CHAPTER IV

DISCUSSION

Lectin has been purified from many plants (2-7), but its biological function has remained controversial. In some leguminous plants, lectin seemed to be involved in rather specific symbiosis between plant and nitrogen-fixing rhizobia (10). In rice (Oryza sativa), it has been newly discovered that Klebsiella oxytoca, a nitrogen-fixer isolated from the rhizosphere can also have symbiotic relation with the rice plant (26). Though purification of rice bran lectin has been reported by Tsuda since 1979, the role of lectin in the association of rice plant and nitrogen -fixing bacteria found in the rhizosphere has never been investigated.

The first step in this study was to purify lectin from rice bran locally obtained in this country. It was found that our local rice bran contained only 3.5% total protein (Table 1) comparing to 6.16% reported by Tsuda (25).

When our crude PBS extract was precipitated with saturated $(NH_4)_2SO_4$, 0-60% and further purified by 2 schemes of chromatographic methods (Table 3),1) purification by ion exchange chromatography, the lectin yield was about 714 mg/kg of rice bran weight and 2.04% according to crude PBS extract weight 2) purification by affinity chromatography, the lectin was about 591.5 mg/ml of rice bran weight and 1.69% according to crude PBS extract weight. While Tsuda's crude

PBS extract was further purified by precipitation with saturated 0-60%, Ovomucoid-Sepharose column, CM - cellulose column I and II, the lectin yield was about 60 mg/kg of rice bran weight and 0.097% according to crude PBS extract weight.

From these results, although total protein in our crude PBS extract was lower than total protein in Tsuda's crude PBS extract but our rice bran lectin yield was higher than that reported by Tsuda.

When our crude PBS extract was assayed for hemagglutination activity with erythrocytes of human A, B, AB, O and rabbit by the microtiter test, the hemagglutination activity was not detectable in crude PBS extract with ordinary red cells. Therefore, erythrocytes of human A, B, AB, O and rabbit were previously treated with trypsin in order to remove glycoproteins from the cell surface (21) and enhance the agglutinability of the erythrocytes. This is caused by increasing both the steric availability of glycolipids and the mobility of components in the membrane. When trypsinized erythrocytes suspension was used in the screening test for hemagglutination activity of crude PBS extract, the results (Table 2) showed that our PBS extract could agglutinate trypsinized rabbit erythrocytes most effactively showing the titer/mg protein of 0.77. While Tsuda's crude PBS extract was assayed for hemagglutination activity with erythrocytes of human A, B, AB, O and rabbit, it could agglutinate rabbit erythrocytes most effectively amang various erythrocytes showing titer/mg protein of 4 which is 5 fold compared to our crude PBS extract. Our lectin from both schemes of separation (Table 3) show the specific hemagglutination activity of 20 only with trypsinized rabbit erythrocytes. While lectin

from the final step of purification according to Tsuda gives the specific hemagglutination activity with rabbit erythrocytes of 768.

From the results, our rice bran lectin and Tsuda's rice bran lectin were both nonspecific for human blood groups ABO but markedly specific for N-acetyl-D-glucosamine on the surface of rabbit erythocytes. However the specific hemagglutination activity of the purified lectin was significantly different by nearly 40 fold.

Our rice bran lectin from 2 schems of purification gave a single band on polyacrylamide gel electrophoresis (Fig 6), and a single symmetrical peak on Sephadex G-75 column (Fig 7) at the same position. Thus our rice bran lectin from 2 schemes of purification were homogenous and should be the same protein. Our rice bran lectin is not glycoprotein, having sugar specific for the N-acetyl-D-glucosamine residue (Fig 8) and acidic protein by passing DEAE-Sephadex A-50 column (Fig 2) which are different from Tsuda's rice bran lectin (25). The molecular weight of our rice bran lectin was estimated to be around 40,000 by gel filtration (Fig 7). Dissociation into subunits of our rice bran lectin were detected by treating the intact lectin with 2-mercaptoethanol and subjected to SDS-gel electrophoresis in the presence of 2-mercaptoethanol in the gel resulting in 4 diffused, fast moving bands with approximate molecular weights of 24,000, 18,000 14,000 and 6,000 respectively (Fig 11). The molecular weight of Tsuda's rice bran lectin has been estimated to be around 37,000-44,000 by ultracentrifugation and SDS-polyacrylamide gel electrphoresis in the absence of 2-mercaptoethanol which is more or less the same as our rice bran lectin. However intact Tsuda's rice bran lectin was reported to be a dimer, in which the M.W. of monomer lectin was about 20,000.

Intact lectin subjected to SDS gel electrophoresis in the presence of 2-mercaptoethanol-dissociated into two nonidentical subunits having M.W. around 12,000 without hemagglutination activity.

Thus the difference in subunits structure of our rice bran lectin and that of Tsuda's might explain for the difference in hemagglutination activity. Besides difference in varieties between Thai and Japanese rice might be another cause for different lectins. Even in one variety such as wheat germ, 3 active proteins (isolectins) were separated from the same source, all were specific for N-acetyl-D-glucosamine (6,7)

Our rice bran lectin and WGA, fraction I (6,7) are lectins from graminous plants, which are not glycoprotein having sugar specific for the N-acetyl-D-glucosamine but different in M.W. and number of subunits (M.W. 35,000 and 2 subunits for WGA). Our rice bran lectin and WGA (6,7) were both nonspecific for human blood groups.

Our results (Fig 9) demonstrated that purified rice bran lectin agglutinated nitrogen-fixing rhizospheric bacteria (No 5, 6, 15, 17 and 18) with similar specific titer of 20. Purified rice bran lectin could not agglutinate Escherichia coli, although they were gram negative having lipopolysaccharide on cell surface. Among sugars tested (Fig 10) only N-acetyl-D-glucosamine inhibited the agglutination of nitrogen-fixing rhizospheric bacteria by purified rice bran lectin indicating that interaction of the binding site of purified lectin with N-acetyl-D-glucosamine residues should be present in lipopolysaccharide on the cell surface of these nitrogen-fixing

bacteria but not present in lipopolysaccharide on the cell surface of Escherichia coli. It is suggested that rice bran lectin might involve in the establishment of symbiotic relationship between nitrogen-fixing bacteria and rice roots, which corresponded to many evidences. For example, in 1974 Bohlool and Schmidt (11) reported that soybeam lectin labeled with a fluorescent stain binds to 22 of 25 strains of Rhizobium japonicum, which infects soybeans, but not any 23 strains from five species of Rhizobia that infect other legumes. Furthermore, Bauer (27) performed binding experiments similar to the former experiments and obtained similar results. In addition, they showed that a sugar known to be a specific inhibitor of the binding of soybean lectin to red blood cells also inhibits binding of soybean lectin to Rhizobium japonicum.

Albersheim and Wolpert (12) used a some what different approach; they isolated lectins from the seeds of four legumes (soybean, pea, red kidney bean and jack bean) and lipopolysaccharides from the four corresponding Rhizobial species. In all cases, the bacterial lipopolysaccharide interacted only with the lectin from the legume which the bacterium forms a symbiotic relationship.

More recently, Jitrakornwunkul (28) using the crude PBS extract and dialysate of 0-60% ($(NH_4)_2SO_4$ precipitate from rice root (Oryza sativa R.D. 7) to assay for the hemagglutination activity with trypsenized rabbit erythrocyte and reported the specific titer of 1.37 and 2.03 respectively. These results also suggest that lectin from rice root might also agglutinate N_2-fixing rhizospheric bacteria.

All these findings suggest that, rice bran lectin might

be involed in the recognition process between N_2-fixing rhizospheric bacteria and the rice root.

In this research only some physicochemical and biological properties of local rice bran lectin was investigated. The author wish to suggest the following extensive studies; 1) comparison of purified lectin from rice root and rice bran lectin, 2) mitogenic effect of rice lectins, and 3) the ability of rice lectins to agglutinate malignant transformed cells.