintenance and cultivation of the bacteria. The agar medium for maintenance consisted of 0.5 g of dipotassium hydrogen phosphate, 0.2 g of magnesium sulfate heptahydrate, 0.1 g of sodium chloride, 3.0 g of calcium carbonate, 1.0 g of yeast extract, 10 g of mannitol and 15 g of agar per liter. *R. japonicum* and *R. leguminosarum* were maintained on agar slants at 11°C.

The bacteria were cultivated with shaking (150~200 rpm) at 25°C in a liquid medium containing (per

* Abbreviations: lipopolysaccharide, LPS; exopolysaccharide, EXPS; cetavlon-ethanol precipitate, CEP; 0.02 M phosphate buffer-0.155 M saline, PBS; peak I, P-I; peak II, P-II; sodium dodecyl sulfate, SDS; periodic acid-Schiff, PAS; soybean lectin, SBA; pea lectin, PA; fluorescein isothiocyanate, FITC; 2-keto-3-deoxyoctonate, KDO; concanavaline A, Con A; wheat germ agglutinin, WGA.

liter): dipotassium hydrogen phosphate (0.6 g), potassium dihydrogen phosphate (0.2 g), sodium chloride (0.1 g), potassium nitrate (0.4 g), magnesium sulfate pentahydrate (0.1 g), calcium nitrate (0.05 g), yeast extract (1 g), mannitol (10 g), boric acid (1 mg), zinc sulfate heptahydrate (1 mg), cupric sulfate pentahydrate (0.5 mg), manganese chloride (0.5 mg), ferric-ethylene-diaminetetraacetic acid (1 mg iron) and sodium molybdate (0.01 mg).

Test for nodulating activity of Rhizobium. The growth pouch (Scientific Products, Div. of American Hospital Supply Corp.) containing the nitrogen-free nutrient solution¹⁶⁾ was autoclaved (120°C, 20 min) and one or two surface-sterilized seeds¹⁷⁾ were set on. After germination in the dark at 25°C, the pouch was moved into the biotron (daytime 25°C, nighttime 20°C, natural light). Four ml of *Rhizobium* suspension (approximately $10^7 \sim 10^8$ cells/ml) was aseptically inoculated to soybeans at eight to ten days and to peas at six to eight days respectively, after the germination. They were grown together with plants (controls) without inoculation.

Isolation and purification of Rhizobial cell surface lipopolysaccharide (LPS).*

(1) *Pretreatment of bacteria and isolation of exopolysaccharide (EXPS).* *Rhizobia* collected from the liquid shaking culture (*R. japonicum*, 7 days; *R. leguminosarum*, 4 to 5 days) were suspended in 0.155 M saline (30 ml per 5 g of wet cells) and washed by centrifugation (20,000 × *g*, 10 min). Washing was repeated until no sugar was detected in the supernatant. The combined supernatants were sterilized with a membrane filter and then dialyzed against water. The resulting solution was stored at 4°C.

(2) *Isolation of LPS.* LPS was extracted according to the method of Westphal¹³⁾ by adding an equal volume of 90% phenol to the suspension of washed cells (obtained under (1)) and stirring the mixture at 65~70°C for 10~20 min. The extraction was repeated 2 times. After cooling, the emulsion was centrifuged and the combined aqueous layers were dialyzed against water.

(3) *Cetavlon treatment and ethanol precipitation.* Crude LPS obtained above was concentrated under reduced pressure (35~40°C) and then made to a 0.5~1% LPS solution in 0.25 M saline. Cetavlon (cetyltrimethyl-ammonium bromide) was added gradually to the stirred solution to make a 1.5% solution and then the mixture was stirred for further 10 min. The precipitate formed was removed by centrifugation and the supernatant was poured into ten volumes of ethanol. It was stirred for 30 min at 4°C and then left still for 2 hr. Precipitated LPS was collected by centrifugation, dissolved in water, and dialyzed. The CEP solution obtained was stored

at 4°C.

(4) *DNase-RNase treatment.* Crude LPS (obtained under (2)) was treated with DNase and RNase (Sigma, bovine pancreas, each 10~100 µg/ml) in 0.05 M Tris-hydrochloric acid buffer (pH 7.5) containing 0.01 M magnesium chloride at 37°C for 16 hr. After the treatment, the reaction mixture was dialyzed against water and centrifuged to remove any precipitate formed.

Gel filtration. Sepharose 6B (Pharmacia Fine Chemicals) was equilibrated with PBS (pH 7.0) and the partially purified LPS (obtained under (3) or (4)) was applied to the column, followed by elution with PBS at 4°C. Total carbohydrate was measured in each fraction and sugar-containing fractions were collected and dialyzed against water. The resulting solutions (P-I and P-II) were concentrated under reduced pressure and stored as a solution or as a lyophilized preparation at 4°C.

*SDS-polyacrylamide gel electrophoresis of LPS.*¹⁹⁾

After the heat treatment of LPS with a disintegration reagent (10 mM Tris buffer, pH 8, 1 mM EDTA, 4% SDS, 30% sucrose, 2.5% dithioerythritol) for 5 min at 100°C according to Jann *et al.*, LPS (100 µg) was subjected to SDS-polyacrylamide gel electrophoresis at 6 mA per gel at room temperature and sugar was located by the PAS method.²⁰⁾

Extraction and purification of lectins

*Soybean lectin (SBA).*²¹⁾ Ground soybean seeds were defatted by n-hexane and extracted with 0.155 M saline (250 ml per 50 g powder). The extract was fractionated with ammonium sulfate (0.4~0.8 saturation) to prepare crude SBA.

Crude SBA (0.5~1 g) was purified by affinity chromatography on a column (2.5 × 20 cm) of affinity adsorbent equilibrated with PBS containing 0.02% sodium azide. SBA was eluted specifically with 0.2 M D-galactose in the same buffer. Two affinity adsorbents were used: one was aminoethyl Bio-Gel P-150 (Bio-Rad Laboratories) coupled with lactose monohydrate by the method of Gray *et al.*²²⁾ and the other Sepharose 6B treated with 0.2 M hydrochloric acid.²³⁾

*Pea lectin (PA).*²⁴⁾ Ground pea seeds were extracted with water (3 l per 240 g powder) and the protein precipitated at the isoelectric point (pH 4.6) was removed. The protein remaining in the supernatant was fractionated with ammonium sulfate (0~0.6 saturation) to prepare crude PA. Crude PA (about 1.5 g) was applied to a Sephadex G-100 column (3.4 × 25 cm) equilibrated with PBS and the column was washed with PBS. PA was eluted by 0.2 M D-glucose in PBS.

Other lectins. Purified Con A and WGA were gifted by Dr. Y. Nagata, the Institute of Applied Microbiology, the University of Tokyo, Bunkyo-ku, Tokyo.

Polyacrylamide gel electrophoresis of lectins. According to the method of Davis *et al.*²⁵⁾, electrophoresis was carried out in 7.5% polyacrylamide gel (pH 9.4) by applying 10~50 μ g sample and 3 mA per gel at 4°C. Protein was dyed with Brilliant Coomassie Blue.²⁶⁾

Assay of hemagglutinating activity of lectins. By using an equal volume of 3% trypsinized human erythrocyte suspension of type A and 0.1% lectin (freeze-dried) solution in 0.155 M saline, the activity of lectin was determined as the maximum dilution multiple (2^x , titer) of the lectin solution which hemagglutination was recognized by the serial double dilution method.

Preparation of fluorescein isothiocyanate (FITC) labeled lectin. Fifty μ g of FITC per mg of lectin was dissolved in 1 ml of 0.5 M sodium carbonate buffer (pH 9.5) and the solution was immediately added to nine volumes of 1% lectin solution. The mixture was stirred gently for one hour at room temperature and then at 4°C for 18 hr. The reaction mixture was then applied to a column (2.5 $\times$ 40 cm) of Bio-Gel P-6 equilibrated with PBS (pH 7.0) and eluted by the same buffer. The absorbance of the eluate at 280 and 500 nm was measured and fractions of labeled lectin were collected and stored frozen.

Binding reaction between fluorescent lectins and *Rhizobia*. Fully grown *Rhizobia* were suspended in PBS and to 0.5 ml of this suspension (10^8 cells/ml) was added 1.5 ml of the fluorescent lectin solution (0.1~0.4%) or 1.5 ml of PBS as a control. The mixture was incubated in a shaker at 30°C for 30 min, and the bacteria were washed by centrifugation with PBS and washing was repeated until unreacted lectin was removed completely. The washed cells were smeared on a slide glass, air-dried, mounted in glycerol diluted ten times with 0.5 M sodium carbonate buffer (pH 9.5) and then examined under a fluorescent microscope (Olympus) equipped with HBO-100 lamp, using UG-1 exciter filter and L435 absorbing filter or equipped with AHL-250 lamp using BG-12 exciter filter, FY3 and FY5 absorbing filter.

Measurement of inhibitory activity against the hemagglutinating reaction of lectin. Lectin solution should be assayed for hemagglutinating activity just before use, and it should be adjusted at a titer of 4. To double dilution series in 0.155 M saline solution of the sample to be tested was added an equal volume of the lectin solution and the mixture was left still for 2 hr at room temperature. Then, one volume of 4% trypsinized human A type erythrocyte suspension was added to two volumes of this mixture in order to assay the hemagglutinating activity. The inhibitory activity was expressed as the minimum concentration of the sample for complete inhibition of the hemagglutinating activity of the lectin solution.

Quantitative analysis

Determination of total carbohydrate. Total carbohydrate was measured by the phenol-sulfuric acid method²⁷⁾ using glucose as a standard.

Determination of 2-keto-3-deoxyoctonate (KDO). The sample was hydrolyzed in 0.01 N sulfuric acid in a boiling water bath for 30 min and then neutralized with barium hydroxide. The precipitate was removed by centrifugation and then KDO in the supernatant was determined by the periodate-thiobarbituric acid method,²⁸⁾ with ammonium KDO (Sigma) as a standard. When the sample contains DNA, 2-deoxyribose would give the color (the maximum absorption for 2-deoxyribose is at 532 nm and that for KDO is at 549 nm); therefore the amount of KDO was obtained by measuring the absorbance at 532 nm and 549 nm.

Determination of nucleic acid. The amount of nucleic acid was shown by OD₂₆₀ of the sample multiplied by the total volume (ml) of the sample and expressed as E₂₆₀. The existence of nucleic acid in the sample was assayed by the ratio of OD₂₆₀ to OD₂₃₀.

Determination of protein. The amount of protein in the sample was determined either by the absorbance at 280 nm or by the Lowry method²⁹⁾ with bovine serum albumin as a standard.

RESULTS

Extraction and purification of LPS

Since *Rhizobia* produced much exopolysaccharide, this was removed by washing the cells with 0.155 M saline prior to extraction of LPS. The amount of total carbohydrate in the aqueous layer obtained by the Westphal method was 50 to 100 times as much as that in the phenol layer.

Since the crude extract contains, in addition to LPS, nucleic acids, exopolysaccharides and lipid A-free polysaccharide degradation products of LPS, LPS was purified by removing these materials, using KDO, which is specific and characteristic to LPS, as an indicator. Nucleic acids were removed by cetavlon-ethanol treatment or DNase-RNase treatment, and the residual nucleic acids and lower molecular weight polysaccharides were removed by gel filtration on Sepharose 6B.

Table I summarises the purification of LPS from 41 g wet wt. of *R. japonicum* 509. By gel filtration of LPS on Sepharose 6B (Fig. 1), two major carbohydrate-containing peaks were obtained; P-I seemed to have a molecular

TABLE I. PURIFICATION OF LPS FROM *R. japonicum* 509

Purification step	TC ^a (μ mol)	E_{260}	OD ₂₆₀ /OD ₂₃₀	TC/KDO	E_{260} /KDO	KDO	
						μ mol	Yield (%)
Crude LPS	2111	8512	1.6	8.9	35.8	238	100
CEP	1280	288	0.7	7.1	1.6	180	76
P-I	197	45	0.1	1.8	0.4	110	46
P-II	363	16	0.1	35.7	1.6	10	4

^a TC, total carbohydrate. FIG. 1. Gel Filtration of LPS from *R. japonicum* 509 on Sepharose 6B.

The column was eluted with PBS (pH 7.0). Each fraction (6 ml) was assayed for total carbohydrate by the phenol-sulfuric acid method at 490 nm.

weight larger than 1,000,000 and P-II seemed to be polysaccharides of lower molecular weight. P-I seemed to be a purified preparation of LPS free of nucleic acids or other polysaccharides, judging from its macromolecular state (LPS aggregates in aqueous solution to become a macromolecule), the ratio of OD₂₆₀ to OD₂₃₀ and the molar ratio of total carbohydrates to KDO (approximately four times as great as that of CEP). P-II may be a mixture of polysaccharides including polysaccharide degradation products of LPS, because this fraction contains a small amount of KDO.

SDS-polyacrylamide gel electrophoresis of LPS (P-I)

The chemical heterogeneity of LPS (P-I) was analysed¹⁹⁾ by SDS-polyacrylamide gel electrophoresis after dissociating macromole-

FIG. 2. Patterns of SDS-Polyacrylamide Gel Electrophoreses with P-I.

(A) P-I obtained from *R. japonicum* 509; (B) P-I obtained from *R. leguminosarum* J357.

cular LPS.

The P-I obtained from *R. japonicum* 509 gave only one band, but the P-I obtained from *R. leguminosarum* J357 gave two major and two minor bands (Fig. 2). The latter result may be interpreted as showing that the P-I from *R. leguminosarum* J357 contains four kinds of LPS molecules aggregating mutually; however, there is a possibility that the preparation is not sufficiently purified. Therefore, further study is needed to establish the purity of this preparation.

Extraction and purification of lectins

Lectins were extracted from seeds of soybean and pea. In analysis of the purified lectins with polyacrylamide gel electrophoresis, SBA gave one major band and three minor bands and PA gave two bands. Both results corresponded to the reports^{24,30,31)} which showed the existence of isolectins in soybean and pea seeds.

Binding reaction between lectin and *Rhizobium*

In order to confirm the hypothesis that lectin obtained from the seeds of the host plant binds specifically with the corresponding strains of *Rhizobium*, the lectin labeled with FITC was mixed with *Rhizobia* and bacterial cells were observed under a fluorescent microscope.

TABLE II. BINDING OF STRAINS OF *R. japonicum* AND *R. leguminosarum* WITH LECTINS AND INFECTIVITY

Species	F-SBA ^a	F-PA ^b	F-SBA + 509-P-I	F-PA + J357-P-I	Infectivity ^c	
					soybean	pea
<i>R. japonicum</i>						
509	+	+	—	NT	+	—
5033	+	—	NT ^d	NT	+	—
501	+	—	NT	NT	+	—
<i>R. leguminosarum</i>						
J357	—	+	NT	—	—	+
J2080	—	+	NT	NT	—	+

^a F-SBA, FITC labeled soybean agglutinin.^b F-PA, FITC labeled pea agglutinin.^c Determined by test for nodulating activity of bacterial strains.^d NT, not tested.

Table II shows the results of the binding reaction between 5 strains of *R. japonicum* and *R. leguminosarum* on the one hand and SBA and PA on the other, in addition to the infectivity of these bacterial strains to the host plants.

All of the three *R. japonicum* strains infected soybean roots and bound to F-SBA as well; two strains did not bind to F-PA.

Both of the two strains of *R. leguminosarum* infected pea roots and bound to F-PA; these did not bind to F-SBA.

Interaction between P-I and lectin

The host-symbiont specificity between leguminous plants and corresponding *Rhizobial* strains is well confirmed by the cross-inoculation test. Some workers showed that lectins from the host plants might be responsible for this specificity.^{6~9)} If this assumption is correct, there might also be specific lectin-receptors on the cell surface of corresponding *Rhizobia*. It is possible that cell surface polysaccharides of *Rhizobium*, especially LPS, may play the role of lectin-receptor in this specific cell-cell recognition phenomena. This possibility was examined as follows.

Inhibitory activity of P-I towards hemagglutinating activity of lectins. By measuring the inhibitory activity against the hemagglutinating reaction of a lectin, it is possible to know if a certain sugar binds to lectin or not, and also the intensity of the reactivity. Accordingly,

the inhibitory activity against SBA of CEP, P-I and P-II obtained from *R. japonicum* 509 as well as N-acetyl-D-galactosamine and D-galactose, specific sugars to SBA, were measured. The results are shown in Table III. The inhibitory activity of P-I, the purified LPS, was approximately four times as great as that of CEP (partially purified LPS). This corresponded to the degree of purification as shown by the molar ratio of total carbohydrate to KDO (1.8 for P-I and 7.1 for CEP; see Table I). This result shows that LPS (P-I) binds to lectin and inhibits the activity of it. P-II has a much weaker inhibitory activity than that of P-I. P-I showed extremely strong inhibitory activity in comparison with N-acetyl-D-galactosamine and D-galactose.

To know the specificity between the lectins from host plants and cell surface polysaccharides from corresponding *Rhizobia*, the inhibitory activity of P-I, P-II and EXPS obtained from both *R. japonicum* 509 and *R. legumino-*

TABLE III. MINIMUM CONCENTRATION FOR INHIBITION OF SBA

Preparations	Concentration (μ mol/ml) ^a
CEP	0.087
P-I	0.022
P-II	5.6
D-Gal	5.6
GalNAc	0.28

^a Calculated by amount of total carbohydrate with D-glucose as a standard.

sarum J357 against four kinds of lectin, SBA, PA, Con A and WGA was measured respectively. As shown in Table IV, P-I from *R. japonicum* and *R. leguminosarum* has the strongest inhibition against the lectin obtained from the corresponding host plant, P-II also showed specific inhibition but was less active. There are reports that exopolysaccharide (capsular polysaccharide) is related to the symbiotic recognition;^{8,15)} however, EXPS obtained from both *R. japonicum* and *R. leguminosarum* in this experiment showed no inhibitory activity against the lectin from corresponding host plants and the strongest against Con A.

TABLE IV. INHIBITORY ACTIVITY OF VARIOUS POLYSACCHARIDES ($\mu\text{mol/ml}$)^a TOWARDS LECTINS^b

Species	Preparations	SBA	PA	Con A	WGA
<i>R. japonicum</i> 509	EXPS	—	—	0.50	—
	P-I	0.0065	0.052	0.21	0.052
	P-II	1.6	6.4	6.4~13	—
<i>R. leguminosarum</i> J357	EXPS	—	—	13	—
	P-I	0.58	0.14~ 0.29	0.58~ 1.2	0.58
	P-II	—	11	—	23

^a Refer to the foot note in Table III.

^b Results of two to four tests for each sample.

Inhibition of the binding reaction between lectin and Rhizobia. Equal volumes of fluorescent SBA (titer 256) and 509-P-I (6.7 $\mu\text{mol/ml}$) and also of fluorescent PA (titer 32) and J357-P-I (4.6 $\mu\text{mol/ml}$) were mixed and left still for 2 hr at room temperature. Then, *R. japonicum* 509 and *R. leguminosarum* J357 were added to each mixture and examined under fluorescent microscope.

As is shown in Table II, SBA and PA, after preincubation with 509-P-I and J357-P-I respectively, were inhibited from binding to the corresponding *Rhizobium*.

Lectin activity (hemagglutinating activity) of roots of host plants

The host plants were grown in the biotron under the same conditions for tests of nodulating activity. The soybean roots were collected at 17 days and pea roots at 15 days after

germination. The roots were ground in a blender and extracted overnight at 4°C with 0.155M saline and then concentrated with 90% saturated ammonium sulfate to obtain the crude extract. The activity of lectin in the root extract was measured using trypsinized rabbit erythrocyte suspension, and the existence of the lectin activity was confirmed (Table V).

TABLE V. HEMAGGLUTINATING ACTIVITY OF ROOT EXTRACTS

Plants	Titer ^a of 1% solution of lyophilized preparations	Yield
		g/100 g roots ^b
Soybean	64	0.050
Pea	16	0.038

^a Measured by using 3% trypsinized rabbit erythrocyte suspension.

^b Expressed as fresh weight.

DISCUSSION

It is generally assumed that the specificity between the leguminous plants and *Rhizobial* species is due to the recognition and binding by *Rhizobial* cells of the specific materials existing on the root surface of corresponding host plants. Since Schmidt and Bohlool⁶⁾ showed that 22 out of 25 strains of *R. japonicum*, but not other *Rhizobial* species, bound to soybean lectin labeled with FITC, lectins attracted attention as a probable component on the side of the plant in the process of symbiotic recognition.

In this report, it was shown that (1) *Rhizobia* bound to lectin of the host plant, (2) LPS from the cell surface of *Rhizobium* played the role of the lectin-receptor in this reaction and (3) the LPS reacted specifically with the lectin of the host plant. These results support the lectin theory on the mechanism of specific recognition process in symbiotic nitrogen fixation of leguminous plants.

Incidentally, some reports against the lectin theory have been submitted.^{32~34)} However, it seems premature to deny immediately the role of lectins in the symbiont recognition process. The problems to be re-considered

may include: the possibility of the tested lectin to be inadequate, the possibility of the lectin-receptor to change or disappear during the growth cycle of the bacteria,¹²⁾ and the effect of the difference in cultural and environmental conditions.³⁵⁾ Another criticism against the lectin theory is that the lectin used was obtained from the seeds but not from the roots, to which *Rhizobial* cells bind. In this report, it is shown that the root has the lectin activity, although the chemical identity between the root and seed lectins or the interaction between the root lectin and the bacterial LPS have not yet been studied. Also, since there may be many kinds of sugar on the bacterial cell surface and some may easily bind to the lectin, it is possible for a certain *Rhizobial* cell to bind to the lectin of the plant other than its host. In this report, *R. japonicum* 509 bound also to pea lectin, and it may be possible, in considering some other evidences, to assume that the cell surface of this strain is modified to form a surface structure somewhat different from that of ordinary soybean *Rhizobial* strains and thus binds to pea lectin.

It is not always true that a *Rhizobial* cell starts symbiosis with a plant once it binds to the root hair of the plant. First, the difference in the strength and specificity of binding between lectin and bacteria is important in the establishment of recognition between a *Rhizobium* and a host. Second, although the binding of the *Rhizobia* to the root hair cells seems to be the most important factor in determining the host specificity, there are three additional stages to pass in the root nodule formation process; attraction of *Rhizobial* cells to the root hairs prior to binding, invasion following binding, and differentiation of bacterial cells to the bacteroids. Taking all these into consideration, it is more advisable to think that lectin is no more than one of the factors for deciding the host specificity.

Providing that lectins play an important role in the recognition process, a question will be raised—what is the lectin binding entity of *Rhizobial* cells? There are two views on this problem; one assumes exopolysaccharide or

capsular polysaccharide^{8,15)} and the other LPS.⁹⁾ This report supports the latter view. P-II showed a specific interaction with the lectin in a similar way to that of P-I; however, P-II contained a small amount of polysaccharide degradation products of LPS in addition to the exopolysaccharides which could not be removed by saline-washing. Also, as shown in Table IV, the exopolysaccharide does not give a specific interaction with the lectin. P-I from *R. leguminosarum* J357 gave four bands in SDS-polyacrylamide gel electrophoresis. It is possible that there may be two kinds of LPS, one with the lectin-receptor activity and thus has a function in the host recognition process and the other without this activity. If it is true, this may be related to the fact that J357-P-I has a weaker activity than 509-P-I (Table IV). However, it is not excluded that the purified preparation of J357-P-I still contains polysaccharides other than LPS. Further purification and analysis are needed to establish the lectin-lipopolysaccharide recognition hypothesis in leguminous root nodule formation process.

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