Microbiology, Vol. 73, No. 4, 2004, pp. 389–391. Translated from Mikrobiologiya, Vol. 73, No. 4, 2004, pp. 461–464.

EXPERIMENTAL ARTICLES

The Effect of the Carbohydrate Components of Pea Roots on the Enzymatic Activity of the Surface Agglutinins of *Rhizobium leguminosarum* bv. *viciae* 252

L. V. Karpunina*, M. S. Smiyan*, and L. V. Kosenko**

**Saratov State Agrarian University, Teatral'naya pl. 1, Saratov, 410600 Russia*
***Zabolotnyi Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, ul. Zabolotnogo 154, Kiev, 03143 Ukraine*

Received August 12, 2003; in final form, February 24, 2004

Abstract—The interaction of the surface agglutinins of *Rhizobium leguminosarum* bv. *viciae* 252 with the carbohydrate components of host pea roots alters the β-glucosidase and proteolytic activities of the agglutinins.

Key words: Rhizobium, agglutinins, enzymes, pea roots.

Plant receptors for bacterial lectins and the role of these lectins in the early stages of bacterial cell attachment to plant cells have thus far been investigated insufficiently. Our recent studies showed that the protein and carbohydrate components of wheat roots may serve as receptors for the lectins of soil nitrogen-fixing bacilli [1, 2] and that the protein components of pea roots may serve as receptors for rhizobial agglutinins [3]. At the same time, very little information is available as to the role of the carbohydrate components of plant roots in their interaction with bacterial lectins possessing enzymatic activity and the formation of nitrogen-fixing associations.

The aim of this work was to study the interaction of the surface agglutinins of *Rhizobium leguminosarum* bv. *viciae* 252 with the carbohydrate components of pea roots and the effect of this interaction on the enzymatic (β-glucosidase and proteolytic) activity of the agglutinins.

MATERIALS AND METHODS

Experiments were carried out with the parent strain *Rhizobium leguminosarum* bv. *viciae* 252, obtained from the collection of rhizobia at the All-Russia Research Institute of Agricultural Microbiology in St. Petersburg–Pushkin, and its nitrosoguanidine-derived mutant strain *Rh. leguminosarum* bv. *viciae* 252/7, deficient in hemagglutinating activity [5].

The strains were grown in a liquid synthetic medium at 28°C for three days [4].

Surface agglutinating and nonagglutinating proteins were prepared as described previously [3].

Exopolysaccharide (EPS) and lipopolysaccharide (LPS) were prepared from the cells of strain 252 [6].

Root exocomponents were prepared from the roots of 4-day-old seedlings of the pea cultivar Uladovskii yubileinyi. Pea beans were germinated aseptically in sterile distilled water. The carbohydrate fraction of the exocomponents was prepared by removing proteins with 40% trichloroacetic acid. The carbohydrate content of this fraction was determined with the phenol–sulfuric acid reagent [7].

The preparation of rhizobial agglutinins (20 μg/ml) was incubated with the preparations of root carbohydrates, EPS, and LPS (1%) at 28°C for 30 min. The preparations were mixed at a volume ratio of 1 : 1.

The hemagglutinating activity of proteins was determined by using a 2% suspension of trypsinized rabbit erythrocytes.

β-Glucosidase activity was assayed as described by Kwon *et al.* [8]. One unit of this activity was defined as the amount of *p*-nitrophenol (mmol) produced in 1 min per milligram protein.

Proteolytic activity was assayed as described by Preston *et al.* [9]. One unit of this activity was defined as the amount of alanine (mg) produced in 1 min per 1 mg protein.

The experimental data were statistically processed as described by Oivin [10].

RESULTS AND DISCUSSION

Our recent studies showed that two agglutinins (R_1 and R_2) of *Rh. leguminosarum* bv. *viciae* 252 are nonfibrillar glycoproteins that are distributed uniformly over the surface of bacterial cells [3] and have an affinity for autogenous exopolysaccharide and lipopolysaccharide [11]. Some microbial lectins possess not only

0026-2617/04/7304-0389 © 2004 MAIK "Nauka/Interperiodica"

Table 1. The enzymatic activity of agglutinins from the parent strain *Rh. leguminosarum* bv. *viciae* 252 and its agglutination mutant 252/7

Enzymatic activity	*R. leguminosarum* 252 $M \pm m$		*R. leguminosarum* 252/7 $M \pm m$		P
Proteolytic activity, mg alanine/(min mg protein)	R_1	0.038 ± 0.004	R_1'	0.038 ± 0.008	<0.1
	R_2	0.062 ± 0.008	R_2'	0.023 ± 0.0001	<0.001
β-Glucosidase, mmol/(min mg protein)	R_1	0.150 ± 0.0005	R_1'	0.280 ± 0.002	<0.01
	R_2	0.050 ± 0.003	R_2'	0.220 ± 0.020	<0.01

hemagglutinating but also enzymatic activity. These are the toxins of enteropathogenic bacteria [12–14], as well as the nontoxic lectins of bacilli [15, 16].

The surface agglutinins R_1 and R_2 of *Rh. leguminosarum* bv. *viciae* 252 were also found to possess both hemagglutinating and enzymatic activities (β-glucosidase and proteolytic) (Table 1). The β-glucosidase activity of the nonagglutinating proteins R_1' and R_2' of the mutant strain 252/7 was, respectively, 1.9 and 4.4 times higher than that of R_1 and R_2, whereas the proteolytic activity of R_1' was the same and that of R_2' was 2.7 times lower than in the case of R_1 and R_2.

These data suggest that there might be a relationship between the hemagglutinating and enzymatic activities of the R_1 and R_2 lectins. In a recent work [16], such a relationship was studied by inhibiting the hemagglutinating activity of bacillar lectins with specific carbohydrates. Similar experiments performed in this study showed that the incubation of the R_1 agglutinin with EPS diminished its β-glucosidase activity from 0.15 to 0.12 mmol/(min mg protein), whereas the β-glucosidase activity of the R_2 agglutinin increased in this case from 0.05 to 0.08 mmol/(min mg protein) (Tables 1, 2). The incubation of the R_1 and R_2 agglutinins with LPS augmented their β-glucosidase activity from 0.15 to 0.21 and from 0.05 to 0.11 mmol/(min mg protein), i.e., by 1.4 and 2.2 times, respectively. Unlike the β-glucosidase activity, the proteolytic activity of R_1 and R_2 incubated with EPS and LPS decreased in all cases, the decrease varying from 1.3 to 2.6 times (Tables 1, 2).

Table 2. The effect of EPS, LPS, and root exocomponents (RECs) on the enzymatic activity of agglutinins from *Rh. leguminosarum* bv. *viciae* 252

Agglutinins and carbohydrate receptors	Proteolytic activity, mg alanine/(min mg protein)	β-Glucosidase, mmol/(min mg protein)
R_1 + EPS	0.023 ± 0.003	0.120 ± 0.001
R_2 + EPS	0.048 ± 0.010	0.080 ± 0.002
R_1 + LPS	0.025 ± 0.003	0.210 ± 0.002
R_2 + LPS	0.024 ± 0.002	0.110 ± 0.002
R_1 + RECs	0.027 ± 0.003	0.200 ± 0.0001
R_2 + RECs	0.032 ± 0.005	0.070 ± 0.0010

Note: EPS and LPS are autoglycoreceptors. RECs are exogenous glycoreceptors. $P < 0.05$ relative to the control (R_1 and R_2 without carbohydrates).

These data suggest that the rhizobial lectins may have two active centers, one being responsible for hemagglutinating activity and the other being responsible for enzymatic activity. The blocking of the hemagglutinating centers of the lectins by the polysaccharides obviously leads to conformational alterations in their enzymatic centers, which may either increase or decrease the enzymatic activity of the lectins. Previously, similar suggestions have been made with respect to bacillar lectins [16], the α-galactosidase of *Cephalosporium acremonium* [17], and the α-mannanase of *Rhodococcus erythropolis* [18].

The agglutinins of *Rh. leguminosarum* bv. *viciae* 252 may act as adhesins during the formation of legume–rhizobial symbiosis [5]. The specific contacts between the carbohydrate components of roots and microbial receptors occur in the carbohydrate-containing mucigel that covers root hairs [19, 20]. It can be suggested that the interaction of rhizobial agglutinins with some carbohydrates in the mucigel may change the enzymatic activity of the agglutinins.

To prove this suggestion, we studied the effect of the exocomponents of pea seedling roots on the enzymatic activity of the rhizobial agglutinins. In [11], we showed that these exocomponents contain glucosamine (52%), uronic acids (8%), and neutral sugars (9% glucose, 4% galactose, 2% mannose, and approximately 0.5% each arabinose, fucose, rhamnose, and ribose).

The incubation of the R_1 and R_2 agglutinins of *Rh. leguminosarum* bv. *viciae* 252 (agglutination titer 1 : 4) with the exocomponents of pea roots subjected to hydrolysis did not affect the hemagglutinating activity of the agglutinins, which is in agreement with our earlier finding that the specific carbohydrate receptors for microbial agglutinins are complex rather than simple sugars [11]. At the same time, the incubation of R_1 and R_2 with the unhydrolyzed root exocomponents augmented the β-glucosidase activity of R_1 and R_2 by 1.3 and 1.4 times, respectively (Tables 1, 2). In contrast, the proteolytic activity of the R_1 and R_2 agglutinins decreased in the presence of the root exocomponents by 1.4 and 1.9 times, respectively. Consequently, the car-

bohydrate fraction of the exocomponents probably contains haptens specific for R_1 and R_2. Similar results were obtained earlier during the study of the interaction of lectins from nitrogen-fixing bacilli with the carbohydrate fraction of wheat root exocomponents [2].

The β-glucosidase and proteolytic activities of rhizobial agglutinins, together with their adhesive properties [5], allow the suggestion to be made that these agglutinins are essential in establishing the symbiosis between nitrogen-fixing bacteria and higher plants. Due to their affinity for carbohydrates, rhizobial agglutinins can recognize respective receptors in the mucigel of root hairs. The interaction of the agglutinins with the receptors blocks the agglutinating center, which alters the conformation of the agglutinins and changes their enzymatic activities (in particular, increases β-glucosidase activity). It would be tempting to suggest that the increased β-glucosidase activity of rhizobial agglutinins enhances the degradation of the polysaccharide complexes of plant cell walls, thus promoting the penetration of rhizobial cells into plant roots and the formation of plant–rhizobial symbiosis.

REFERENCES

1. Karpunina, L.V., Mel'nikova, U.Yu., Vishnevetskaya, O.A., and Nikitina, V.E., The Lectins of *Bacillus polymyxa*: Localization and Interaction with Different Parts of Wheat Roots, *Mikrobiologiya*, 1993, vol. 62, no. 2, pp. 301–313.
2. Karpunina, L.V., Mel'nikova, U.Yu., and Konnova, S.A., Biological Role of Lectins from the Nitrogen-Fixing *Paeniibacillus polymyxa* Strain 1460 during Bacterial–Plant Root Interactions, *Curr. Microbiol.*, 2003, vol. 47, no. 5, pp. 376–378.
3. Karpunina, L.V., Savenkova, N.N., Vladimirova, M.V., Koltunova, E.F., and Nikitina, V.E., The Agglutinins of *Rhizobium leguminosarum* and Their Role in the Interactions with Plants, *Izv. Ross. Akad. Nauk, Ser. Biol.*, 1996, no. 6, pp. 698–704.
4. Kovalevskaya, T.M., The Dynamics of Carbohydrate Accumulation by Different *Rhizobium leguminosarum* Strains, *Microbiol. Zh.*, 1984, vol. 46, no. 1, pp. 39–42.
5. Karpunina, L.V., Savenkova, N.N., Vishnevetskaya, O.A., Mashkina, A.B., and Nikitina, V.E., Role of Cell Surface Agglutinins of *Rhizobium leguminosarum* in the Attachment to Pea Roots, *Mikrobiologiya*, 1996, vol. 65, no. 4, pp. 512–516.
6. Kosenko, L.V., Patseva, M.A., and Zakharova, I.Ya., The Monosugar Composition of the Surface Polysaccharides of Pea Rhizobacteria, *Mikrobiologiya*, 1990, vol. 59, no. 2, pp. 289–295.
7. Dubois, M., Gilles, K.A., Hamilton, J.K., Rebera, P.A., and Smitch, T., Colorimetric Method for Determination of Sugars and Related Substances, *Anal. Chem.*, 1956, vol. 28, no. 3, pp. 350–356.
8. Kwon, K.-S., Kang, H.G., and Hak, Y.C., Purification and Characterization of Two Extracellular β-Glucosidases from *Aspergillus nidulans, FEMS Microbiol. Lett.*, 1992, vol. 97, pp. 149–154.
9. Preston, K.R. and Kruger, J.E., Physiological Control of Exo- and Endoproteolytic Activities in Germinating Wheat and Their Relationship to Storage Protein Hydrolysis, *J. Plant Physiol.*, 1978, vol. 64, pp. 450–454.
10. Oivin, I.A., The Statistical Processing of Experimental Results, *Patol. Fiziol. Eksperim. Terap.*, 1960, no. 4, pp. 76–89.
11. Karpunina, L.V., Nikitina, V.E., Koltunova, E.F., Kosenko, L.V., Patseva, M.A., and Cherneva, I.N., The Interaction of Agglutinin from *Rhizobium leguminosarum* 252 with Autogenous and Plant Receptors, *Mikrobiol. Zh.*, 1995, vol. 57, no. 1, pp. 64–70.
12. Draper, R.K., Chin, D., and Simon, M.I., Diphtheria Toxin Has the Properties of a Lectin, *Proc. Natl. Acad. Sci. USA*, 1978, vol. 75, no. 1, pp. 261–265.
13. Dotevall, H., Jonson-Stromberg, G., Sanyal, S., and Holmgren, J., Characterization of Endotoxin and Soluble Haemagglutinin from *Vibrio mimicus*: Identity of *Vibrio cholerae* 01 Toxin and Haemagglutinin, *FEMS Microbiol. Lett.*, 1985, vol. 27, no. 1, pp. 17–22.
14. Keusch, G.T., Yacevicz, M., and Donohue-Rolfe, A., Pathogenesis of *Shigella diarrhea*: Evidence for N-Linked Glycoprotein *Shigella* Toxin Receptor Modulation by β-Galactosidase, *J. Infect. Dis.*, 1986, vol. 153, no. 2, pp. 238–248.
15. Lakhtin, V.M., Simonenko, I.A., and Budanov, M.V., The Purification and Some Properties of the Extracellular Sialo-Specific Lectin of *Bacillus subtilis* 316M, *Prikl. Biokhim. Mikrobiol.*, 1993, vol. 29, no. 3, pp. 389–397.
16. Mel'nikova, U.Yu., Karpunina, L.V., Ostakhina, N.V., and Ignatov, V.V., Enzyme Activity of Lectins from the Nitrogen-Fixing Soil Bacterium *Bacillus polymyxa, Curr. Microbiol.*, 2000, vol. 41, pp. 246–249.
17. Lakhtin, V.M. and Zaprometova, O.M., β-Galactosidase from *Cephalosporium acremonium* 237 and Its Lectin Properties, *Biokhimiya*, 1988, vol. 53, no. 8, pp. 1270–1277.
18. Zacharova, I.Ya., Tamm, V.Y., and Pavlova, I.N., α-Mannanase from *Rhodococcus erythropolis, Methods Enzymol.*, 1988, vol. 160, pp. 620–626.
19. Longmann, D. and Callow, J.A., Specific Saccharide Residues Are Involved in the Recognition of Plant Root Surfaces by Zoospores of *Phytium aphanidermatum, Physiol. Mol. Plant Pathol.*, 1987, vol. 30, pp. 139–150.
20. Moody, S.F., Clarke, A.E., and Basic, A., Structural Analysis of Secreted Slime from Wheat and Cowpea Roots, *Phytochemistry*, 1988, vol. 27, no. 9, pp. 2857–2861.