CHAPTER 4

Discussion

4.1 Purification of rice lectin

The purification of embryo lectin of two rice cultivars, RD 7 and RD 25, in this research differed from the procedure used by Limpananont(1987) in 2 aspects; firstly extraction was conducted at low pH of 2 which was suitable, since the stability range of pH 2-10 was reported(Limpananont,1987) and resulted in slightly clear supernatant. The ammonium sulfate precipitation did not significantly increase the purity of lectin, it simultaneously precipitated the minute particle of starch, but the chitin column increased the purity of lectin to 160-420 fold, and nearly homogeneous protein band was observed on PAGE. Secondly, the elution of lectin from chitin column was the most important step, it was efficiently eluted at pH 3.8 (Stinissen et al,1984) with chitin hydrolysate. After dialysis of the pool fractions against acetate buffer pH 3.8 some precipitated proteins were observed, but there was no lost in hemagglutinating activity. The purity of lectin maximally

increased after the step of ion exchange chromatography on SP-Sephadex column, the elution profile also indicated the differene between lectin of RD 7 and RD 25 (Fig 3.2 a and b). The first eluted protein peak of RD 7 contained hemagglutinating activity, whereas RD 25 did not showed lectin actvity in the first peak. The major peak of lectin activity from RD 25 was coincidental with RD 7 but other two isoforms were eluted at higher salt concentration. After the fourth steps of purification, the total activity recovering was about 80-90%.

In the past lectin was extracted in different pH of solution, such as pH 7.4 (Tsuda,1979) pH 3.8 (stinissen et al,1983) and pH 1.3 (Tabary et al,1987), from all pH range of 1.3 - 7.4, the extracts contained hemagglutinating activity. Extraction at low pH has more advantage, because some proteins which are denatured and precipitated will be eliminated.

4.2 Antibody against rice lectin

Sera from only one of four rabbits immunized with rice lectin exhibited high titer after 5 months. It specifically interacts with the antigens, lectin RD 7, and completely

cross-reacted with lectin RD 25 and some of other rice varieties.Since antibody against lectin RD 7 was able to react with lectin of all tested cultivars, it was useful to apply this antisera in many aspects; to study the relationship among rice cultivars by immunodiffusion patterns, to locate lectin in tissues of rice plant by immunofluorescent technique, and finally to quantitate the amount of lectin in various tissues (embryo,root and leaf) by a sensitive ELISA. It was noted that lectin of RD 7 contain at least one antigenic determinant which is common to lectin in all tested cultivars, including 2 wild rice species. Since lectin was a sugar specific binding protein, the reaction between lectin and antibody may occur in two possible ways; firstly as a lectin-immunoglobulin (glycoprotein) interaction, and secondly as antigen(lectin)-antibody reaction, but the first possibility was rejected because lectin did not give any reaction with control sera taken before immunization, and the interaction was not affected by adding Ø.1 M GlcNAC (Mishkind et al,198Ø). Antibody was able to react with denatured antigen(caused by SDS) such as rice lectin, and WGA (Kolberg and Sollid,1985;Kolberg et al,1987),it implied that denatured form of antigen did not affect so much to the reaction between antigen and antibody.

4.3 Distribution of lectin in different tissues of rice

Lectin distribution was found in root and leaf, in seedling and adventitious root of adult plant, but in matured leaf lectin was not found.

Localization of lectin by this method was not feasible for the intracellular lectin, but suitable for the lectin which located on cell surface and opening areas, because intact sample were used for lectin localization, in the other works(Cammue et al,1986) using fluorescent antibody technique to localized lectin in coleoptile of 3 day old seedling, they found that lectin distributed throughout this organ which corresponding to the lectin content ,quantitated by modified ELISA.In the root, lectin was found in different positions and quantity, which similar to the previous report(Cammue et al, 1986) that lectin in root gradually decreased in cereal plants and they did not detect lectin in adventitious root of adult rice plants (Stinissen et al, 1985). In this research lectin was located on the root tip (Fig 3.5 E) of adventitious root even in the flowering stage. When the role of lectin was considered as an asscociative factor between rice and N_2-fixing bacteria(Klebsiella spp.), not only the lectin was found in root, but it was also found in leaf which

might played in the role of associative factor. According to Smanta and Sen(1986) and Sengupta et al(1981) who sprayed the suspsension of nitrogen-fixing bacteria, (Klebsiella pneumonianiae) on leaf of rice and wheat seedlings, they reported that associated N_2-fixing bacteria on the leaf enhanced the dry weight, cholorophyll, and total N content of plants more than 50%. However on the surface of the old leaf, lectin was absent, this might suggest the role of lectin in cell differentiation, since the old leaf was fully mature, then lectin was not necessary for the differentiation furthermore.

4.4 Relationship of lectin among various rice varieties

Antibody against rice (RD 7) lectin is very useful for studying the relationship among rice varieties, lectin from RD 7 was a common antigen within 30 rice cultivars and 2 species of wild rice investigated. These rice varieties possibly evolved from a common ancester, and lectin gene was conserved throughout the period of evolution, which suggested that lectin might have the unknown functions which should be verified. The pattern and density of precipitation bands refered to their relationship and contents, the completely fused ring of lectin in gel would

encounter for the antigenic identity between these lectins. The extraband might refered to the isoform of lectin . The varieties that contained low amount of lectin by ELISA , KTH 17, PTL 60and BMT 370 exhibited the diffusive band in agar gel immumodiffusion. Besides, rice lectin shared at least one antigenic determinants which was common to WGA, lectin from the wheat of the genus Triticum , and it was also reported by Peumans et al., (1983), Korlberg et al., (1987), and Tabary et al (1987) that anti-WGA cross-reacted with rice lectin. In addition, lectin from rye and barley were serologically indistinguishable to WGA, since they completely cross-reacted with anibody against WGA (Cammue et al.,1985b, Peumans et al.,1982a), those plants are the members of the tribe Triticeae, and should have the same evolutionary ancester likewise the rice. All these results suggest that rice and wheat should have the same origin of evolutionary ancester.

4.5 Development of ELISA method

Antibody against lectin RD 7 was valuable in indirect ELISA too, both in normal and modified method, with the sensivity of 10 ng lectin/ml, and the high accuracy (Table 3.4). The normal and modified method are different in binding mechanism, the

modified method is suitable and usful for quantitation of lectin in crude extracts which contain high concentration oftotalprotein (1mg/ml), and normal method is suitable for samples with low total protein concentration (Ø.Ø5-Ø.2mg/ml). The ELISA method developed in this research requires, the first antibody at the dilution of 1:1ØØ,ØØØ, which is 1:1ØØ of that reported by Shimazaki and Pratt, (1985), in which the dilution of the first antibody required was 1:1,ØØØ,to quantitate lectin in wheat. It indicates that, this lectin antibody obtained was much hihgher in titer than the previous one obtained in wheat. This ELISA method developed was different from the one used by Raikhel et al.(1984), which was the sandwich type achieved to quantitate WGA-like protein in wheat, and Raikhel and Pratt (1987), which using double sandwich ELISA. However, the mechanism of modified ELISA method used in this research was similar to the sandwich ELISA, which are suitable for the quantitation of lectin in crude extract. The modified ELISA procedure is better than the previous ELISA method, since it extended wider suitable range of lectin concentration (1Ø-1ØØ ng/ml) than the former ELISA procedure (1Ø-6Ø ng/ml)

4.6 Lectin content in embryo

Lectin content of all 28 rice cultivars was able to be detected by normal ELISA. Lectin quantity correlated with the double gel immunodiffusion. The amount of embryos lectin depend upon the cultivars of rice because its grain and embryo were different in size and weight. Cammue et al (1986) quantitated lectin content in embryo of japonica rice by HPLC and reported the values of 250 ng/coleoptile which is 2.5 fold higher than lectin of NSPT (103 ng/embryo was lower than 2.5 fold.

4.7 Lectin in developing rice seedling and effect of NH_4Cl

Lectin in rice seedling (4-7 day old) was quantitated by modified ELISA, in root and leaf and found that leaf lectin decreased after germination. Lectin content in developing leaf and root of 8 cultivars of rice differed from each other in corresponding with the content of lectin in embryo. The amount of lectin in other graminae seedlings was quantitated by different method and given different values of lectin. Raikhel et al.(1984) using sandwich ELISA procedure to quantitate wheat germ agglutinin like lectin in 7 day old seedlings obtained 100ng/ shoot base, comparing to rice (RD 7) lectin in leaf (leaf+leaf

sheath) of 7 day old seedling was 15±2 ng/plant which was 6 fold lower than wheat , however lectin in different plant might contained lectin in different amount.

Effect of NH_4Cl to lectin content in rice seedling,at 2Ø mM MH_4Cl root lectin was significantly lower when compared to the absence of NH_4Cl, it was noted that root system did not fully develop when 2ØnM NH_4Cl was present which should result in low lectin content in root. In contrary, the presence of N_2-fixing bacteria enhanced root proliferation, but the induction of lectin by these N_2-fixer have not been tested.

The observation that content of embryo lectin correlates with the resistance of rice to blast disease caused by fungi (Pyricularia oryzae was raised from the finding that KDML 1Ø5, which contained lectin in very low amount (7ng/embryo) was susceptible to blast didssease Table 4.1 whereas NMS4 which contained high lectin 91ng/embryo was resistant to blast disease (สถาบันวิจัยข้าว,2529). Futher studies were required whether, lectin content in embryo might be used as a factor for selection of blast resistant lines. Futhermore, Gibson et al. (1982) reported in soybean seed that cultivars resistance to Phytophthera megasperma var. sojae (Race1) contained soybean agglutinin (SBA)

approximately twice of susceptible cultivars, which was similar to rice that cultivars

Table 4.1 Resistance and susceptible cultivars of rice with respect to blast disdease cause by Pyricularia oryzae

Cultivars	Blast infection
PL 111	R
NMS 1	MR
LPN 111	MR
PTL 60	MR
RD 25	MR
RD 7	MR
LPT 123	MR
BMT 370	S
KDML 105	S
KTH 17	S

Source : Rice Research Institute, Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkhen, Bangkok, Thailand

R=Resistance MR=Moderate resistance

S=Susceptible

contained lectin in high level were moderately resistant to blast (Table 4.1) and susceptible cultivars contained lectin in low level. There are reports in tropical rice fields, that when high rate of nitrogen fertilizer were applied, it can promote the virulence of blast disease even resistant cultivars can be more susceptible to blast infection (วีระแพทย์, 2526) which might be correlate to this result that the presence of high exogenuos NH_4Cl concentration can be deplete lectin content in leaf and root of rice seedlings