ISSN 1068-1620, Russian Journal of Bioorganic Chemistry, 2009, Vol. 35, No. 1, pp. 54–61. © Pleiades Publishing, Ltd., 2009.
Original Russian Text © A.M. Zakharenko, M.I. Kusaikin, Bui Min Li, Fam Van Huen, Hyun Hoang Khan, V.V. Sova, T.N. Zvyagintseva, 2009, published in Bioorganicheskaya Khimiya, 2009, Vol. 35, No. 1, pp. 62–69.

Catalytic Properties of Endo-1,3-β-*D*-glucanase from the Vietnamese Edible Mussel *Perna viridis*

A. M. Zakharenko[a], M. I. Kusaikin[a,1], Bui Min Li[b], Fam Van Huen[b], Hyun Hoang Khan[b], V. V. Sova[a], and T. N. Zvyagintseva[a]

[a] *Pacific Institute of Bioorganic Chemistry, Far East Division, Russian Academy of Sciences, pr. Stoletiya Vladivostoka 159, Vladivostok, 690022 Russia*

[b] *Nyachang Institute of Scientific and Applied Technologies, VANT, Nyachang, Vietnam*

Received February 20, 2008; in final form, July 2, 2008

Abstract—A β-1,3-glucanase with a molecular mass of 33 kDa was isolated in the homogeneous state from a crystalline stalk of the commercially available Vietnamese edible mussel *Perna viridis.* It hydrolyzes β-1,3-bonds in glucans and is capable of catalyzing the transglycosylation reaction. The β-1,3-glucanase has a K_m value of 0.3 mg/ml for the hydrolysis of laminaran and shows a maximum activity in the pH range from 4 to 6.5 and at 45°C. Its half-inactivation time is 180 min at 45°C and 20 min at 50°C. The enzyme was ascribed to glucan-endo-(1 ⟶ 3)-β-*D*-glucosidases (EC 3.2.1.39). The enzyme could be used in the structure determination of β-1,3-glucans and enzymatic synthesis of new carbohydrate-containing compounds.

Key words: endo-β-1,3-glucanase, mussel, Perna viridis, crystalline stalk, laminaran

DOI: 10.1134/S1068162009010075

INTRODUCTION

β-1,3-Glucanases (laminarinases) belong to *O*-glycosyl hydrolases, the key enzymes of carbohydrate metabolism. They cleave *O*-glycoside bonds in β-1,3-glucans. These enzymes are widely distributed in various organisms, from archebacteria to eukaryotes, and are involved in many physiological processes [1]. In particular, β-1,3-glucanases participate in the degradation of polysaccharides, which are utilized by bacteria as an energy source. In fungi, these enzymes catalyze the lysis of the intrinsic cell matrix during the development of cells. In plants, they take part in cell differentiation, the defense of cells against pathogenic fungi, and the degradation of the β-1,3-glucan layer of the envelope of seeds during their germination. β-1,3-Glucanases play an important role in the digestion of sea invertebrates. They are assumed to be involved in the fertilization and early embryogenesis of sea urchins.

Sea invertebrates, which are represented by a great number of taxons being at different stages of evolution and differing from one another in the mode of nutrition and living, are rich and relatively accessible sources of various *O*-glycosyl hydrolases [2, 3]. Some sea invertebrate species serve as objects of mariculture. *O*-Glycosyl hydrolases are most often found in the alimentary tracts of sea animals, which are brought upon industrial processing of animals into wastes.

[1] Corresponding author; phone: (4232) 31-07-05; fax: (4232) 31-40-50; e-mail: mik@piboc.dvo.ru.

We have shown previously that β-1,3-glucanases occur widely in sea invertebrates inhabiting different regions of the World ocean. The highest value of activity of β-1,3-glucanases is observed in the alimentary organs of sea mollusks and crustaceans; the enzymes are particularly abundant in crystalline stalks of bivalves [4]. Some β-1,3-glucanases have been isolated in the homogeneous state from crystalline stalks of mollusks *Spisula sachalinensis* (LIV) [5], *Chlamys albidus* (L0) [6], and *Mizuhopecten yessoensis* (LV) [7], and their physicochemical properties and the mechanisms of action have been studied. By now the genes and amino acid sequences of LIV and LV β-1,3-glucanases [7, 8] have been determined, which permitted one to assign these β-1,3-glucanase to the 16th family of *O*-glycosyl hydrolases. A distinguishing feature of the catalytic action of β-1,3-glucanases from sea mollusks is their high transglycosylating activity, which is successfully used in the enzymatic synthesis of novel compounds and the transformation of natural glucans with the aim of increasing their biological activity [9–11].

The goal of this work was to isolate and study the properties of β-1,3-glucanase from the crystalline stalk of the marketable species of the mussel *Perna viridis*, which inhabits the coastal zone of the Socialist Republic of Viet-Nam.

RESULTS AND DISCUSSION

A search for *O*-glycosyl hydrolases (β-1,3-glucanases, fucoidanases, and glycosidases) in 14 most

Table 1. Composition of *O*-glycosyl hydrolases from a crystalline stalk of *P. viridis*

Enzyme	Substrate*	Type of bond	Activity, %
β-1,3-Glucanase (laminarinase)	Laminaran	β-1,3; β-1,6	100
β-1,6-Glucanase (pustulanase)	Pustulan	β-1,6	0.2
Amylase	Amylopectin	α-1,4	6.4
Cellulase	CM-cellulose	β-1,4	4
α-Fucosidase	Fuc*p*α-ONp	α	0
β-Glucosidase	Glc*p*β-ONp	β	0
β-Galactosidase	Gal*p*β-ONp-	β	0
α-Mannosidase	Man*p*α-ONp-	α	0

Note: * Np, *p*-nitrophenyl.

widely occurring species of sea invertebrates of the South Chinese Sea has been performed. Fucoidanases and glycosidases (α-fucosidases, β-glucosidases, β-galactosidases, and α-mannosidases were found in the alimentary tracts and oocytes of sea urchins. β-1,3-Glucanase was contained in the alimentary organs of all species examined (sea urchins, mollusks, worms, and goloturians). The maximum activity of β-1,3-glucanase was detected in the crystalline stalk of the mussel *P. viridis*.

An analysis of the content of *O*-glycosyl hydrolases in an extract of the crystalline stalk of the mussel *P. viridis* showed that β-1,3-glucanase is the major enzyme. The activity of the enzymes catalyzing the hydrolysis of amylopectin, CM-cellulose, and pustulan was insignificant (6.4; 4; 0.2%, respectively) compared with the activity of β-1,3-glucanase toward laminaran (100%). The extract contained no glycosidases (Table 1). We previously have shown in studies of the distribution of *O*-glycosyl hydrolases in sea invertebrates that the crystalline stalk of sea bivalves contains highly active β-1,3-glucanases. Other hydrolases: lipases, proteases, and nucleases were not found in this organ [12].

The purification of β-1,3-glucanase involved the stages of cation-exchange chromatography on CM-cellulose and gel filtration on G-75 Sephadex (Table 2). The high concentration of β-1,3-glucanase and the almost complete absence of other enzymes in the starting extract made it possible to obtain, with a low degree of purification (6.4 times), an enzyme, which was homogeneous, as indicated by SDS electrophoresis, and had a molecular mass of 33 kDa (Fig. 1). The yield of the enzyme was 10.6%.

β-1,3-Glucanase exhibited a maximum activity in a wide pH range, from 4 to 6.5 (Fig. 2), and at a temperature of 45°C (Fig. 3). The time of half-inactivation (a 50% decrease in activity) at 45°C was 180 min; at 50°C, it decreased to 20 min (Fig. 4). The K_m value for the laminaran hydrolysis was calculated by the Lineweaver-Burk method to be 0.3 mg/ml (Fig. 5), a value characteristic for β-1,3-glucanases from various sources: 0.3 mg/ml for β-1,3-glucanase from the marine fungus *Trichoderma aureviride* [13] and 0.7, 0.25, and 0.6 mg/ml for the β-1,3-glucanases from sea mollusks L0, LIV, and LV, respectively [7, 14].

A study of the specificity of β-1,3-glucanase from *P. viridis* showed that it cleaves β-1,3-bonds in mixed 1,3;1,6-β-*D*-glucans: it hydrolyzed with a high rate soluble laminaran and translam, and the poorly soluble yeast glucan (Table 3). However, β-1,3-glucanase has no capacity inherent in lytic enzymes of this kind for degrading high-molecular-weight and poorly soluble 1,3;1,6-β-*D*-glucans pachyman and aubasidan. Glucans with other types of bonding, such as pustulan (β-1,6-glycoside bond), amylopectin (α-1,4-glycoside bond), and CM-cellulose, were also not hydrolyzed by this enzyme. A possible explanation of the difference in the rates of hydrolysis of readily soluble glucans laminaran and translam by β-1,3-glucanase is the structural features of these glucans. In molecules of translam synthesized by the enzymatic transformation of natural laminaran [11], β-1,6-bonded glucose residues (about 25%) occur as both inclusions in the main chain of glucan and branches and are concentrated at the nonreducing terminus of the molecule. In a laminaran molecule, β-1,6-bonded glucose residues (about 10%) exist as branches and are relatively uniformly distributed throughout the β-1,3-glucan chain. Presumably, the structure of natural laminaran is preferable for β-1,3-glucanase under study.

A modification of terminal glucose residues in a laminaran molecule (periodate-oxidized laminaran) did not change the rate of its hydrolysis by β-1,3-glucanase from *P. viridis* (Table 3) as compared with starting laminaran, which is characteristic of endoenzymes that cleave the internal bonds of the glucan molecule. As

Table 2. Purification of β-1,3-glucanase from a crystalline stalk of *P. viridis*

Purification stage	Total protein, mg	Total activity; activity units	Specific activity, act.units/mg protein	Purification degree (times)	Yield, %
Extraction	89.1	22.6	0.25	1	100
Ion-exchange chromatography on CM-cellulose	11.7	11.6	1.0	4	51.2
Gel filtration on G-75 Sephadex	1.5	2.4	1.6	6.4	10.6

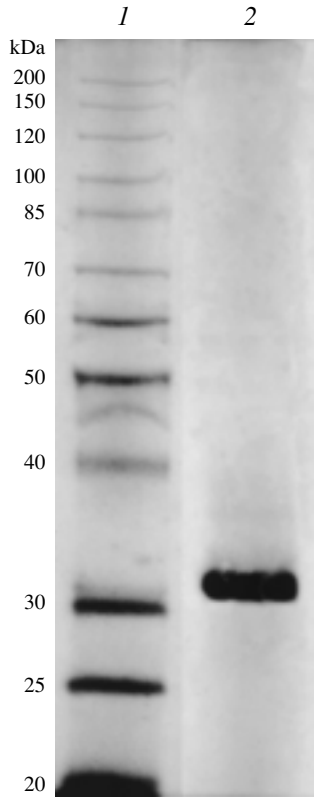

Fig. 1. Electrophoregram of proteins in 15% PAG (reducing conditions): *1*, markers; *2*, Purified 1,3-β-*D*-glucanase from a crystalline stalk of *P. viridis*.

known, exo-β-1,3-glucanases cleaving sequentially glucose (more rarely disaccharide) from the nonreducing terminus of glucan do not hydrolyze periodate-oxidized laminaran [15].

The products of laminaran hydrolysis by β-1,3-glucanase from *P. viridis* were analyzed by MALDI-TOF mass spectrometry (Fig. 6). The composition of the products also confirmed the endotype of the action of the enzyme. With the extent of substrate hydrolysis being about 20%, glucose and oligosaccharides of different polymerization degree were found in the reaction products. The spectra showed the major peaks with *m/z* $[M + Na]^+$: Glc_1 (203.3), Glc_2 (365.2), Glc_3 (527.2), Glc_4 (689.2), Glc_5 (851.3), Glc_6 (1013.5), Glc_7 (1175.7), Glc_8 (1338.0), Glc_9 (1501.0), Glc_{10} (1662.3), Glc_{11} (1825.5), Glc_{12} (1987.6), Glc_{13} (2150.6), Glc_{14} (2312.4).

The degree of cleavage of laminaran on exhaustive hydrolysis by β-1,3-glucanase from *P. viridis* reached 74%. According to HPLC data, the major products with this degree of hydrolysis were glucose and di-, tri-, and tetra-saccharides (Fig. 7).

The specificity of the isolated enzyme toward the hydrolysis of β-1,3-bonds was confirmed by comparing the ^1H NMR spectra of laminaran and the products of its enzymatic hydrolysis. It was shown that the spectra of laminaran contain a doublet of the anomeric proton

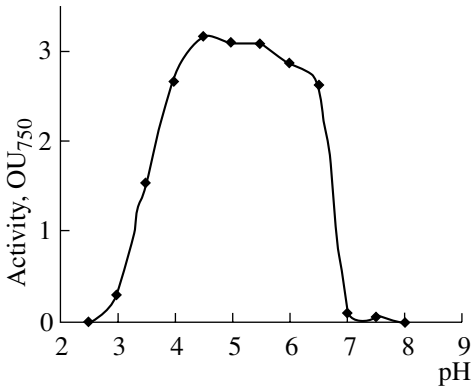

Fig. 2. Determination of pH optimum for the effect of1,3-β-*D*-glucanase from a crystalline stalk of *P. viridis*.

H1 4.98 ppm (*J* 7.9 Hz), which is assigned to β-1,3-bonded glucose residues, and a group of signals in the region of 4.44-4.35 ppm, which corresponds to the anomeric protons of β-1,6-bonded glucose residues (Fig. 8a). The spectra of the products of enzymatic hydrolysis of laminaran showed an increased intensity of signals from the reducing glucose residues α (5.32 ppm) and β (4.76 ppm) (Fig. 8b), which are poorly pronounced in the spectrum of native laminaran, indicating the cleavage of the polysaccharide molecule. In addition, the number of signals in the region of 5.01–4.95 ppm that correspond to the anomeric protons of β-1,3-bonded glucose residues involved in the polysaccharides formed, increases after the enzymatic hydrolysis. The signals of the anomeric protons of β-1,6-bonded glucose residues in the region of 4.44–4.35 ppm do not change after the enzymatic hydrolysis of laminaran.

We showed that, like typical hydrolases, endo-β-1,3-glucanases from sea mollusks have an enhanced, as compared with enzymes from overland sources, capacity for transglycosylation reactions, i. e., reactions in

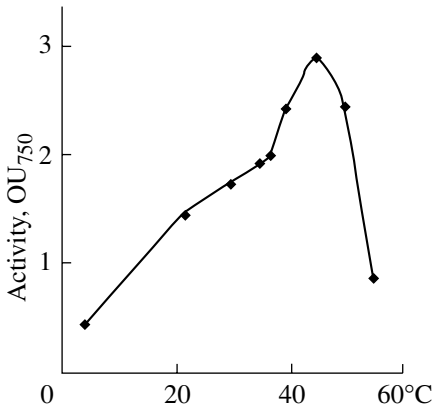

Fig. 3. Determination of temperature optimum for the effect of 1,3-β-*D*-glucanase from a crystalline stalk of *P. viridis*.

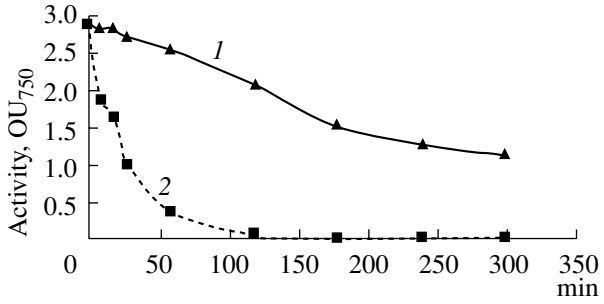

Fig. 4. Determination of thermal stability of 1,3-β-*D*-glucanase from a crystalline stalk of *P. viridis* at (*1*) 45 and (*2*) 50°C.
Key: 1. Activity, OU@750; 2. min

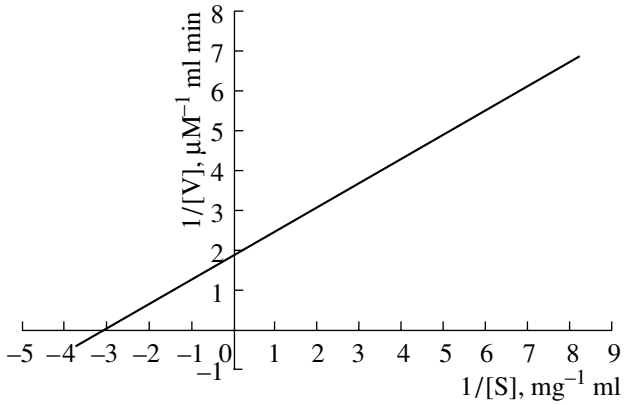

Fig. 5. Lineweaver–Burk plot for determining the Michaelis constant (K_m) for 1,3-β-*D*-glucanase from a crystalline stalk of *P. viridis*.

which the glycon moiety of the substrate is transferred not only to water (hydrolysis) but also to hydroxyl-containing compounds (transglycosylation) [4]. The ratio of the transglycosylation and hydrolysis rate constants k_T/k_T for the endo-β-1,3-glucanases LIV and LO, with glycosyl residues of laminaran as a donor and *p*-nitrophenyl-β-*D*-glucoside as an acceptor, is 2×10^4. For amylases and lysozyme from overland sources, this ratio is approximately 10^2 [9].

We found by HPLC that β-1,3-glucanase from *P. viridis* also effectively catalyzes transglycosylation proceeding with the formation of novel carbohydrate-containing compounds, *p*-nitrophenyl oligosides (Fig. 9). An analysis of the composition of the products at different stages of the reaction showed that the situation that arises is characteristic for the action of endoenzymes: first the formation of larger *p*-nitrophenyl oligosides (NpG_5, NpG_4, and NpG_3; Fig. 9a) and then their transformation to shorter products (NpG_1 and NpG_2; Figs. 9b and 9c) during the secondary hydrolysis take place [16].

It has earlier been found that sulfated polyoxysteroids, to which halistanol sulfate from the tropic sponge of the Halihondriidae family belongs, irreversibly inhibits endo-β-1,3-glucanases from sea mollusks but weakly activates exo-β-1,3-glucanase from the overland mollusk. The results of spectral studies showed that the binding of halistanol sulfate to endo-β-1,3-glucanases induces a conformational transition in molecules of this enzyme [17]. A model of the complex of endo-β-1,3-glucanase LV from *M. yessoensis* and halistanol sulfate was constructed. It was assumed that the inhibition results from the electrostatic interaction of two sulfate groups of halistanol sulfate with arginine residues located near the catalytic domain of the enzyme [7]. β-1,3-Glucanase from *P. viridis* was inhibited by halistanol sulfate (I_{50} 1.3×10^{-4} M). It should be noted that β-1,3-glucanase from the tropic mollusk *P. viridis* is more resistant to this natural metabolite; the concentration of halistanol sulfate required to decrease its activity by 50% is by one order of magnitude higher than for β-1,3-glucanases from sea mollusks inhabiting the colder waters of the Sea of Japan and the Sea of Okhots [17].

Thus, from a crystalline stalk of the marketable Vietnamese mussel *P. viridis*, we isolated β-1,3-glucanase in the homogeneous state, which hydrolyzes β-1,3-bonds in glucans and is capable of catalyzing the transglycosylation reaction. The enzyme, classed as endo-(1 ⟶ 3)-β-*D*-glucosidase (EC 3.2.1.39), can be used to determine the structure of β-1,3-glucans and the enzymatic synthesis of novel carbohydrate-containing compounds.

Table 3. Specificity of β-1,3-glucanase from a crystalline stalk of *P. viridis*

Substrate	Type of bond, ratio	Molecular mass, kDa; solubility*	Activity, %
Laminaran	β-1,3; β-1,6 90 : 10	5–6 P	100
Periodate-oxidized laminaran	β-1,3; β-1,6 90 : 10	5–6 P	100
Translam	β-1,3; β-1,6 75 : 25	8–10 P	32.1
Yeast glucan	β-1,3; β-1,6 90 : 10	>200 CP	1.6
Pachyman	β-1,3; β-1,6 98 : 2	50–120 CP	0
Aubasidan	β-1,3; β-1,6 50 : 50	500–550 CP	0
Pustulan	β-1,6	30 P	0
Amylopectin	α-1,4	2000 P	0
CM-cellulose	β-1,4	>2000 P	0
Glc*p*β-ONp	β	0.301 P	0

Notes: * S, soluble.
** PS, poorly soluble.

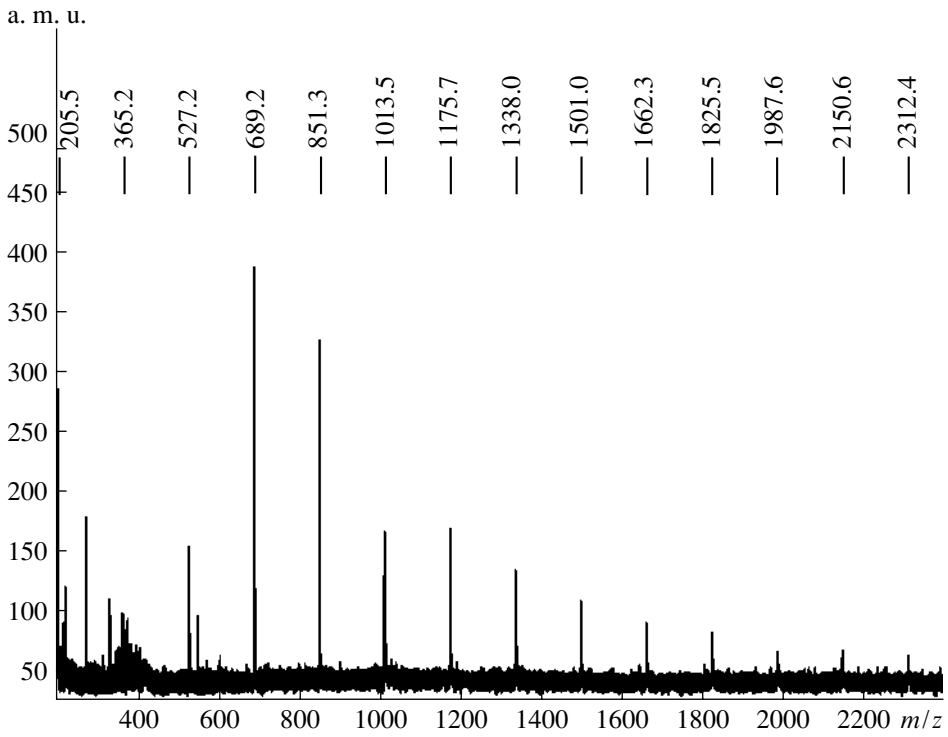

Fig. 6. MALDI TOF mass spectrum of the products of laminaran hydrolysis by 1,3-β-*D*-glucanase from a crystalline stalk of *P. viridis*. The degree of substrate hydrolysis is about 20%.

EXPERIMENTAL

Sea mollusks *P. viridis* were harvested in the coastal zone of the South Chinese Sea (the Socialist Republic of Viet-Nam) in July 2007. Crystalline stalks were stored frozen. Laminaran and fucoidan from brown algae *Laminaria cichorioides* (Phaeophyceae) were isolated by the method described [18], and periodate-oxidized laminaran was obtained as described previously [15]. Translam was obtained by enzymatic transformation of laminaran from *L. cichorioides* [11]. Pustulan from the lichen *Umbilicaria rossica*, yeast glucan from the cell wall of baker's yeast, pachyman from *Poria cocos*, aubasidan from *Aureobasidium pullulans*, and halistanol sulfate from the tropic sponge of the Halihondriidae family were kindly provided by the research workers of the Pacific Institute of Bioorganic Chemistry (Far East Division, Russian Academy of Sciences). Carriers for chromatography, CM-cellulose, amylopectin, and *p*-nitrophenyl derivatives of sugars were the commercial preparations from Sigma (United States).

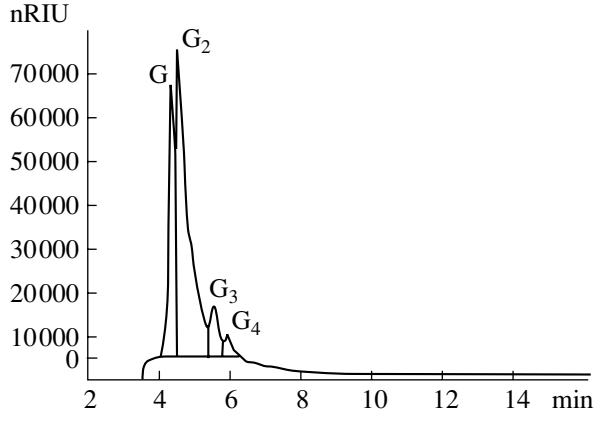

Fig. 7. HPLC of the products of exhaustive hydrolysis of laminaran by 1,3-β-*D*-glucanase from a crystalline stalk of *P. viridis*. G, glucose; G_2, biose; G_3, triose; G_4, tetraosa.

Determination of enzyme activity. A standard reaction mixture contained a 0.1% substrate solution (200 μl) and an enzyme solution (50 μl) in 0.025 M sodium acetate buffer, pH 5.2. The incubation was carried out for 20 min at 37°C. The activity during the hydrolysis of polysaccharides was determined from an increase in the amount of reducing sugars by the method of Nelson using glucose as a standard [19]. The activity during the hydrolysis of *p*-nitrophenyl derivatives of sugars was determined from the amount of released *p*-nitrophenol. A unit of enzyme activity was defined as that amount of the enzyme required to catalyze the formation of 1 μmol of glucose (or *p*-nitrophenol) in 1 min under standard conditions. The specific activity was expressed as unit/mg protein. Protein concentration in solution was determined by the method of Bradford [20] and during chromatography, from absorption at 280 nm.

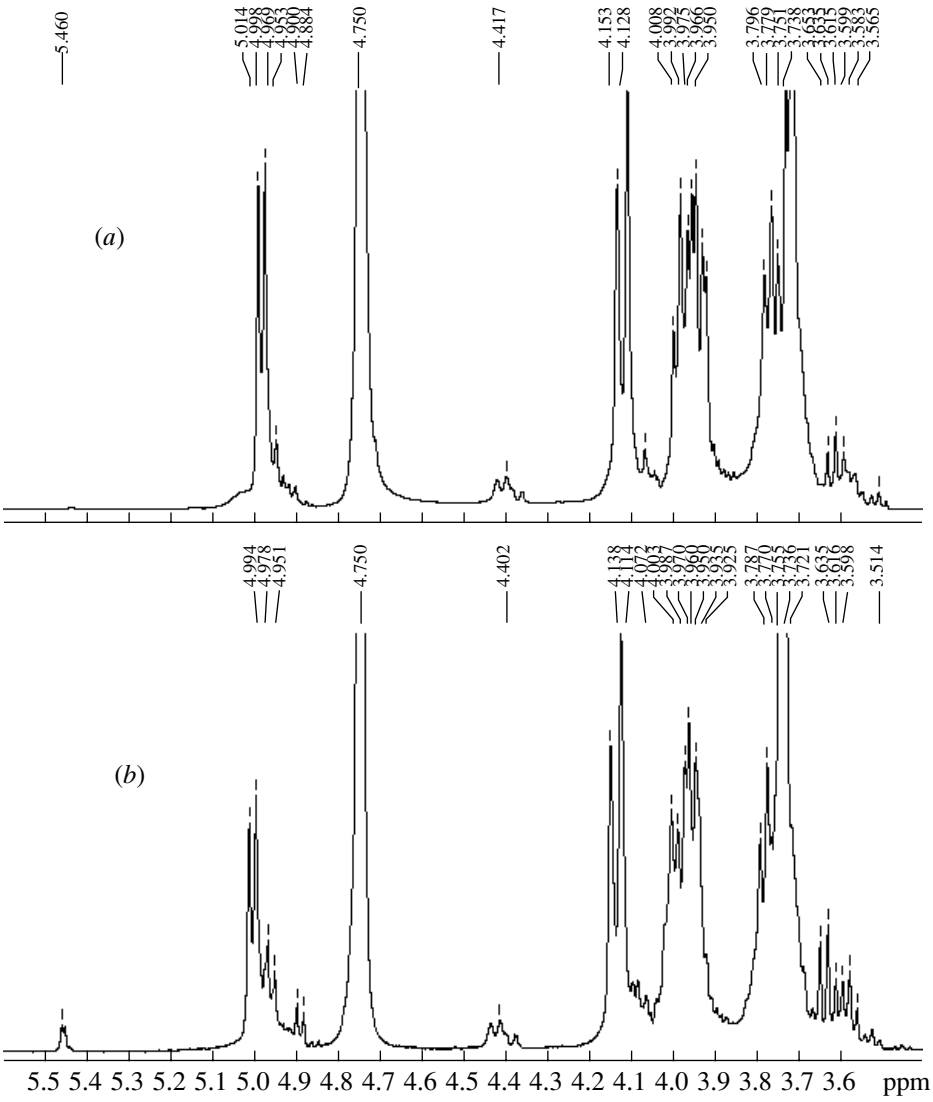

Fig. 8. ^{1}H NMR spectra of (*a*) laminaran and (*b*) the products of its hydrolysis by 1,3-β-*D*-glucanase from a crystalline stalk of *P. viridis*.

MALDI-TOF mass spectra of laminaran and the products of its enzymatic hydrolysis were recorded on a Bruker Biflex III MALDI-TOF spectrometer (Germany).

^{1}H NMR spectra were recorded on a Bruker DRX 500 spectrometer (Germany).

The composition of the hydrolysis products was determined by HPLC on an Agilent 1100 Series chromatograph (United States) using a Zorbax-NH_2 column (4.6 × 250 mm, Germany). Elution was with acetonitrile–water 80 : 20, and detection was performed using a refractometer.

Transglycosylation products were analyzed by HPLC on an Agilent 1100 Series chromatograph (United States) using a C_8 column (4.6 × 150 mm, Zorbax, Germany). The products were eluted using a gradient of acetonitrile concentration in water (from 0 to 80%) at a flow rate of 1 ml/min. The yield of products was registered by absorption at 302 nm.

Isolation and purification of β-1,3-glucanase. Crystalline stalks (6.6 g) were homogenized in 70 ml of cooled 0.025 I sodium acetate buffer, pH 5.2, at 4°C. The homogenate was centrifuged for 15 min at 12000 rpm. The supernatant was applied to a column with CM-cellulose (1.5 × 10 cm) equilibrated with the same buffer. The elution was performed using a linear gradient of NaCl concentration in buffer (from 0 to 1 M; the total volume 300 ml) at a flow rate of 0.5 ml/min. The protein content in fractions (from absorption at 280 nm) and the β-1,3-glucanase activity were determined as described above. Fractions (3.5 ml) containing β-1,3-glucanase were combined, concentrated to 10.5 ml by ultrafiltration on a pH-10 membrane (Amicon, Netherlands). An enzyme preparation was applied to a Sephadex G-75 column (1.5 × 18 cm)

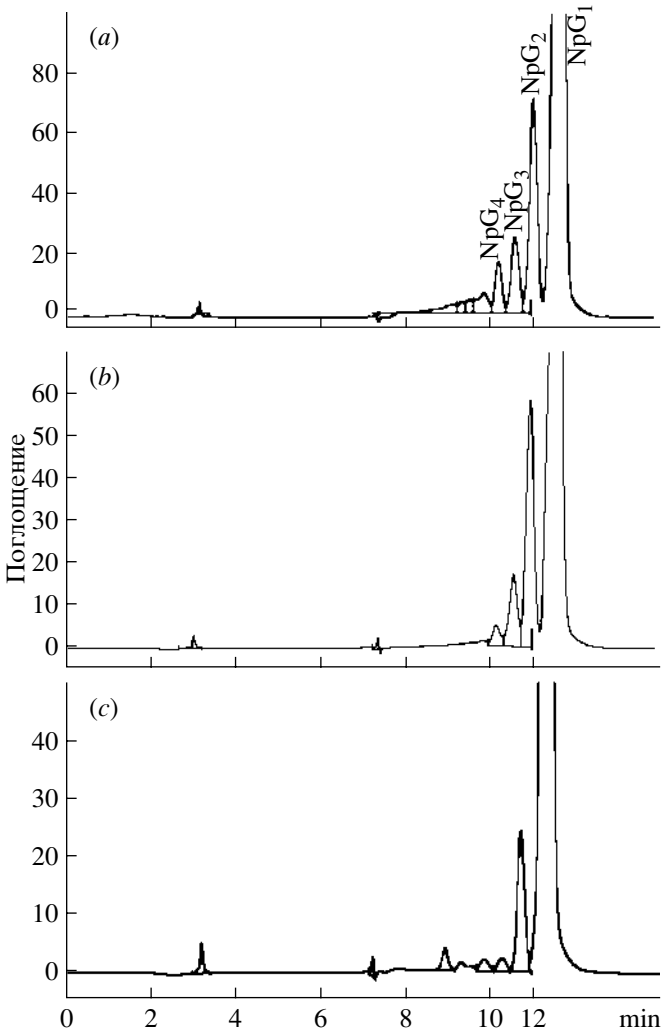

Fig. 9. HPLC of the products of transglycosylation catalyzed by 1,3-β-*D*-glucanase from a crystalline stalk of *P. viridis*. NpG_1, *p*-nitrophenyl-β-*D*-glucopyranoside; NpG_n, *p*-nitrophenyl oligosides. Reaction time (*a*) 10 min, (*b*) 30 min, and (*c*) 24 h.

equilibrated with 0.025 I acetate buffer, pH 5.2. Elution was performed with buffer at a flow rate of 0.3 ml/min; the volume of fractions was 1 ml. Fractions containing β-1,3-glucanase were combined, concentrated by ultrafiltration, and stored in a refrigerator at 4°C.

The homogeneity and the molecular mass of β-1,3-glucanase were established by SDS electrophoresis in 15% PAG under reducing conditions [21].

Effect of β-1,3-glucanase on different substrates. The reaction mixture contained 50 μl of an enzyme solution in buffer (enzyme activity 10^{-2} units) and 200 μl of a substrate solution (1 mg/ml). Incubation with laminaran and translam was performed for 20 min, and incubation with other substrates, for 3-24 h. The rates of hydrolysis of the substrates were expressed in percent relative to the rate of laminaran degradation.

The Michaelis constant (K_m) for the laminaran hydrolysis was calculated by the Lineweaver-Burk method. The initial rates of the hydrolysis of laminaran (concentrations from 0.01 to 0.1%) by purified β-1,3-glucanase (10^{-2} units) under standard conditions were determined.

Obtaining the products of hydrolysis and transglycosylation by endo-1,3-β-*D*-glucanase. An enzyme solution (100 μl; 2×10^{-2} units) was added to 400 μl of a laminaran solution (1 mg/ml), and the mixture was incubated at 37°C. At definite intervals, samples (50 μl) were taken. The reaction was terminated by boiling. The products were analyzed by MALDI-TOF mass spectrometry.

Transglycosylation products were obtained by adding the enzyme (50 μl; 10^{-2} units) in buffer to 1 ml of a solution containing laminaran (2 mg) and *p*-nitrophenyl-β-*D*-glucopyranoside (2 mg). The incubation was performed at 37°C. At definite intervals, 50 μl aliquots were taken, and acetonitrile (50 μl) was added to the aliquots. The samples were analyzed by HPLC.

Opimum pH. A reaction mixture containing 50 μl of the enzyme solution (10^{-2} units) and 200 μl of a laminaran solution (1 mg/ml) in 0.2 I citrate-phosphate buffer of different pH values (from 2.5 to 8.0) was incubated for 20 min at 37°C, and the reducing capacity in samples was determined by the method of Nelson [18].

Temperature optimum. A standard mixture of the enzyme and laminaran was incubated for 20 min at temperatures from 4 to 60°C, and the reducing capacity in samples was determined [18].

Thermostability. An enzyme solution (400 μl; enzyme activity $(8 \times 10^{-2}$ units) was kept at different temperatures (from 40 to 60°C), and 50 μl aliquots were taken at definite intervals. Then laminaran (1 mg/ml, 200 μl) was added to cooled samples, the samples were incubated for 20 min at 37°C, and the residual activity of the enzyme was determined.

Effect of halistanol sulfate on endo-1,3-β-*D*-glucanase. A halistanol sulfate solution (50 μl) of different concentration (from 10^{-5} to 10^{-3} M) was added to a solution of the enzyme in buffer (50 μl; enzyme activity 10^{-2} units), the mixture was kept for 20 min at room temperature, 200 μl of a laminaran solution (1 mg/ml) was added, and the residual activity of the enzyme was determined. The I_{50} value was determined from the activity vs. inhibitor concentration dependence.

ACKNOWLEDGMENTS

The authors thank S.D. Anastyuk (Pacific Institute of Bioorganic Chemistry, Far East Division, Russian Academy of Sciences) for recording MALDI-TOF spectra and V.A. Denisenko for recording NMR spectra.

This work was supported by the Russian Foundation for Basic Research (project no. 07-04-90010), a grant

of CRDF (REC-003), and the program of the Russian Academy of Sciences Molecular and Cellular Biology.

REFERENCES

1 1. Mackay, R.M., Baird, S., Dove, M.J., Gines, M., Moranelli, F., Nasim, A., Willick, G.E., Yaguchi, M., and Seligy, V.L., *Biosystems*, 1985, vol. 18, pp. 279–292.
2. Burtseva, Yu.V., Kusaikin, M.I., Sova, V.V., Shevchenko, N.M., Skobun, A.S., and Zvyagintseva, T.N., *Biol. Morya*, 2000, vol. 26, pp. 429–432.
3. Kusaikin, M.I., Burtseva, Yu.V., Svetasheva, T.G., Sova, V.V., and Zvyagintseva, T.N., *Biokhimiya,* 2003, vol. 68, pp. 384–392.
4. Zvyagintseva, T.N., Sova, V.V., Bakunina, I.Yu., Sundukova, E.V., Shevchenko, N.M., Ermakova, S.P., and Elyakova, L.A., *Khim. Interesah Ustoich. Razvit.,* 1998, no. 6, pp. 417–426.
5. Privalova, N.M. and Elyakova, L.A., *Comp. Biochem. Physiol.*, 1978, vol. 60, pp. C. 225–228.
6. Sova, V.V., Elyakova, L.A., and Vaskovsky, V.E., *Biochim. Biophys. Acta*, 1970, vol. 212, pp. 111–115.
7. Kovalchuk, S.N., Sundukova, E.V., Kusaykin, M.I., Guzev, K.V., Anastiuk, S.D., Likhatskaya, G.N., Trifonov, E.V., Nurminski, E.A., Kozhemyako, V.B., Zvyagintseva, T.N., and Rasskazov, V.A., *Comp. Biochem. Physiol.*, 2006, vol. 143, pp. 473–485.
8. Kozhemyako, V.B., Rebrikov, D.V., Lukyanov, S.A., Bogdanova, E.A., Marin, A., Mazur, A.K., Kovalchuk, S.N., Agafonova, E.V., Sova, V.V., Elyakova, L.A., and Rasskazov, V.A., *Comp. Biochem. Physiol.*, 2004, vol. 137, pp. 169–178.
9. Zvyagintseva, T.N., Makar'eva, T.N., Ermakova, S.P., and Elyakova, L.A., *Bioorg. Khim.,* 1998, vol. 24, pp. 219–223.
10. Borriss, R., Krah, M., Brumer, H., Kerzhner, M.A., Ivanen, D.R., Eneyskaya, E.V., Elyakova, L.A., Shishlyannikov, S.M., Shabalin, K.A., and Neustroev, K.N., *Carbohydr. Res.*, 2003, vol. 338, pp. 1455–1467.
11. Zvyagintseva, T.N., Elyakova, L.A., and Isakov, V.V., *Bioorg. Khim.*, 1995, vol. 21, pp. 218–225.
12. Sova, V.V., Elyakova, L.A., and Vaskovsky, V.V., *Comp. Biochem. Physiol.*, 1970, vol. 32, pp. 459–464.
13. Burtseva, Yu.V., Verigina, N.S., Sova, V.V., and Zvyagintseva, T.N., *Prikl. Biokhim. Mikrobiol.*, 2003, vol. 39, pp. 542–548.
14. Elyakova, L.A. and Zvyagintseva, T.N., *Bioorg. Khim.*, 1981, vol. 7, pp. 680–685.
15. Nelson, T.E., Scarletti, J.V., Smith, F., and Kirkwood, S., *Can. J. Chem.*, 1963, vol. 245, pp. 1671–1678.
16. Zvyagintseva, T.N., Nazarova, N.I., and Elyakova, L.A., *Bioorg. Khim.*, 1984, vol. 10, pp. 1342–1346.
17. Bakunina, I.Yu., Sova, V.V., Elyakova, L.A., Makar'eva, T.N., Stonik, V.A., Permyakov, E.A., and Emel'yanenko, V.I., *Biokhimiya* (Moscow), 1991, vol. 56, pp. 1397–1405.
18. Zvyagintseva, T.N., Shevchenko, N.M., Popivnich, I.B., Isakov, V.V., Scobun, A.S., Sundukova, E.V., and Elyakova, L.A., *Carbohydr. Res.,* 1999, vol. 322, pp. 32–39.
19. Nelson, N., *J. Biol. Chem.*, 1944, vol. 153, pp. 375–381.
20. Bradford, M., *Anal. Biochem.*, 1976, vol. 72, pp. 248–254.
21. Laemmli, U.K., *Nature*, 1970, vol. 227, pp. 680–685.

SPELL: 1. Поглощение