Microbiology, Vol. 70, No. 5, 2001, pp. 557–559. Translated from Mikrobiologiya, Vol. 70, No. 5, 2001, pp. 647–650.
Original Russian Text Copyright © 2001 by Alen'kina, Nikitina, Borisova-Golovko.

EXPERIMENTAL ARTICLES

Investigation of the Relationship between the Lectin Activity of Azospirilla and Their α-Glucosidase, β-Glucosidase, and β-Galactosidase

S. A. Alen'kina, V. E. Nikitina, and M. V. Borisova-Golovko

Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, pr. Entuziastov 13, Saratov, 410015 Russia

Received April 20, 2000; in final form, January 24, 2001

Abstract—The activities of α-glucosidase, β-glucosidase, and β-galactosidase were studied during the isolation and purification of lectins from *Azospirillum brasilense* Sp7 and *Azospirillum lipoferum* 59b cells. These enzymatic activities were revealed in crude extracts of surface proteins, protein fraction precipitated with ammonium sulfate or ethanol–acetone mixture, and protein fraction obtained by gel filtration on Sephadex G-75. The distribution of the enzymes between different protein fractions varied for the azospirilla studied. The cofunction of the *A. brasilense* Sp7 lectin and β-galactosidase on the cell surface is assumed. A strong interaction between the *A. lipoferum* 59b lectin and glucosidases was revealed. The lectin from *A. lipoferum* 59b may possess saccharolytic activity.

Key words: azospirilla, α-glucosidase, β-glucosidase, β-galactosidase, lectin activity.

Azospirilla, widespread soil rhizosphere bacteria, are known to occur in the cortex of grass roots [1–3]. The inoculation of various cereals with these bacteria was found to increase the crop yield [4, 5]. The capacity of azospirilla for nitrogen fixation and production of plant hormones [6, 7] appeared to be responsible for the increase in the grass yield [8].

The hypothesis that the penetration of *Azospirillum* cells into the root cortex initiated the studies of bacteria–root associations. The penetration of bacteria into the root cortex occurs through mechanically injured zones. This process can be facilitated by bacterial polysaccharide-modifying enzymes. The binding of various enzymes to immobilized or free lectins were described [9, 10]. At the same time, the so-called hemagglutinating enzymes, which possess both enzymatic and hemagglutinating activities, are known [11, 12].

In view of the foregoing, we suggested that the lectins of azospirilla either possess hydrolytic activity or may be associated with some hydrolytic enzymes.

MATERIALS AND METHODS

The type strain *Azospirillum brasilense* Sp7 was obtained from the Institute of Microbiology, Russian Academy of Sciences (Moscow); the strain *A. lipoferum* 59b (B-1519) was obtained from the All-Russia Collection of Microorganisms; and the strain *A. brasilense* Sp7.2.3 with eliminated lectin activity was derived from the strain *A. brasilense* Sp7 by transposon mutagenesis [13]. Azospirilla were grown in the synthetic medium described by Sadasivan and Neyra [14] at 37°C for 18 h.

The agglutinating activity of cells was assayed using a 2% suspension of trypsin- treated rabbit erythrocytes [15].

Lectins were isolated from the cell surface by the Echdat method [16].

Proteins were purified by gel filtration on a column (30 × 2.2 cm) with Sephadex G-75 (40–120 μm) using 0.1 M CH_3COOH (pH 4.8) and or a buffer containing 0.15 M NaCl (pH 7.0) as eluents. The elution was carried out at a rate of 1.5 ml/min. The absorbance of the eluate was recorded at 278 nm using a Uvicord SII monitor (LKB).

α-Glucosidase, β-glucosidase, and β-galactosidase were assayed by measuring the amount of nitrophenol formed from 4-nitrophenyl-α-D-glucopyranoside, 4-nitrophenyl-β-D-glucopyranoside, and 4-nitrophenyl-β-D-galactopyranoside, respectively [17]. The amount of the nitrophenol produced was determined spectrophotometrically at 425 nm. One unit of enzymatic activity was defined as the amount of enzyme required for the hydrolysis of 1 μmol of substrate per 1 min at pH 4.6 at 37°C. The specific activity of enzymes was expressed as units/mg protein.

The results were statistically processed in terms of Studentís *t*-test statistics [18].

0026-2617/01/7005-0557$25.00 © 2001 MAIK "Nauka/Interperiodica"

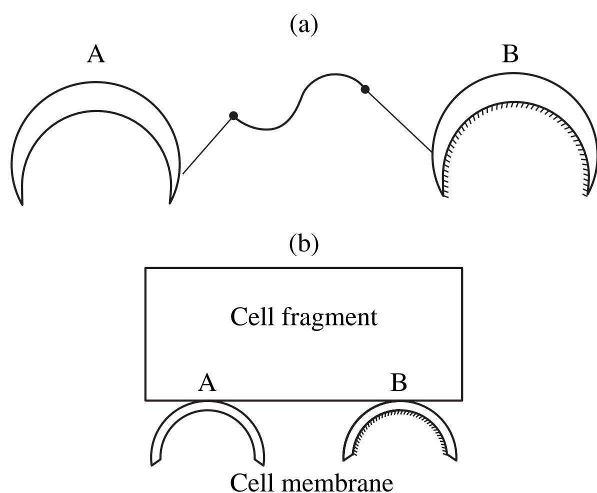

Fig. 1. (a) Lectin (A) and hydrolytic (B) moieties of the same molecule and (b) functionally related lectin (A) and hydrolytic enzyme (B) on the cell surface.

RESULTS AND DISCUSSION

According to Gilboa-Garber and Garber [19], the main function of lectins in microorganisms, plants, and animals is their cofunction with hydrolytic enzymes. Lectins recognize carbohydrate-containing receptors, attach to them, and induce hydrolytic activities. In this case, there are two possibilities: either the lectin molecule contains a moiety with an enzymatic activity (see Fig. 1a) or the lectin and enzymatic activities belong to different molecules, which, because of their close spatial arrangement on the membrane, may cofunction (see Fig. 1b).

To clear up the lectin interrelations with α-glucosidase, β-glucosidase, and β-galactosidase, it was necessary to determine whether or not hemagglutinating and enzymatic activities are linked during the isolation of lectins from the cell surface, how do these activities change as the lectins are purified, and whether or not the lectins possess enzymatic activity.

The isolation and purification of lectins were performed in several steps. At the first stage, the crude extract of cell-surface proteins was obtained. At the second stage, proteins were precipitated by two methods: (1) with ammonium sulfate at 55% saturation; and (2) with a mixture of ethanol and acetone in a ratio of 1 : 1. At the final stage, proteins were purified by gel filtration.

The crude extracts of the cell-surface proteins of *A. brasilense* Sp7 and *A. lipoferum* 59b possessed all three enzymatic activities. β-Galactosidase activity prevailed in the extracts of both species, being an order of magnitude lower in the extract of *A. lipoferum* 59b cells (see table). Changes in β-galactosidase activity during lectin purification were different for the two species studied. In the case of *A. brasilense* Sp7, the specific β-galactosidase activity increased as lectins were purified, to be almost 2.5-fold higher in the preparation obtained by gel filtration on Sephadex G-75 than in the crude extract. By contrast, the activity of the *A. lipoferum* 59b β-galactosidase decreased by 1.6 times at the

α-Glucosidase, β-glucosidase, and β-galactosidase activities of protein fractions during the isolation and purification of lectins from azospirilla

Purification step	α-Glucosidase, units/mg protein			β-Glucosidase, units/mg protein			β-Galactosidase, units/mg protein		
	A. brasilense		*A. lipoferum*	*A. brasilense*		*A. lipoferum*	*A. brasilense*		*A. lipoferum*
	Sp7	Sp7.2.3	59b	Sp7	Sp7.2.3	59b	Sp7	Sp7.2.3	59b
Crude extract of cell-surface proteins	6.1 ± 0.4	8.0 ± 0.4	2.00 ± 0.01	7.0 ± 0.3	4.2 ± 0.4	2.8 ± 0.1	32.1 ± 2.3	30.2 ± 1.1	4.9 ± 0.1
Precipitation with:									
Ammonium sulfate	1.50 ± 0.07	8.3 ± 0.4	8.10 ± 0.02	2.6 ± 0.1	1.4 ± 0.1	14.2 ± 0.2	35.0 ± 1.2	46.0 ± 2.0	4.7 ± 0.8
Ethanol–acetone mixture	2.2 ± 0.1	5.4 ± 0.6	3.30 ± 0.05	3.70 ± 0.02	3.5 ± 0.1	6.3 ± 0.1	42.1 ± 2.1	31.3 ± 3.0	3.5 ± 0.1
Gel filtration on Sephadex G-75:									
Fraction precipitated with ammonium sulfate	2.3 ± 0.1	2.0 ± 0.1	11.7 ± 2.0	1.24 ± 0.10	1.0 ± 0.1	15.7 ± 0.1	66.3 ± 3.3	45.2 ± 4.4	4.8 ± 1.0
Fraction precipitated with ethanol–acetone mixture	1.0 ± 0.1	0.5 ± 0.01	13.1 ± 2.5	0.44 ± 0.02	1.5 ± 0.2	14.0 ± 0.1	76.0 ± 6.2	57.0 ± 2.5	3.0 ± 0.1

Note: Data are the means of five replicated measurements followed by the standard deviations from the means.

final stage of lectin purification. The activities of α- and β-glucosidases in the crude extracts of cell-surface proteins from *A. brasilense* Sp7 cells were almost the same and considerably higher than in the case of *A. lipoferum* 59b (see table). As lectins were purified, glucosidase activities either decreased (*A. brasilense* Sp7) or increased (*A. lipoferum* 59b).

Earlier, we described the *A. brasilense* Sp7.2.3 mutant lacking lectin activity [13]. In spite of the absence of this activity, mutant cells were found to contain lectin molecules on their surface, which had the same molecular mass and carbohydrate specificity as in the case of the parent cells but lacked the ability to interact with lectin-specific antibodies.

As can be seen from the table, the tendencies in the enzymatic activities during the isolation and purification of lectins from the cell surface of the parent and mutant cells were similar: the activity of β-galactosidase increased and that of both glucosidases decreased. No enzymatic activities were revealed in the lectin preparation of *A. brasilese* Sp7 subjected to additional purification by electrophoresis (data not presented). At the same time, the electrophoresis of the protein fractions obtained by precipitation with ammonium sulfate or solvent mixture showed the presence of proteins with different molecular masses, which exhibited α-, β-glucosidase and β-galactosidase activities.

Thus, the lectin isolated from *A. brasilese* Sp7 cells exhibited no activities of α-glucosidase, β-glucosidase, and β-galactosidase. However, the results presented indicate that this lectin may cofunction, to a greater or lesser degree, with these enzymes. The *A. brasilense* Sp7 lectin is most closely associated with β-galactosidase, probably because of the close arrangement of these proteins on the cell surface or due to the lectin specificity for D-galactose [20]. The lectin activity of *A. lipoferum* 59b is related to α- and β-glucosidases more closely than to β-galactosidase. This may be indicative of the functioning of lectin–enzyme complexes or of the glucosidase activity of the *A. lipoferum* 59b lectin.

REFERENCES

1. Baldani, V.L.D. and Dobereiner, J., Host Plant Specificity in the Infection of Cereals with *Azospirillum* spp., *Soil Biol. Biochem.*, 1980, vol. 12, pp. 433–439.
2. Gafny, R., Okon, Y., Kapulnik, Y., and Fischer, M., Adsorption of *Azospirillum brasilense* to Corn Roots, *Soil Biol. Biochem.*, 1986, vol. 18, pp. 69–75.
3. Levanony, H. and Bashan, Y., Active Attachment of *Azospirillum brasilense* to the Root Surface of Cereal Plants and to Sand Particles, *Plant Soil*, 1991, vol. 137, pp. 91–97.
4. Kapulnik, Y., Sarig, S., Nur, I., and Okon, Y., Effect of *Azospirillum* Inoculation on the Yield of Field-Grown Wheat, *Can. J. Microbiol.*, 1983, vol. 29, pp. 895–899.
5. Kapulnik, Y., Kigel, J., Okon, Y., *et al.*, Effect of *Azospirillum* Inoculation on Some Growth Parameters and the N-Content of Wheat, Sorghum, and Panicum, *Plant Soil*, 1981, vol. 61, p. 65.
6. Fallik, E., Okon, Y., Epstein, E., Glodman, A., and Fischer, M., Identification and Quantification of IAA and IBA in *Azospirillum brasilense*-inoculated Maize Roots, *Soil Biol. Biochem.*, 1989, vol. 21, pp. 147–153.
7. Murty, M.G. and Ladha, J.K., Influence of *Azospirillum* Inoculation on the Mineral Uptake and Growth of Rice under Hydroponic Conditions, *Plant Soil,* 1988, vol. 108, pp. 281–285.
8. Patriquin, D.G., Dobereiner, J., and Jain, D.K., Sites and Processes of Association between Diazotrophs and Grasses, *Can. J. Microbiol.*, 1983, vol. 29, pp. 900–915.
9. Satav, I.G., Johnston, R.F., Monk, B., and Criddle, R.S., Inhibition of Yeast Mitochondrial ATPase by Concanavalin A, *Arch. Biochem. Biophys.*, 1980, vol. 199, pp. 110–116.
10. Igbal, G. and Saleemuddin, M., Activity and Stability of Glucose Oxidase and Invertase Immobilized on Concanavalin A–Sepharose: Influence of Lectin Concentration, *Biotechnol. Bioeng.*, 1983, vol. 25, pp. 3191–3195.
11. Kovalenko, E.A. and Buglova, T.T., Galactose-Specific Lectin from *Fusarium graminearum* UMB-1060, *Tez. dokl. II Vsesoyuzn. nauchn. konf. "Rezul'taty i perspektivy nauchnykh issledovanii mikrobnykh polisakharidov"* (Proc. 2nd All-Union Conf. "Results and Prospects of the Investigation of Microbial Polysaccharides"), Leningrad, 1984, p. 66.
12. Dey, P.M. and Pridham, J.B., *Vicia faba* α-Galactosidases with Lectin Activities: An Overview, *Lectins Biology, Biochemistry, and Clinical Biochemistry,* Bog-Hansen, T.C. and van Driessche, E., Eds., Berlin: Walter de Gruyter, 1986, vol. 5, pp. 161–170.
13. Alen'kina, S.A., Petrova, L.P., and Nikitina, V.E., Isolation and Characterization of a Lectin-Deficient Mutant from *Azospirillum brasilense* Sp7, *Mikrobiologiya*, 1998, vol. 67, no. 6, p. 782.
14. Sadasivan, L. and Neyra, C.A., Flocculation in *Azospirillum brasilense* and *Azospirillum lipoferum, J. Bacteriol.*, 1985, vol. 163, pp. 716–723.
15. Lis, H. and Sharon, N., Soya Bean Agglutinin, *Methods Enzymol.*, 1972, Part B, pp. 360–368.
16. Echdat, Y., Ofek, I., Yachow-Yan, Y., Sharon, N., and Mirelman, D., Isolation of Mannose-Specific Lectin from *E. coli* and Its Role in the Adherence of Bacteria to Epithelial Cells, *Biochem. Biophys. Res. Commun.*, 1978, vol. 85, pp. 1551–1559.
17. *Carbohydrate Analysis*, Chaplin, M.E. and Kennedy, J.E., Eds., Oxford: IRL, 1986.
18. Rokitskii, P.F., *Biologicheskaya statistika* (Statistics in Biology), Minsk, 1973.
19. Gilboa-Garber, N. and Garber, N., Microbial Lectins Cofunction with Lytic Activities as a Model for a General Basic Lectin Role, *FEMS Microbiol. Rev.*, 1989, vol. 63, pp. 211–222.
20. Nikitina, V.E., Alen'kina, S.A., Ital'yanskaya, Yu.V., and Ponomareva, E.G., Isolation and Comparison of Lectins from the Cell Surface of Hemagglutinating and Non-Hemagglutinating Azospirilla, *Biokhimiya*, 1994, vol. 59, no. 5, pp. 656–662.