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Purification and Characterization of β-N-Acetylgalactosaminidase from *Bacillus* sp. AT173-1

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β-N-Acetylgalactosaminidase [EC 3.2.1.53] was purified to homogeneity from the culture media of *Bacillus* sp. AT173-1. The enzyme has a molecular weight of 48,000 as estimated by SDS-PAGE under reducing conditions and an isoelectric point of 4.3. The enzyme requires dithiothreitol as an activator and is most active at pH 6.0. Analysis of its substrate specificity using 2-aminopyridine-labeled oligosaccharides as substrates revealed the enzyme specifically hydrolyzes β-N-acetylgalactosaminyl linkages of GalNAcβ1-4Galβ1-4Glc, GalNAcβ1-3Galα1-4Galβ1-4Glc, and N-glycans terminating with β-N-acetylgalactosamine residues but not those with β-N-acetylglucosamine residues. The enzyme is thus a novel β-N-acetylgalactosaminidase with practically no β-N-acetylglucosaminidase activity.

Key words: β-N-acetylgalactosaminidase, β-N-acetylglucosaminidase, β-N-acetylhexosaminidase, *Bacillus* sp. AT173-1.

β-N-Acetylgalactosamine (β-GalNAc) is found in the carbohydrate moieties of glycosphingolipids (*1*), serum-type glycoproteins (*2-18*), and mucin-type glycoproteins (*19, 20*). β-N-Acetylhexosaminidase (β-HexNAc'ase) [EC 3.2.1.52] is widely used in elucidating the biological functions and structures of β-N-acetylgalactosaminylated oligosaccharides (*6-9, 13, 21-24*). However, because β-HexNAc'ase hydrolyzes both β-GalNAc and β-N-Acetylglucosaminyl (β-GlcNAc) linkages, a digest with β-HexNAc'ase gives unclear results when used to elucidate a GalNAc-containing structure. In such cases, β-N-acetylgalactosaminidase (β-GalNAc'ase) [EC 3.2.1.53] without β-N-acetylglucosaminidase (β-GlcNAc'ase) activity will give clearer results than β-HexNAc'ase. β-GalNAc'ases from calf brain (*25*), and bovine brain and liver (*26*) have been described. The enzyme from calf brain had approximately 9% β-GlcNAc'ase activity relative to the β-GalNAc'ase activity; the enzymes from bovine brain and liver have not been purified and characterized. Hence, no β-GalNAc'ase is presently available for the investigation of glycoconjugates and oligosaccharides. We therefore searched for a microbial β-GalNAc'ase without β-GlcNAc'ase activity and detected the enzyme in the culture media of *Bacillus* sp. AT173-1 isolated from a soil sample. Here, we describe the purification and some properties of the *Bacillus* β-GlcNAc'ase produced by this strain.

MATERIALS AND METHODS

Materials—Polypeptone S was purchased from Nihon Seiyaku (Tokyo) and yeast extract from Difco Laboratories (Detroit MI, USA). *p*-Nitrophenyl-N-acetyl-β-galactosaminide (PNP-β-GalNAc) and *p*-nitrophenyl-N-acetyl-β-glucosaminide were obtained from Sigma (St. Louis, MO, USA). QAE-Toyopearl 550C was purchased from Tosoh (Tokyo), Phenyl-Sepharose HP, Q-Sepharose FF, Sephacryl S-200, and Resource PHE were from Pharmacia LKB Biotechnology (Uppsala, Sweden). β-HexNAc'ase from jack bean was obtained from Seikagaku Kogyo (Tokyo). Other chemicals were analytical grade reagents.

Enzyme Production—*Bacillus* sp. AT173-1 was grown in a medium containing 0.625 g of GalNAc, 10 g of Polypeptone S, 10 g of yeast extract, 1 g of K_2HPO_4, and 0.2 g of $MgSO_4 \cdot 7H_2O$ per liter of water. The bacteria were grown aerobically in 500-ml flasks containing 100 ml of medium on a reciprocal shaker at 30°C for 48 h. The culture broth was centrifuged to remove the cells and the supernatant was used as the crude enzyme source.

Enzyme Assay—β-GalNAc'ase solution for enzyme assay was diluted, if necessary, with 50 mM acetate buffer, pH 6.0, containing 0.2% BSA, and was preincubated at room temperature for 1 h with 0.1% DTT in 50 mM acetate buffer, pH 6.0, to activate the enzyme (see "RESULTS"). β-GalNAc'ase activity was assayed by incubating the solution for 10 min with 2.0 mM PNP-β-GalNAc at 37°C in 0.5 ml of 50 mM acetate buffer, pH 6.0. The other exoglycosidase activities were assayed by incubation for 1 h with the corresponding *p*-nitrophenylglycoside under the same conditions as those used for the β-GalNAc'ase assay, after which 2.5 ml of 2% Na_2CO_3 solution was added to the reaction mixture to stop the reaction, and the absorbance was measured at 420 nm. One unit (U) of activity was defined as the amount of enzyme required to release 1 μmol of *p*-nitrophenol per min under the assay conditions described above.

Protein Determination—The protein concentration was determined by the method of Lowry *et al.* (*27*) with BSA as a standard.

Purification of the Enzyme—All chromatographic steps were carried out at 0-8°C.

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Abbreviations: BSA, bovine serum albumin; DTT, dithiothreitol; PA, pyridylaminated; PAGE, polyacrylamide gel electrophoresis.

Step 1. Ammonium sulfate fractionation: The crude enzyme solution was brought to 80% saturation with ammonium sulfate. The precipitate was collected by centrifugation and dialyzed against 50 mM Tris-HCl buffer, pH 8.0.

Step 2. QAE-Toyopearl 550C column chromatography: The enzyme solution was applied to a QAE-Toyopearl 550C column (2.6×33 cm) equilibrated with 50 mM Tris-HCl buffer, pH 8.0, containing 0.1 M NaCl. The column was washed thoroughly with the same buffer, then the enzyme was eluted with a 2-liter linear gradient of 0.1–1.0 M NaCl. The fractions containing the activity were pooled.

Step 3. Phenyl-Sepharose HP column chromatography: The enzyme solution was brought to 25% saturation with ammonium sulfate and applied onto a Phenyl-Sepharose HP column (1.6×11 cm) equilibrated with 50 mM Tris-HCl buffer, pH 8.0, containing 25% saturation ammonium sulfate. The column was washed thoroughly with the same buffer, then the enzyme was eluted with a 400-ml linear gradient of ammonium sulfate (25 to 0% saturation). The active fractions were pooled and dialyzed against 50 mM acetate buffer, pH 6.0.

Step 4. Q-Sepharose FF column chromatography: The dialyzed enzyme solution was applied onto a Q-Sepharose FF column (1.6×11 cm) equilibrated with 50 mM acetate buffer, pH 6.0. The column was washed thoroughly with the same buffer, then the enzyme was eluted with a 400-ml linear gradient of 0.1–0.5 M NaCl. The activity-containing fractions were pooled and concentrated to about 1 ml by ultrafiltration with a Millipore UFP2-LCC membrane.

Step 5. Sephacryl S-200 HP column chromatography: The concentrated enzyme solution was applied onto a Sephacryl S-200 column (1.6×60 cm) equilibrated with 50 mM Tris-HCl buffer, pH 8.0, containing 0.15 M NaCl. The enzyme was eluted with the same buffer at a flow rate of 0.4 ml/min. The fractions containing activity were pooled.

Step 6. Resource PHE column chromatography: The enzyme solution was brought to 25% saturation with ammonium sulfate and applied onto a Resource PHE column (1 ml) equilibrated with 50 mM Tris-HCl buffer, pH 8.0, containing 25% saturation ammonium sulfate. The column was washed thoroughly with the same buffer, then the enzyme was eluted with a 40-ml linear gradient of ammonium sulfate (25 to 0% saturation). The activity-containing fractions of the later activity-containing peak were pooled and concentrated by ultrafiltration.

Electrophoresis—SDS-PAGE was carried out on 10% polyacrylamide gel by the method of Laemmli (*28*), and native-PAGE on 6% polyacrylamide gel, pH 8.8, by the method of Davis (*29*). Isoelectric focusing was done using the Phast System (Pharmacia) using ready-made gels (PharstGel IEF 3-9) according to the protocol, and the protein was detected using a silver staining kit (Pharmacia). The molecular weight and isoelectric point of the enzyme were estimated by SDS-PAGE and isoelectric focusing, respectively, using standard proteins obtained from Pharmacia.

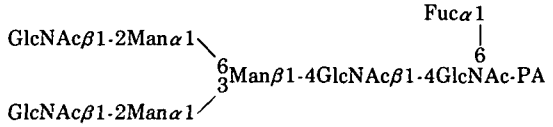
Analysis of the Enzyme Reaction Products—PA-oligosaccharides **a, b, d, f,** and **j**, and PA-lactose were purchased from Takara Shuzo (Kyoto), and PA-oligosaccharides **e**, **h**, and **i** were from Ajinoki (Handa, Aichi). PA-oligosaccharide **c** was made from PA-oligosaccharide **f** by digestion with β-galactosidase from *Aspergillus oryzae* (Toyobo, Osaka); 20 pmol PA-oligosaccharide **f** was incubated with 10 mU β-galactosidase in 4 μl of 25 mM acetate buffer, pH 5.0, at 37°C for 3 h. The structures of PA-oligosaccharides **a**–**e** are shown in Table IV, and those of PA-oligosaccharides **f**–**j** in Table I.

The products formed by the β-GalNAc'ase were compared with those obtained from jack bean β-HexNAc'ase. The following buffer solutions were used for the PA-oligosaccharide reactions: 50 mM acetate buffer, pH 6.0, containing 0.2% BSA and 0.1% DTT for β-GalNAc'ase; and 40 mM acetate buffer, pH 5.0, containing 0.2% BSA for β-HexNAc'ase. The enzyme solution was incubated with a PA-oligosaccharide (2 μM for PA-oligosaccharides **a, b,** and **c**, and 1 μM for **d** and **e**) at 37°C in 10 μl buffer. After incubation, 40 μl of 1% trifluoroacetic acid solution was added to stop the reaction. A portion containing 4 pmol of the PA-oligosaccharide was then analyzed by HPLC with a Shimadzu LC-10A chromatograph using columns of two kinds. Size-fraction HPLC was done with an amino-silica column (Nakanopak Amide-A, 4.6×250 mm, Ajinoki). Elution was at a flow rate of 1.0 ml/min at 40°C with two solvents, A and B. Solvent A was a 35 : 65 (v/v) mixture of 0.2 M triethylamine acetate buffer, pH 7.3, and acetonitrile. Solvent B was a 50 : 50 (v/v) mixture of 0.2 M triethylamine acetate buffer, pH 7.3, and acetonitrile. The column was equilibrated with solvent A. After injection of the sample, the ratio of solvent B to A was increased linearly to 60 : 40 in 30 min. Reversed-phase HPLC was done with a C_{18}-silica column (Nakanopak ODS-A, 6.0×150 mm, Ajinoki). Elution was at a flow rate of 1.0 ml/min at 55°C with two elution modes, isocratic and gradient. Isocratic elution was done with 10 mM sodium phosphate buffer (pH 3.8) containing 0.02% 1-butanol, and gradient elution with two solvents, C and D. Solvent C was 10 mM sodium phosphate buffer (pH 3.8), and solvent D was 10 mM sodium phosphate buffer (pH 3.8) containing 0.5% 1-butanol. The column was equilibrated with a mixture of solvents D and C, 20 : 80 (v/v). After injection of the sample, the ratio of solvent D to C was increased linearly to 50 : 50 in 60 min. PA-oligosaccharides were detected by fluorescence, with the excitation and emission wavelengths of 320 and 400 nm, respectively.

TABLE I. **Structures of oligosaccharides f-j.**

f Galβ1-4GlcNAcβ1-3Galβ1-4Glc-PA

g Galα1-4Galβ1-4Glc-PA

h


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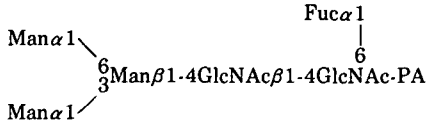

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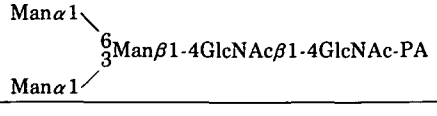


RESULTS

Activation of the Enzyme—β-GalNAc'ase activity in the crude enzyme solution decreased gradually at 4°C when the activity was assayed without preincubation with DTT, and after 8 days it had dropped to 25% of the initial activity. When DTT was added to this enzyme solution to a final concentration of 0.1%, the initial activity of β-GalNAc'ase was recovered. As shown in Fig. 1, the crude and purified enzyme were almost completely activated in the presence of 0.08 and 0.02% DTT, respectively.

β-GalNAc'ase and β-GlcNAc'ase Activities in the Crude Enzyme Solution—The β-GalNAc'ase activity of the crude enzyme was 88.0 mU/ml; the β-GlcNAc'ase activity was less than 0.2 mU/ml.

Purification of β-GalNAc'ase from Bacillus sp. AT173-1—Typical purification results are shown in Fig. 2 and Table II. In the final purification step, two β-GalNAc'ase active peaks appeared on Resource PHE chromatography (Fig. 2C). The peak that eluted earlier gave multiple protein bands on isoelectric focusing, one of which was the same as the peak that eluted later. This later peak gave a single protein band on SDS-PAGE, native-PAGE (Fig. 3), and isoelectric focusing. On native-PAGE, this single band retained β-GalNAc'ase activity (Fig. 3). Purification of the enzyme resulted in an approximately 8,700-fold increase in specific activity with a yield of 1.5%. The specific activity of purified enzyme was 154 U/mg protein.

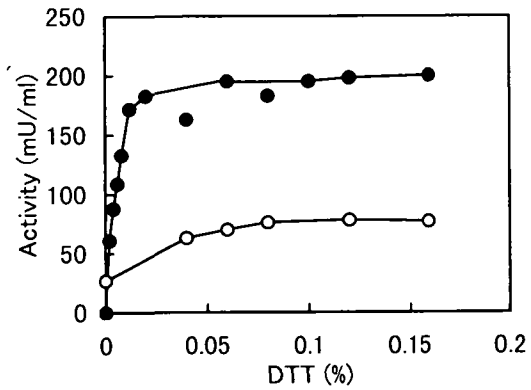
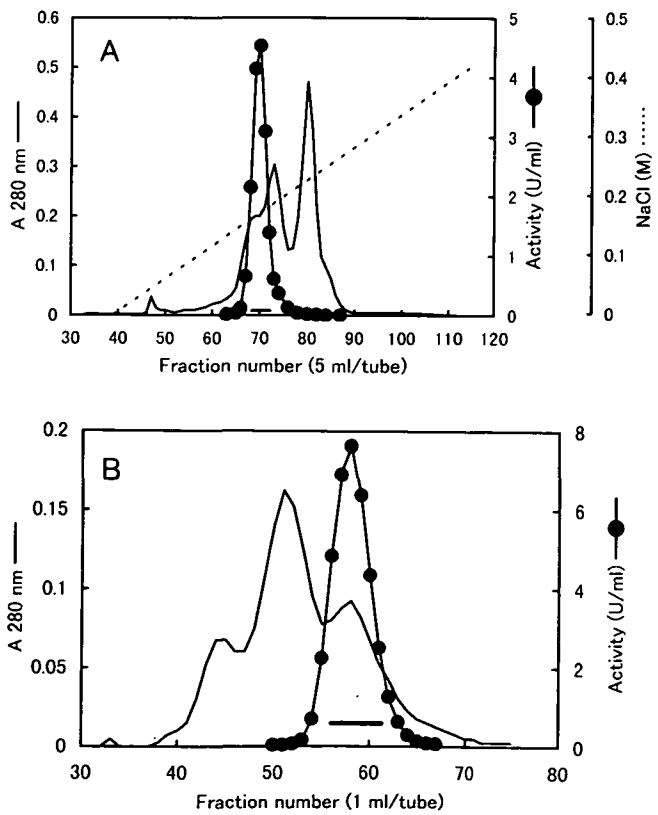


Fig. 1. **Activation of β-GalNAc'ase by DTT.** The crude (○) and purified enzyme (●) solutions were preincubated with various concentrations of DTT for 1 h at room temperature. After preincubation, the enzyme activity was assayed.

TABLE II. **Purification of β-GalNAc'ase from *Bacillus* sp. AT173-1.**

Purification step	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Yield (%)	Purification (fold)
Culture supernatant	36,500	647	0.0177	100	1
$(NH_4)_2SO_4$ precipitation	1,290	432	0.335	66.8	19
QAE-Toyopearl 550C	255	299	1.17	46.2	66
Phenyl-Sepharose HP	21.9	180	8.23	27.8	465
Q-Sepharose FF	8.75	76.5	8.74	11.8	494
Sephacryl S-200 HP	1.15	31.2	27.1	4.8	1,530
Resource PHE	0.0620	9.56	154	1.5	8,700



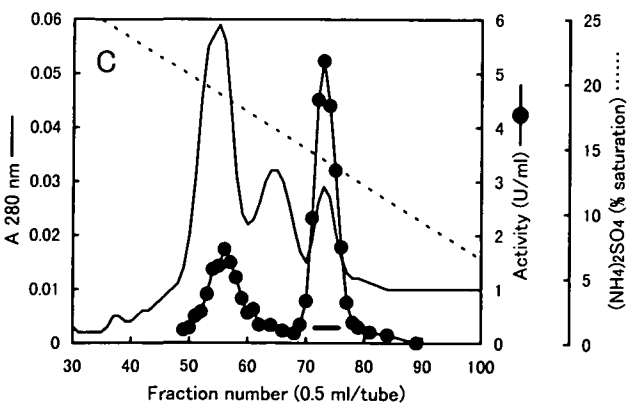


Fig. 2. **Purification of β-GalNAc'ase from *Bacillus* sp. AT173-1.** Purification was carried out as described under "MATERIALS AND METHODS." (A) Q-Sepharose FF chromatography. (B) Sephacryl S-200 chromatography. (C) Resource PHE chromatography. The fractions indicated by bars were collected for further purification.

Molecular Weight and Isoelectric Point—The molecular weight of the enzyme denatured with SDS and 2-mercaptoethanol was estimated to be about 48,000 by SDS-PAGE, and that of the native enzyme about 54,000 by Sephacryl S-200 gel filtration. The isoelectric point was estimated to be about 4.3 by isoelectric focusing.

K_m and k_{cat} Values—The K_m and k_{cat} values for the reaction with PNP-β-GalNAc as a substrate were 1.0 mM and 970 s^{-1}, respectively.

Effect of Temperature and pH on the Stability and Activity of the Enzyme—The enzyme retained more than 80% of its activity up to 40°C when heated for 15 min in 50 mM acetate buffer, pH 6.0 (open circles in Fig. 4). When the enzyme was assayed at various temperatures, the maximum activity was observed at 45°C (filled circles in Fig. 4). When the enzyme was assayed at various pHs, the maximum activity was observed at pH 6.0 (Fig. 5A). The enzyme retained more than 80% of its activity in the pH range from 5.5 to 9.5 (Fig. 5B).

Effects of Various Reagents on β-GalNAc'ase Activity—Table III summarizes the effects of various reagents on the β-GalNAc activity. The enzyme activity was completely inhibited in the presence of 10 mM Cu^{2+}, Zn^{2+}, and Fe^{2+}. The activity was reduced by 50% in the presence of 10 mM GalNAc but was hardly lowered in the presence of 10 mM GlcNAc. The presence of the protein denaturant urea reduced the enzyme activity by almost 70%.

Substrate Specificity toward p-Nitrophenylglycosides—The enzyme solution purified to homogeneity, with β-GalNAc'ase activity of 2 U/ml, was examined for β-GlcNAc'ase, α-fucosidase, α-galactosidase, β-galactosidase, α-GalNAc'ase, α-mannosidase, and β-mannosidase activities toward *p*-nitrophenylglycosides. β-GlcNAc'ase activity was detected up to 0.05% of β-GalNAc'ase activity; the other exoglycosidase activities were not detected.

Substrate Specificity toward PA-Oligosaccharides—β-GalNAc'ase hydrolyzed PA-oligosaccharides **a** and **b** in the same manner as β-HexNAc'ase, as shown in Fig. 6, A and B. However, as shown in Fig. 6C and Table IV, β-GalNAc'ase hydrolyzed PA-oligosaccharide **c** much more slowly than did β-HexNAc'ase. β-GalNAc'ase did not hydrolyze PA-oligosaccharide **d**, whereas β-HexNAc'ase did so (Fig. 6D and Table IV). As shown in Fig. 6E, β-GalNAc'ase and β-HexNAc'ase hydrolyzed PA-oligosaccharide **e** but gave different reaction products; β-GalNAc'ase liberated the β-GalNAc residues of the nonreducing termini from this

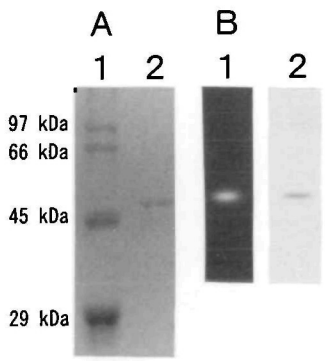


Fig. 3. **SDS and native-PAGE of purified β-GalNAc'ase.** SDS-PAGE (A): Molecular weight marker proteins (A-1) and β-GalNAc'ase (A-2); native-PAGE (B): After electrophoresis, the gel was dipped into 100 ml of the substrate solution containing 4 mg of 4-methylumbelliferyl-β-GalNAc and 0.1 g of DTT in 50 mM acetate buffer, pH 6.0, for 5 min. β-GalNAc'ase activity was detected under the ultraviolet light (B-1). After detecting the β-GalNAc'ase activity, this gel was stained with Coomassie Brilliant Blue R-250 (B-2).

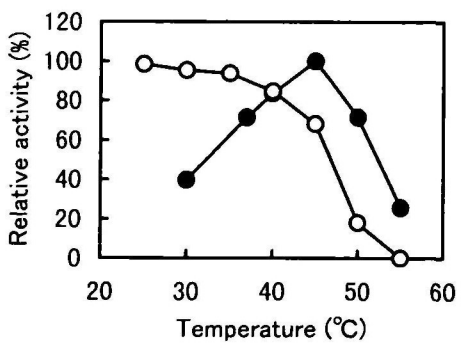


Fig. 4. **Effects of temperature on β-GalNAc'ase.** β-GalNAc'ase activity (●) was assayed at the indicated temperatures. Residual activity (○) was measured after incubation of the enzyme (0.14 U/ml) at the indicated temperatures for 15 min in 50 mM acetate buffer, pH 6.0, containing BSA (0.2%).

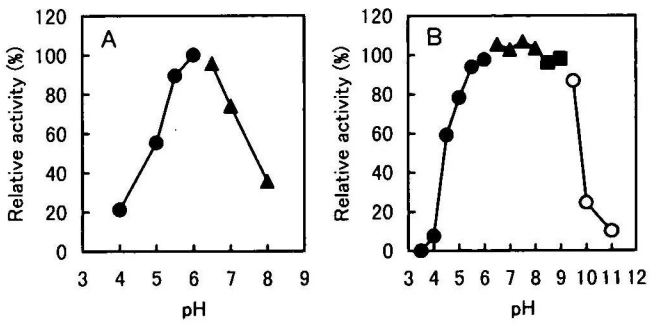


Fig. 5. **Effects of pH on β-GalNAc'ase.** Effects of pH on the activity (A) and stability (B). A: β-GalNAc'ase activity was assayed in 25 mM acetate buffer of pH 4.0-6.0 (●) and in 25 mM phosphate buffer of pH 6.5-8.0 (▲), as described under "MATERIALS AND METHODS." B: The enzyme (0.97 U/ml) was incubated at 25°C for 1 h in the following buffers with various pHs containing BSA (0.04%): 50 mM acetate buffer for pH 3.5-6.0 (●), 50 mM phosphate buffer for pH 6.5-8.0 (▲), 50 mM phosphate-borate buffer for pH 8.5-9.0 (■), and 50 mM borate-NaOH buffer for pH 9.5-11.0 (○). Residual activity of the enzyme was measured.

TABLE III. **Effects of various compounds on β-GalNAc'ase activity.**

Compound	Concentration	Relative activity (%)
No addition		100
$MgSO_4$	10 mM	104
$MnSO_4$	10 mM	97
$CuSO_4$	10 mM	0
$ZnSO_4$	10 mM	0
$CaSO_4$	10 mM	100
$FeSO_4$	10 mM	0
EDTA	1 mM	99
GalNAc	10 mM	50
	50 mM	12
GlcNAc	10 mM	98
	50 mM	95
Triton X-100	0.1%	90
Urea	2 M	32

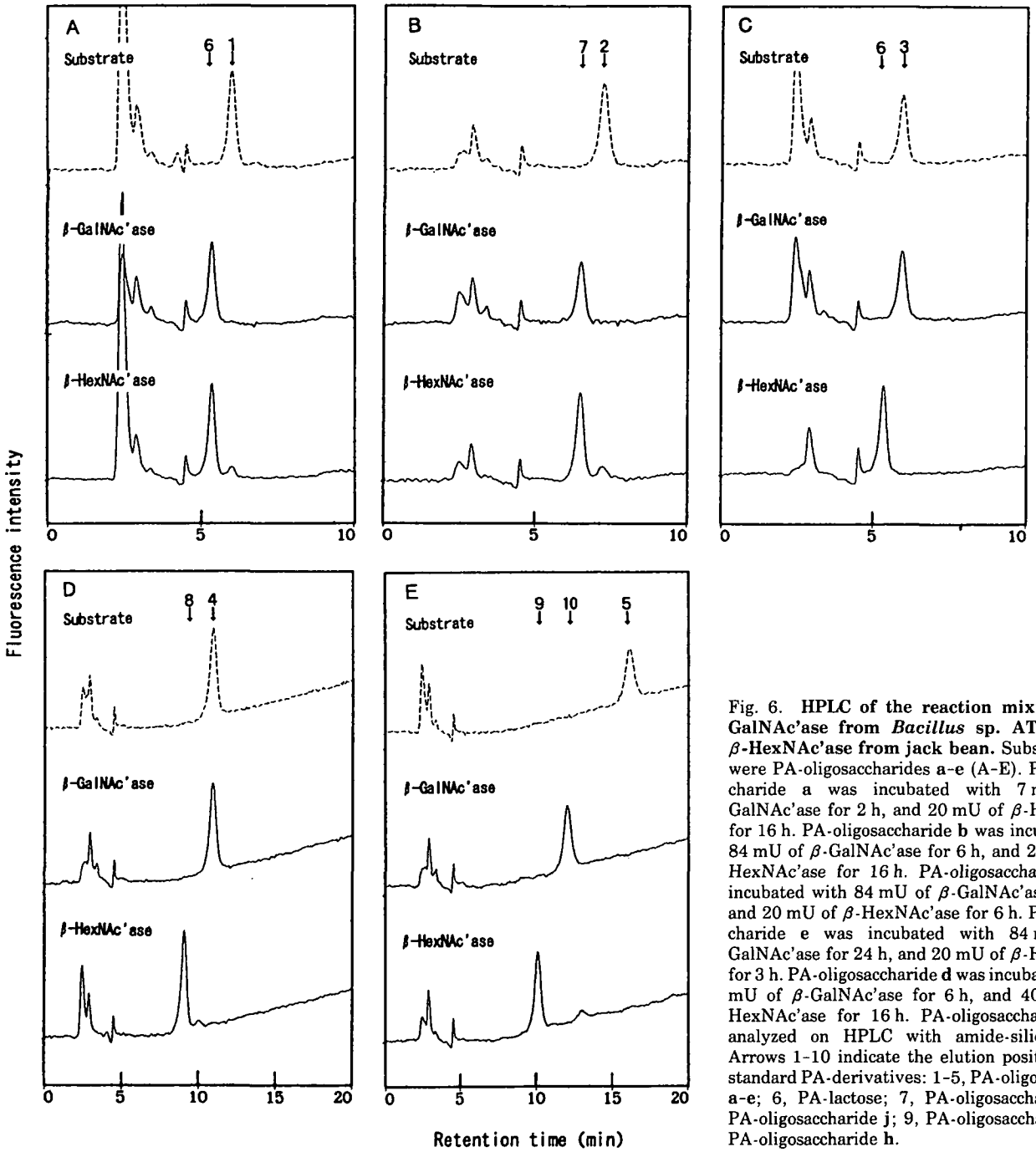


Fig. 6. **HPLC of the reaction mixture of β-GalNAc'ase from *Bacillus* sp. AT173-1 and β-HexNAc'ase from jack bean.** Substrates used were PA-oligosaccharides **a**-**e** (A-E). PA-oligosaccharide **a** was incubated with 7 mU of β-GalNAc'ase for 2 h, and 20 mU of β-HexNAc'ase for 16 h. PA-oligosaccharide **b** was incubated with 84 mU of β-GalNAc'ase for 6 h, and 20 mU of β-HexNAc'ase for 16 h. PA-oligosaccharide **c** was incubated with 84 mU of β-GalNAc'ase for 24 h, and 20 mU of β-HexNAc'ase for 6 h. PA-oligosaccharide **e** was incubated with 84 mU of β-GalNAc'ase for 24 h, and 20 mU of β-HexNAc'ase for 3 h. PA-oligosaccharide **d** was incubated with 84 mU of β-GalNAc'ase for 6 h, and 40 mU of β-HexNAc'ase for 16 h. PA-oligosaccharides were analyzed on HPLC with amide-silica column. Arrows 1-10 indicate the elution positions of the standard PA-derivatives: 1-5, PA-oligosaccharides **a**-**e**; 6, PA-lactose; 7, PA-oligosaccharide **g**; 8, PA-oligosaccharide **j**; 9, PA-oligosaccharide **i**; 10, PA-oligosaccharide **h**.

PA-oligosaccharide, whereas β-HexNAc'ase liberated the β-GalNAc and β-GlcNAc residues successively in the sequence GalNAcβ1-4GlcNAcβ1-2Man.

With β-GalNAc'ase, the rate of hydrolysis of PA-oligosaccharide **b**, which has a GalNAcβ1-3 linkage, was slower than that of PA-oligosaccharides **a** and **e**, which have GalNAcβ1-4 linkages, and the rate of hydrolysis of PA-oligosaccharide **e**, which has two terminal β-GalNAc residues per molecule, was about half that of PA-oligosaccharide **a** (Table IV).

DISCUSSION

We have found a novel β-GalNAc'ase in the culture medium of *Bacillus* sp. AT173-1. Although the enzyme was gradually inactivated in solution at 4°C, it was reactivated by incubation with DTT, a reagent that protects SH residues of the active sites of enzymes from oxidation (*30*). This result suggests that the enzyme has an active site with an easily oxidable SH residue(s) that causes its reversible inactivation.

In the final purification step, two β-GalNAc'ase active peaks appeared (Fig. 2C). Electrophoresis of these peaks

TABLE IV. **Substrate specificity of β-GalNAc'ase from *Bacillus* sp. AT173-1.** PA-oligosaccharides were incubated at 37°C for 24 h with the following amounts of enzyme: PA-oligosaccharide **a**, 0.125 mU; PA-oligosaccharide **b**, 5.0 mU; PA-oligosaccharide **c**, 120 mU; PA-oligosaccharide **d**, 120 mU; PA-oligosaccharide **e**, 0.25 mU. Reaction products were analyzed by HPLC with the following columns: PA-oligosaccharide **a**, **b**, and **e**, amide-silica column; PA-oligosaccharide **c**, C_{18}-silica column (isocratic elution); PA-oligosaccharide **d**, C_{18}-silica column (gradient elution).

	Substrate	Relative activity (%)
a	GalNAcβ1-4Galβ1-4Glc-PA	100[a]
b	GalNAcβ1-3Galα1-4Galβ1-4Glc-PA	5.68
c	GlcNAcβ1-3Galβ1-4Glc-PA	0.0230
d	GlcNAcβ1-2Manα1 6 3Manβ1-4GlcNAcβ1-4GlcNAc-PA GlcNAcβ1-2Manα1	0
e	Fucα1 6 GalNAcβ1-4GlcNAcβ1-2Manα1 6 3Manβ1-4GlcNAcβ1-4GlcNAc-PA GalNAcβ1-4GlcNAcβ1-2Manα1	53.1

[a]Reaction rate was 1.57 pmol (released monosaccharide)/mU β-GalNAc'ase/h.

showed that the later one contained β-GalNAc'ase protein only, while the earlier one contained β-GalNAc'ase and other proteins.

The molecular weight of denatured β-GalNAc'ase was estimated to be about 48,000, and that of the native enzyme about 54,000, indicating that β-GalNAc'ase is a monomer protein, while its isoelectric point of 4.3 suggests the presence of acidic amino acid residues on the β-GalNAc'ase protein surface.

The substrate specificity of the enzyme toward β-GalNAc residues is markedly higher than those of any other β-GalNAc'ases or β-HexNAc'ases described hitherto. The purified enzyme still had β-GlcNAc'ase activity, but it was only 0.05% of the β-GalNAc'ase activity. When PA-oligosaccharides with β-GlcNAc residues at their nonreducing termini were used as substrates, no hydrolyzed products were detected on HPLC analysis using an amide-silica column after 24 h of reaction (Fig. 6), indicating that the enzyme should be assigned as a β-GalNAc'ase [EC 3.2.1.53], and not as a β-HexNAc'ase [EC 3.2.1.52].

The action of the β-GalNAc'ase was influenced by the aglycon structure of the substrate. The results shown in Table IV suggest that the β-GalNAc'ase hydrolyzes GalNAcβ1-4 linkages about 20 times faster than GalNAcβ1-3 linkages.

The findings described above indicate that the enzyme from *Bacillus* sp. AT173-1 is a novel β-GalNAc'ase with practically no β-GlcNAc'ase activity, which should prove useful in studying the structures and functions of glycoconjugates and oligosaccharides in many biological systems.

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