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α-N-Acetylgalactosaminidase from Marine Bacterium *Arenibacter latericius* KMM 426^T Removing Blood Type Specificity of A-Erythrocytes

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Abstract—An α-N-acetylgalactosaminidase IV able to remove blood type specificity of human A(II)-erythrocytes and not effecting B(III)-erythrocytes was isolated from the marine bacterium *Arenibacter latericius* KMM 426^T. The α-N-acetylgalactosaminidase IV preparation exhibits high activity during inhibition of hemagglutination with blood group substance A containing determinants analogous to A-erythrocytes. The enzyme has a pH optimum from 7.0 to 8.0 and completely retains its activity during 30-min heating at 50°C and for a week at 20°C. The enzyme can be stored under the sterile conditions for any length of time at 4°C, but it does not withstand freezing. The α-N-acetylgalactosaminidase is resistant to NaCl; for *p*-nitrophenyl-α-D-galactosaminide, the K_m is 0.38 mM. The molecular mass of the enzyme determined by gel filtration is 84 kD.

Key words: α-N-acetylgalactosaminidase, marine bacteria *Arenibacter latericius*, transformation of erythrocytes

Glycosidases of various specificities are used as instruments in the investigation of the antigenic structure of various biological objects. The directed change of blood type specificity of erythrocytes is a special case of enzymatic modifications of antigens. Enzymatic modification of erythrocytes is of practical importance because it allows obtaining erythrocytes of the universal 0(I) group from erythrocytes of various groups.

Thus, α-N-acetylgalactosaminidases (EC 3.2.49) able to remove at neutral pH α-1,3-bound residues of N-acetylgalactosamine from glycoproteins of blood group substances and A-erythrocytes, converting them into 0(I) group substances are of great practical interest (Scheme 1). A method of transformation of A_2 blood group to 0(I) group by the enzyme isolated from hen liver was patented in the USA [1], but this enzyme has a pH optimum at 4.0, its activity drastically decreasing in the neutral region. This enzyme is also specific only for the rather uncommon blood group A_2 antigen [2]. Bacterial α-N-acetylgalactosaminidases from the bacterium *Clostridium perfingens* [3] and fungi *Aspergillus niger* [4] and *Acremonium* sp. [5] inactivating serologic activity of A-erythrocytes are known, but they have some drawbacks. Thus, the enzyme isolated from *A. niger* is not active against the natural substrates. It is rather difficult to obtain glycosidases of the anaerobic pathogenic bacterium *C. perfingens* in considerable amounts; *C. perfringens* strains also produce toxin. Glycosidases from the soil micromycete *Acremonium* sp. exhibits activity under non-physiological conditions (pH 4.5). α-N-Acetylgalactosaminidase of required specificity is produced into the cultural liquid by surface-originated anaerobic bacteria *Ruminococcus torques* IX-70 strains isolated from human feces [6]. These bacteria need special cultivation conditions and growth factors. So, effective producers of α-N-acetylgalactosaminidase are still unknown. Marine microorganisms and their enzymes have not been studied in this aspect.

As shown earlier, only about 20% of marine as well as freshwater strains studied by us contained α-N-acetylgalactosaminidases [7, 8]. Enzymes able to inactivate serologic activity of A-erythrocytes of human blood are still more uncommon (Kuhlmann, Likhosherstov, and Martynova, unpublished data).

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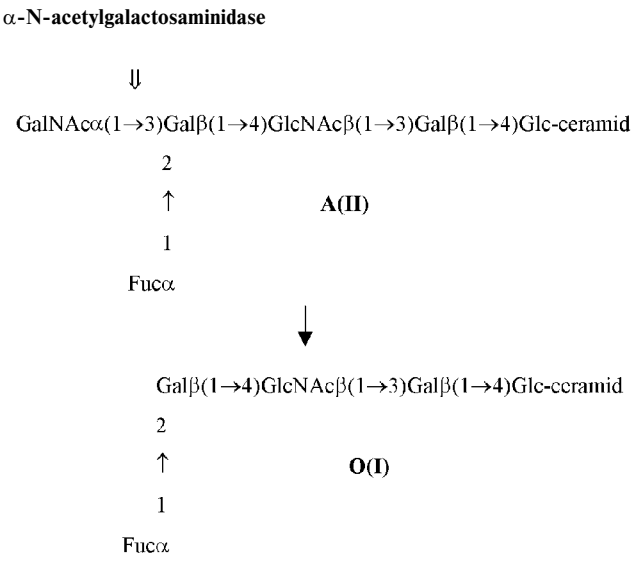

Scheme 1. Modification of blood group substance A by α-N-acetylgalactosaminidase

The α-N-acetylgalactosaminidase of the pigmented bacterium *Flavobacterium* sp. KMM 426 exhibited the maximal activity against not only commercial substrate *p*-nitrophenyl-α-N-acetyl-D-galactosaminide, but also against modification of A-erythrocytes to H-erythrocytes [9]. Taxonomic study showed that these bacteria belong to the *Cytophaga-Flavobacterium-Bacteroides* group. Based on 16S ribosome gene sequencing and pheno-, hemo-, and genotypic characteristics, the isolate was assigned to a new genus and species of marine bacteria *Arenibacter latericius* KMM 426^T [10].

This work presents isolation of α-N-acetylgalactosaminidase from biomass of the marine bacterium *Arenibacter latericius* KMM 426^T and study of its physicochemical and enzymatic properties and specificity of its action on human blood erythrocytes.

MATERIALS AND METHODS

Bacterial isolation and cultivation. *Arenibacter latericius* KMM 426^T strain was isolated from ground sediments sampled in South China Sea from 20-m depth in December 1988 during the 8th expedition of the research ship "Academician Oparin".

KMM 426 strain was cultivated on medium containing 5.0 g/liter bactopeptone from Difco (USA), 1.0 g/liter glucose, 0.2 g/liter K_2HPO_4, 500 ml of distilled water, 500 ml of sea water, pH 7.8. Seeding material was grown on a shaker (150 rpm) in 250-ml shaker flasks (with medium of 50 ml) for 24-30 h at 25°C to the density 10^9 cells/ml. The resulting material was seeded into 1000-ml flasks with 500 ml of the same fermentative medium. Fermentation was performed on a shaker (120 rpm) for 24 h at 25°C.

Assay of activities of accompanying glycoside hydrolases in the total bacterial extract. Glucanase activities were assayed by the appearance of reducing saccharides according to Nelson [11]. To determine the activity of β-1,3-glucanases (laminaranases), laminaran from the brown alga *Laminaria cichorioides* was used as the substrate [12]. Fucoidan hydrolases were tested with fuciodanes of brown algae *Fucus evanescens* and *L. cichorioides* [12]. Activity of β-1,6-glucanases (pustulanases) was assayed in the presence of pustulan from lichen [13]. Commercial reagents—amylose, agarose, alginic acid, carboxymethylcellulose—were used for assaying activity of amylases, agarases, alginases, and cellulases, respectively.

The presence and activity of α-, β-galactosidases, α-N-acetylgalactosaminidase, β-glucosidase, β-N-acetylhexosaminidase, α-fucosidase, α-mannosidase was tested with *p*-nitrophenyl derivatives of corresponding monosaccharides from Sigma (USA) as described earlier [14].

Isolation and purification of α-N-acetylgalactosaminidase. Wet biomass was suspended in 0.01 M phosphate buffer, pH 7.2, the ratio of biomass and buffer not exceeding 0.2 g/ml. Suspension was ultrasonicated (frequency 22 kHz, current 0.4 A) 4-5 times for 40 sec with 10-20 sec intervals using an ultrasonic disintegrator. The homogenate was centrifuged at 10,000*g* for 30-35 min. The supernatant was purified by ion-exchange chromatography first on a DEAE-Sepharose CL-6B (Sigma) column (15.0 × 2.8 cm) and then on a DEAE-Toyopearl 650 M (Toyo Soda, Japan) column (15.5 × 2.0 cm) in 0.01 M phosphate buffer, pH 7.2. The enzyme was eluted with a linear gradient of NaCl (0-1 and 0-0.5 M, with 500 ml volumes, respectively), flow rate 22 ml/h. Fractions containing α-N-acetylgalactosaminidase were concentrated by ultrafiltration and purified by gel filtration on a Sepharose CL-6B (70 × 2.8 cm) column. Finally, the enzyme preparation was rechromatographed on a DEAE-Toyopearl 650 M column.

The standard hydrolytic activity of α-N-acetylgalactosaminidase (U) was assayed after each step of purification with commercial glycoside of *p*-nitrophenyl-α-N-acetyl-D-galactosaminide from Sigma as described earlier [15]. The amount of the enzyme forming 1 μmol *p*-nitrophenol per min under the described conditions was taken as the activity unit.

Specific activity of the enzyme was calculated as the ratio of the standard activity units to protein mass (U/mg). The protein concentration was determined according to Lowry.

Determination of pH optimum. To determine the pH optimum of α-N-acetylgalactosaminidase IV activity, 0.05 ml of enzyme solution and 0.45 ml of *p*-nitrophenyl-

α-N-acetyl-D-galactosaminide solution (1 mg/ml) in corresponding 0.1 M phosphate (pH 5.1-7.9) and borate (pH 7.9-9.0) buffers was incubated for 15 min at 20°C. Then the reaction was stopped by addition of 1 M Na_2CO_3 (2.5 ml) and the optical density was measured at 410 nm, the substrate solution at the same pH being used as a control.

Determination of thermal stability of the enzyme. α-N-Acetylgalactosaminidase IV solution in 0.01 M phosphate buffer (pH 7.2) was incubated for 30 min at certain temperature, cooled to room temperature, and the residual activity was assayed as describer above.

Agglutination tests were performed according to the standard procedure on 96-well plates in a series of double dilutions of corresponding antiserum. Erythrocytes, standard blood group antisera, and monoclonal antibodies (polyclone-A) were obtained from the Hematology Research Center of the Russian Academy of Medical Sciences.

Effect of enzyme on erythrocytes. Of several samples of donor erythrocytes, those with the maximal agglutination efficiency were chosen. Erythrocytes washed with physiological solution were mixed with enzyme solution (2 U/ml) in isotonic phosphate buffer, pH 7.3. After 24 h incubation with stirring at 36°C, the erythrocytes were washed thrice with physiological solution (pH 7.3) and mixed with corresponding sera in a series of double dilutions on the plates. Agglutination titer was read after 1 h incubation at room temperature.

Assay of activity with blood group-specific substance (BGS). BGS were obtained from the Institute of Organic Chemistry of the Russian Academy of Sciences from pig and horse stomach mucosa [16, 17]. After 24 h incubation of BGS with A determinants with the enzyme solution in isotonic phosphate buffer (pH 7.3) at 26°C, inhibition of hemagglutination [18] was performed on a standard plate for immunological reactions. To a series of sequential dilutions of BGS in physiological solution (aliquots of 50 μl) after incubation with the enzyme, corresponding antiserum (aliquots of 50 μl) diluted eight times with physiological solution was added. After 1 h incubation at room temperature, 40 μl of 1% suspension of I, II, or III blood group erythrocytes was added into each well. The results were read after 1 h incubation at room temperature, BGS not treated with the enzyme being used as a control.

Determination of substrate specificity with oligosaccharides and blood group-specific glycoproteins (BGG). Excision of the galactose residue from melibiose (Galα1,3Glc), disaccharide Galα1,3Gal, and trisaccharide Gal1,3(Fucα1,2)Gal by the action of enzyme was studied using a Biotronic LC-2000 carbohydrate analyzer [19]. Excision of N-acetylgalactosamine from BGG A + H was registered by an amino acid analyzer after acidic hydrolysis of low-molecular-weight fraction of the products of fermentolysis obtained by gel chromatography on Sephadex G-15.

Molecular mass of the enzyme was estimated by gel filtration on a Sepharose CL-6B column (100 × 1 cm) in 0.02 M phosphate buffer, pH 7.2. Trypsin (23.7 kD), ovalbumin (43 kD), and BSA (68 kD) were used as protein markers. Yield of proteins was determined according to Lowry and that of α-N-acetylgalactosaminidase by enzymatic activity.

Determination of Michaelis constant (K_m). The reaction mixture contained 0.45 ml of *p*-nitrophenyl-α-N-acetyl-D-galactosaminide of required concentration (from 0.05 to 1.4 mg/ml) in 0.1 M phosphate buffer (pH 7.2) and 0.05 ml of the studied enzyme solution. K_m was calculated by the standard method.

RESULTS AND DISCUSSION

Wet bacterial extract was tested for the presence of glycanases and glycosidases using natural and synthetic substrates from the collection of the Laboratory of Enzyme Chemistry of the Pacific Institute of Bioorganic Chemistry (Far Eastern Branch of the Russian Academy of Sciences). The bacterial source of enzyme chosen by us seems especially convenient because beside α-N-acetylgalactosaminidase it synthesizes only two glycosidases, β-N-acetylhexosaminidase and α-L-fucosidase.

The procedure of the enzyme purification included extraction of disintegrated biomass, ion-exchange chromatography on DEAE-Sepharose CL-6B (Fig. 1), gel filtration on Sepharose CL-6B (Fig. 2), ion-exchange chromatography on DEAE-Toyopearl 650 M (Fig. 3), and then rechromatography on DEAE-Toyopearl 650 M. α-N-Acetylgalactosaminidase I did not adsorb on anion exchanger under the conditions described above. As a result of gel filtration, α-N-acetylgalactosaminidase II gave two activity peaks, III and IV (Fig. 2). After purification we obtained three molecular forms of α-N-acetylgalactosaminidase, I, III, and IV, purified 0.5, 2.3, and 57.5 times with the yield 19, 6.7, and 20%, respectively (Table 1). Of these three molecular forms, only α-N-acetylgalactosaminidase IV purified by double ion-exchange chromatography on DEAE-Toyopearl 650 M transformed human A(II)-erythrocytes into 0(I)-erythrocytes and inactivated group specificity of blood group substance A. Accompanying glycosidases were easily removed during purification (Fig. 1).

The results of immunological study of the native erythrocytes treated with α-N-acetylgalactosaminidase IV are presented in Table 2. As shown, all A-antigens are transformed into 0(I)-antigens by the enzyme because there is no agglutination with anti-A-serum. α-N-Acetylgalactosaminidase IV does not cause nonspecific aggregations of erythrocytes or their hemolysis; this indicates rather high purity of the enzyme preparation.

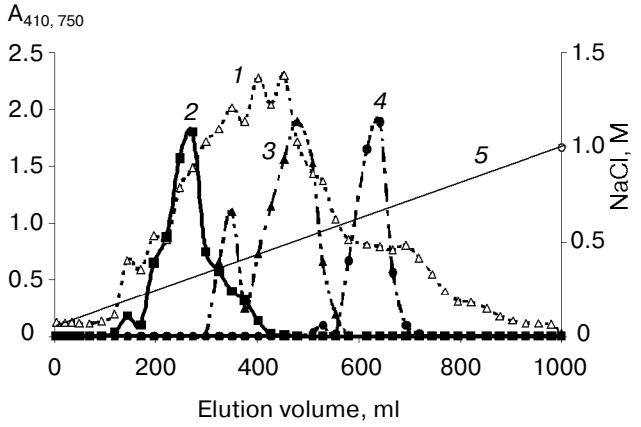

Fig. 1. Elution profile obtained by ion-exchange chromatography of extract of the bacterium *Arenibacter latericius* KMM 426^T on DEAE-Sepharose CL-6B: *1*) protein tested according to Lowry; *2*) α-N-acetylgalactosaminidase II activity; *3*) β-N-acetylglucosaminidase activity; *4*) α-fucosidase activity; *5*) gradient of NaCl concentration. The column (15.0 × 2.8 cm) was equilibrated with 0.01 M phosphate buffer (pH 7.2), flow rate 22 ml/h.

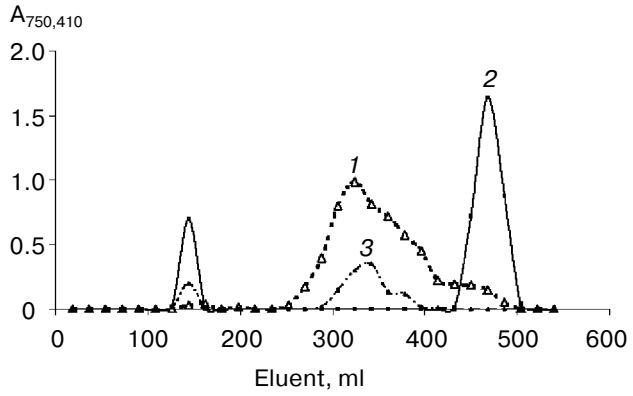

Fig. 2. Gel filtration on Sepharose CL-6B of fractions of *Arenibacter latericius* KMM 426^T enzyme preparation containing α-N-acetylgalactosaminidase II: *1*) protein tested according to Lowry; *2*) α-N-acetylgalactosaminidase III and IV activity; *3*) β-N-acetylglucosaminidase activity. The column (80.0 × 2.5 cm) was equilibrated with 0.01 M phosphate buffer (pH 7.2), flow rate 28 ml/h.

The α-N-Acetylgalactosaminidase IV preparation exhibited high activity in inhibition of hemagglutination with blood group substance A (BGS A + H) containing determinants analogous to A-erythrocytes. To prove that α-N-acetylgalactosaminidase IV is an exoglycosidase, BGG A + H were treated with enzyme preparation, and the products were separated by gel chromatography on Sephadex G-15. In the low-molecular-weight fraction preliminarily subject to acidic hydrolysis, only galactosamine was identified by amino acid analysis. Almost complete absence of other monosaccharides and the presence of free N-acetylgalactosamine in the low-molecular-weight products of fermentolysis indicated that α-N-acetylgalactosaminidase IV is an exoenzyme.

Some physicochemical and enzymatic properties of the α-N-acetylgalactosaminidase IV were studied with commercial substrate *p*-nitrophenyl-α-N-acetyl-D-galactosaminide.

The enzyme has a pH optimum between 7.0 and 8.0 required for reaction of transformation of human A(II)-erythrocytes to 0(I)-erythrocytes under mild conditions. This property provides a significant advantage of the new enzyme over the α-N-acetylgalactosaminidase from hen liver having optimum activity at pH less than 4.5.

The enzyme retains 100% activity during 30 min heating at 50°C and pH 7.2, for an indefinite length of time at 4°C and for a week at 20°C in the presence of 0.1% sodium azide, but it does not tolerate freezing. A higher thermal stability of the studied enzyme compared with glycosidases of marine bacteria studied earlier [14, 15] can probably be explained by the warmer environment of this bacterium. Like many enzymes of marine microorganisms, α-N-acetylgalactosaminidase IV is halotolerant [15, 20] and has activity maximum in 0.5 M NaCl (Fig. 4).

The Michaelis constant (K_m) is 0.38 mM at pH 7.2.

The molecular mass of the enzyme was estimated by gel filtration on a Sepharose CL-6B column calibrated

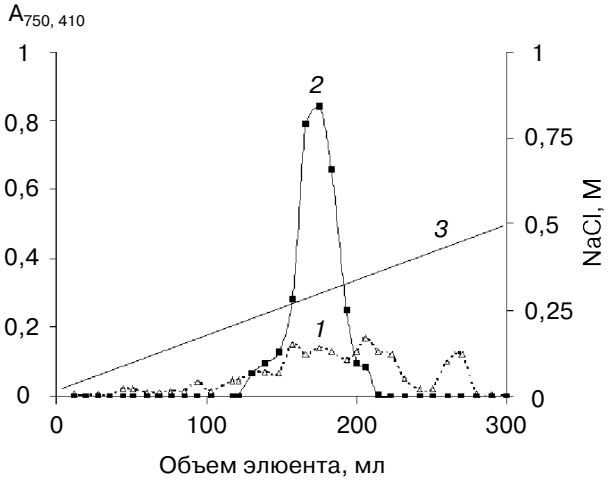

Fig. 3. Ion-exchange chromatography of α-N-acetylgalactosaminidase IV on DEAE-Toyopearl 650 M: *1*) protein assayed according to Lowry; *2*) α-N-acetylgalactosaminidase IV. The column (15.5 × 2.0 cm) was equilibrated with 0.01 M phosphate buffer (pH 7.2), flow rate 22 ml/h.

Table 1. Purification of α-N-acetylgalactosaminidase IV from marine bacterium *Arenibacter latericius* KMM 426^T

No.	Purification stage	Enzyme	Total volume, ml	Total protein, mg	Total activity, units	Yield, %	Specific activity, units/mg	Degree of purification
1	Extraction		400	1570	677	100	0.4	0
2	Anion-exchange chromatography on DEAE-Sepharose CL-6B	α-GalNAc I α-GalNAc II	400 403	770 380	130 547	19 81	0.2 1.4	0.5 3.5
3	Ultrafiltration	α-GalNAc II	74	308	433	64	1.4	3.5
4	Gel filtration on Sepharose CL-6B	α-GalNAc III α-GalNAc IV	166 172	49 35	45 189	6.7 28	0.9 5.4	2.3 14
5	Chromatography on DEAE-Toyopearl 650 M	α-GalNAc IV	24	6	139	20	23	57.5

Table 2. Agglutination titer of donor A(II)-erythrocytes before and after treatment with α-N-acetylgalactosaminidase IV

Polyclone-A	Agglutination titer								
	2	4	8	16	32	64	128	256	512
Anti-A + transformed A-erythrocytes	—	—	—	—	—	—	—	—	—
Anti-A + native A-erythrocytes	+	+	+	+	+	+	+	+	—

with protein markers. Calculations were performed from a plot of elution volume versus molecular masses of the standard proteins. According to the results of gel filtration, the α-N-acetylgalactosaminidase IV has molecular mass 84 kD.

The data on the structure of active centers of α-N-acetylgalactosaminidases from various sources are still very scarce. For most enzymes, the effect of bivalent metal ions and some monosaccharides as competitive inhibitors to enzymatic activity has been studied. The effect of chemical reagents inactivating enzymatic activity via modification of functionally important amino acid residues is almost unstudied. We investigated the role of certain amino acid residues in the catalytic activity of the α-N-acetylgalactosaminidase IV by inhibitory analysis (Table 3). Inhibition of enzymatic activity by *p*-chloromercuribenzoate and N-ethylmaleimide suggests importance of SH groups for enzymatic activity of this

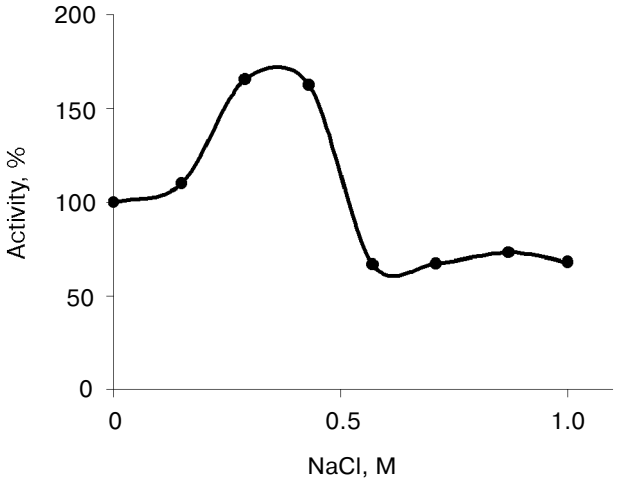

Fig. 4. Enzymatic activity of α-N-acetylgalactosaminidase IV versus NaCl concentration in 0.01 M phosphate buffer, pH 7.2.

Table 3. Effect of chemical reagents on activity of α-N-acetylgalactosaminidase IV

No.	Reagent	Reagent concentration, M	pH of reaction	Residual activity, %
1	*p*-Chloromercuribenzoate	$1.8 \cdot 10^{-3}$	7.2	0
2	N-Ethylmaleimide	$1.8 \cdot 10^{-3}$	7.2	60
3	N-Bromosuccinimide	$1.8 \cdot 10^{-3}$	7.2	0
4	Carbodiimide	$1.8 \cdot 10^{-3}$	7.2	0
5	Acetylimidazole	$5.0 \cdot 10^{-3}$	7.2	30
6	2,2α-Dianisidine	$2.0 \cdot 10^{-3}$	7.2	70
7	Diethylpyrocarbonate	$2.0 \cdot 10^{-3}$	7.2	60
8	EDTA	$1.0 \cdot 10^{-4}$	7.2	100
		$1.0 \cdot 10^{-3}$	7.2	80
9	Ca^{2+}	$1.0 \cdot 10^{-3}$	7.2	0
10	Mg^{2+}	$1.0 \cdot 10^{-3}$	7.2	130
11	Sodium azide	$3.0 \cdot 10^{-2}$	7.2	100
12	Ethanol	2%	7.2	100

Note: 0.05 ml of enzyme solution, 0.1 ml of 0.1 M phosphate buffer (pH 7.2), and 0.05 ml of reagent solution were incubated for 10 min at 20°C and then 0.3 ml of the substrate solution (1 mg/ml) was added. Activity was estimated by the standard procedure with corresponding controls.

enzyme. Selectivity of N-bromosuccinimide in relation to tryptophan residues is exhibited mainly at pH 4.0. Unfortunately, at this pH the studied enzyme is not completely active. Oxidation of α-N-acetylgalactosaminidase IV by N-bromosuccinimide at pH 7.2 resulted in complete loss of its enzymatic activity. Probably this is due to modification of a tryptophan residue. For example, it is found that a tryptophan residue (Trp16) participates in binding the substrate with α-N-acetylgalactosaminidase from yeast *Pichia pastoris* [21]. However, under such conditions imidazole moieties of histidine and free SH groups of cysteine residues can be involved along with tryptophan residues. Under the influence of diethylpyrocarbonate dissolved in ethanol about 60% enzymatic activity retained. Ethanol (2%) taken as a solvent did not inhibit the α-N-acetylgalactosaminidase IV.

To study the effect of bivalent metal ions, the enzyme preparation desalted by gel filtration was used. EDTA slightly decreased the enzymatic activity, Ca^{2+} inactivated it completely, whereas in the presence of Mg^{2+} the enzymatic activity increased by 30% (Table 3).

Thus, an α-N-acetylgalactosaminidase of required specificity isolated from a unique marine bacterium is of large theoretical and practical importance for future investigations. Being relatively efficient with BGS and having a pH optimum in the neutral zone, it may be very promising not only as a biological instrument, but also as an enzyme for obtaining donor erythrocytes unified in ABO specificity.

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REFERENCES

1. Goldstein, J. (1983) US Patent 518990.
2. Phillips, R., Mawhinney, T., Harmata, M., and Smith, D. (1995) *Artif. Cell Blood Sub.*, **23**, 63-79.
3. Levy, G. N., and Aminoff, D. (1980) *J. Biol. Chem.*, **255**, 1737-1742.
4. McDonald, M. J., and Bahl, O. P. (1972) in *Methods in Enzymology*, Academic Press, New York, pp. 734-738.
5. Kadowaki, S., Ueda, T., Yamamoto, K., Kumagai, H., and Tocyikura, T. (1989) *Agr. Biol. Chem.*, **53**, 111-120.
6. Hoskins, L. C., Boulding, E. T., and Larson, G. (1997) *J. Biol. Chem.*, **272**, 7932-7939.
7. Bakunina, I. Yu., Ivanova, E. P., Mikhailov, V. V., Nedashkovskaya, O. M., Gorshkova, N. M., and Parfenova, V. V. (1994) *Mikrobiologiya*, **63**, 847-852.
8. Ivanova, E. P., Bakunina, I. Yu., Nedashkovskaya, O. I., Gorshkova, N. M., Mikhailov, V. V., and Elyakova, L. A. (1998) *Biol. Morya*, **24**, 351-358.
9. Nedashkovskaya, O. I., Bakunina, I. Yu., Mikhailov, V. V., Elyakova, L. A., Kuhlmann, R. A., Likhosherstov, L. M., and Martynova, M. D. (1999) Russian Patent No. 2141526.
10. Ivanova, E. P., Nedashkovskaya, O. I., Chum, J., Lysenko, A. M., Frolova, G. M., Svetashev, V. I., Vysotskii, M. V., Mikhailov, V. V., Hug, A., and Colwell, R. R. (2001) *Int. J. Syst. Evol. Microbiol.*, in press.

11. Nelson, T. E. (1944) *J. Biol. Chem.*, **153**, 375-381.
12. Zvyagintseva, T. N., Shevchenko, N. M., Popivnich, I. B., Isakov, V. V., Scobun, A. S., Sundukova, E. V., and Elyakova, L. A. (1999) *Carbohydr. Res.*, **322**, 32-34.
13. Zvyagintseva, T. N., Elyakova, L. A., Sundukova, E. V., and Mishchenko, N. P. (1986) Author's certificate No. 1227199, *Byull. Izobret.*, No. 16.
14. Bakunina, I. Yu., Ivanova, E. P., Nedashkovskaya, O. I., Gorshkova, N. M., Mikhailov, V. V., and Elyakova, L. A. (1996) *Prikl. Biokhim. Mikrobiol.*, **32**, 624-628.
15. Bakunina, I. Yu., Sova, V. V., Nedashkovskaya, O. I., Kuhlmann, R. A., Likhosherstov, L. M., Martynova, M. D., Mikhailov, V. V., and Elyakova, L. A. (1998) *Biochemistry* (Moscow), **63**, 1209-1215.
16. Likhosherstov, L. M., Derevitskaya, V. A., and Fedorova, V. I. (1969) *Biokhimiya*, **34**, 45-50.
17. Derevitskaya, V. A., Likhosherstov, L. M., Arbatskii, N. P., and Kochetkov, N. K. (1972) *Izv. Akad. Nauk SSSR*, *Ser. Khim.*, No. 12, 2782-2786.
18. Zamprometova, S. M., Ulezlo, I. V., Kuhlmann, R. A., Likhosherstov, L. M., and Martynova, M. D. (1992) *Biokhimiya*, **57**, 797; (1990) *Biokhimiya*, **55**, 2281-2285.
19. Kabat, E. A. (1956) in *Blood Group Substances*, Academic Press, New York, pp. 56-58.
20. Sova, V. V., Elyakova, L. A., Ivanova, E. P., Fedosov, Yu. V., and Mikhailov, V. V. (1994) *Biochemistry* (Moscow), **59**, 1013-1020.
21. Zhu, A., Monahan, C., and Wang, Z. K. (1996) *Biochim. Biophys. Acta*, **1297**, 99-104.