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Purification and Properties of β-*N*-Acetylglucosaminidase from *Escherichia coli*

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β-*N*-acetylglucosaminidase (EC 3.2.1.30) has been purified from *Escherichia coli* K-12 to near homogeneity based on polyacrylamide gel electrophoresis in both 0.5% sodium dodecyl sulfate and in 6 M urea at pH 8.5. The purified enzyme shows a pH optimum of 7.7 and the K_m for *p*-nitrophenyl-β-D-2-acetamido-2-deoxyglucopyranoside is 0.43 mM. The molecular weight of this enzyme, determined by both Sephadex gel filtration and by sodium dodecyl sulfate gel electrophoresis, is equivalent to 36,000. It is shown to be a soluble cytoplasmic enzyme. Studies on the substrate specificities of the purified enzyme indicate that this enzyme is an exo-β-*N*-acetylglucosaminidase.

The growth of a bag-shaped macromolecule (murein sacculus), covalently linked by peptide and glycosidic bonds, requires continuous breakage and reclosure of some of these covalent bonds. For normal growth and division of bacterial cells, the bond-breaking by autolytic enzymes must be closely followed in time and in space by bond-forming reactions. This simple but elegant postulate by Weidel and Pelzer (28) is supported experimentally by the following observations. (i) Murein hydrolases have been found in a variety of species of both gram-positive and gram-negative bacteria (8); (ii) extensive turnover of peptidoglycan has been observed during exponential growth in some bacterial species but not in others (18, 24); (iii) pleiotropic cell envelope mutants have been isolated that show increased dependence on NaCl for growth and division, defects in cell division or separation of daughter cells, and altered activity or aberrant control of autolytic enzymes (2, 5, 6); and finally (iv) cessation of peptidoglycan biosynthesis induced by a variety of experimental conditions is always followed by extensive degradation of pre-existing peptidoglycan (26, 30).

In *Escherichia coli* cells, activities of a number of autolytic enzymes have been detected in the crude extract. They include β-*N*-acetylglucosamine, β-*N*-acetylmuramidase, *N*-acetylmuramyl-L-alanine amidase, endopeptidase, transglycosylase, D-alanine carboxypeptidase I, and D-alanine carboxypeptidase II (10, 11, 13, 18, 22, 23; R. Hartmann, Ph.D. thesis, University of Tübingen, Tübingen, West Germany, 1973; J. V. Höltje, Ph.D. thesis, University of Tübingen, Tübingen, West Germany, 1973). Some of these enzymes are bound to the cell envelope (9, 10; Hartmann, Ph.D. thesis), whereas others are soluble (10; Hartmann, Ph.D. thesis). In recent years, investigations of the physiological functions of autolytic enzymes in *E. coli* have centered around the purification, substrate specificity, subcellular localization, and oscillation of the activity during the cell cycle of *E. coli*. Thus amidase, endopeptidase, and a novel transglycosylase have been purified to varying extents (10, 11, 13; Hartmann, Ph.D. thesis; Höltje, Ph.D. thesis). To date, biochemical genetics has not yet been used to determine the physiological roles of these autolytic enzymes in the cell cycle of *E. coli*.

In this paper, we report the purification and characterization of the β-*N*-acetylglucosaminidase from *E. coli* K-12. The isolation of mutant strains with altered levels of this enzyme will be described in the accompanying note (31).

MATERIALS AND METHODS

Bacterial strains and growth conditions. *E. coli* K-12 strain AB1157 was used as the parental wild-type strain in this study. The compositions of the growth media have been described previously (26, 30). Cells were grown in Erlenmeyer flasks aerobically at 30, 37, or 42 C as specified, with vigorous shaking. Growth was followed by measuring the optical density at 600 nm in a spectrophotometer. Cells for large-scale enzyme purification were grown, at the New England Enzyme Center, Boston, Mass., in L broth containing 0.2% glucose, and the cells were harvested at late exponential growth. This particular batch of AB1157 cells has been kept frozen at −70 C for over 1 year. The activity of β-*N*-acetylglucosaminidase in the extract of the frozen cells was found to be within 10% of that found in freshly grown cells of the same strain.

Preparation of cell-free crude extract. The cells

were washed once with 0.01 M tris(hydroxymethyl)-aminomethane (Tris)-maleate buffer (pH 6.8) and resuspended in the same buffer. The cell suspensions were sonicated repeatedly for short intervals of time (30 s) with intermittent cooling for 2 min at 0 C until more than 90% of the cells had been broken as judged by phase-contrast microscopy. Cellular debris and unbroken cells were removed by centrifugation at 40,000 × *g* for 20 min.

Enzyme assays. β-*N*-acetylglucosaminidase was assayed using *p*-nitrophenyl-β-D-2-acetamido-2-deoxyglucopyranoside as substrate. The reaction mixture contained, in a final volume of 0.8 ml, 400 μmol of Tris-maleate buffer (pH 6.8), 2 μmol of *p*-nitrophenyl-β-D-2-acetamido-2-deoxyglucopyranoside, 20% glycerol, and enzyme. After incubation at 37 C for varying periods of time, the reaction was terminated by the addition of 0.2 ml of 1.25 M K_2CO_3. After centrifugation at 20,000 × *g* for 10 min, the supernatant fluid was used for the optical density measurements at 420 nm in a Zeiss spectrophotometer. The molar extinction coefficient of *p*-nitrophenol, determined under the same condition, was found to be 14,000 with a light path of 1 cm. Controls lacking enzyme or substrate were always included in each assay. Identical conditions were used for determining the enzyme activity on the other artificial substrates.

Enzyme activity on glycopeptide monomer, glycopeptide dimer, and murein-lipoprotein complex were followed by the release of *N*-acetylglucosamine. The reaction mixture contained (in a final volume of 0.1 ml) 10 μmol of potassium phosphate buffer (pH 6.8), 20% glycerol, substrate, and enzyme. Glycopeptide monomer (600 μg), 400 μg of glycopeptide dimer, and 4 mg of murein-lipoprotein complex were used as substrates. After incubation at 37 C for varying periods of time, the reaction was terminated by heating for 2 min at 100 C. The *N*-acetylglucosamine released was determined by the Elson-Morgan assay (4).

Alkaline phosphatase (EC 3.1.3.1) was assayed by the method of Garen and Levinthal (7). Phosphoglucoisomerase (EC 5.3.1.9) was assayed as described previously (29).

Other assays and measurements. Protein was determined by the method of Lowry et al. (16). *N*-acetylglucosaminolactone was prepared by *N*-acetylation of glucosaminic acid, followed by acidification to pH 2 with dilute HCl and evaporation to dryness (14). The concentration of lactone (a mixture of 1,4- and 1,5-lactones) was determined by the hydroxylamine method (15).

Polyacrylamide gel electrophoresis. Polyacrylamide (7.5%) gel electrophoresis in 0.5% sodium dodecyl sulfate was carried out according to the method of Inouye and Guthrie (12). Relative electrophoretic mobilities of purified enzyme and protein standards were calculated according to Weber and Osborn (27). For alkaline urea gel, 7.5% polyacrylamide gels were prepared in 6 M urea and 0.1 M Tris-hydrochloride buffer (pH 8.5), with 25 mM Tris-glycine (pH 9.2) as the running buffer (29). The alkaline urea gels were pre-run overnight.

Procedures for osmotic shock treatment and for spheroplast formation. The procedure of Neu and Heppel (19) and that of Nossal and Heppel (20) were used for releasing periplasmic proteins by osmotic shock. The method of Osborn et al. (21) was used for converting *E. coli* cells to spheroplasts. Lysozyme was added at a final concentration of 200 μg/ml.

Preparation of natural substrates. Murein-lipoprotein complex was isolated according to the procedure of Braun and Sieglin (1). Two grams of murein-lipoprotein complex were mixed with a small amount of highly radioactive murein-lipoprotein complex (labeled with [^{3}H]glucosamine). Glycopeptide monomer and glycopeptide dimer were formed by digesting the murein-lipoprotein complex to completion with 40 mg of egg white lysozyme (25). The suspension was centrifuged at 37,000 × *g* for 2 h and the supernatant was applied to three Sephadex G-25 columns (2.5 by 95 cm each), connected in a series and which had been previously equilibrated with 20 mM pyridinium acetate buffer (pH 5). Chromatography was monitored by measuring ninhydrin, radioactivity, Elson-Morgan, and Ouchterlony tests, using anti-lipoprotein antibody prepared according to Lin and Wu (unpublished data). Two radioactive and ninhydrin-positive peaks were found. The ratio of radioactivity to ninhydrin-positive materials is consistent with the faster migrating peak being glycopeptide dimer and the slower migrating peak being glycopeptide monomer. Most of Ouchterlony-positive materials were found in the void volume; a small amount of it was detected in the faster migrating peak however, none was found in the slower migrating peak. The two peaks were pooled separately, concentrated by lyophilization, and further purified by high voltage paper electrophoresis (pH 1.9) using a unit from Savant Instrument, Inc. The electrolyte was made up of 0.4 M formic acid and 1.4 M acetic acid. The spots were identified by radioactivity and by ninhydrin staining. The glycopeptide monomer and glycopeptide dimer were identified by comparison with standards. The glycopeptide monomer and dimer so prepared were shown to have identical R_f as the standards in two descending chromatographic systems (isobutyric acid-1 M NH_4OH, 5:3; and *n*-butanol-acetic acid-water upper phase, 4:1:5). Whatman 3 MM paper was used. Finally, composition was confirmed by amino acid analysis.

Chemicals. *p*-Nitrophenyl-β-D-2-acetamido-2-deoxyglucopyranoside and glucose-6-phosphate dehydrogenase were from Calbiochem, Los Angeles, Calif. All other artificial substrates were purchased from Koch-Light Laboratories, Ltd., Buckinghamshire, England. Glucosaminic acid was from Schwarz/Mann, Orangeburg, N.Y. Phenylmethyl sulfonyl fluoride was purchased from Sigma Chemical Co., St. Louis, Mo. All other chemicals were reagent grade from commercial sources.

RESULTS

Intracellular localization of β-N-acetylglucosaminidase in E. coli cells. No enzyme activity was found in the culture media after removing the cells by centrifugation. In the crude extract prepared by sonication, this en-

zyme was recovered quantitatively in the supernatant fraction after centrifugation at 100,000 × *g* for 2 h (Table 1). To determine whether this enzyme is located in the periplasmic space, two approaches were taken. The results (Table 1) indicated that by both osmotic shock and spheroplast formation with ethylenediaminetetraacetate-lysozyme treatment, the recovery of β-*N*-acetylglucosaminidase activity in various fractions followed that of a known cytoplasmic enzyme marker, phosphoglucoisomerase, and was very different from that of a periplasmic enzyme, alkaline phosphatase (Table 1).

Purification of β-N-acetylglucosaminidase. Preliminary experiments indicated that the β-*N*-acetylglucosaminidase of *E. coli* K-12 is just as unstable as that of *E. coli* B where most of the activity was lost in a few days after the crude extract was chromatographed through two columns (17); however, we were able to stabilize the β-*N*-acetylglucosaminidase activity with the use of 20% glycerol and consequently were able to extensively purify this enzyme. All steps were performed at 4 C and all buffers used contained 20% glycerol and 10 mg of sucrose per ml. Column fractionation was monitored by measuring optical density at 280 nm and the conductivity of each fraction in a Zeiss spectrophotometer and with a Radiometer conductivity meter, respectively.

(i) Preparation of crude cell extract. Frozen cells (500 g) were washed once with 1 mM sodium phosphate buffer (pH 6.8) and resuspended in the same buffer. The cells were broken with one passage through a French pressure cell set at greater than 10,000 lb/in^2. The supernatant was prepared by centrifugation of the suspension of broken cells at 54,000 × *g* for 30 min.

(ii) Protamine sulfate precipitation. Nucleic acids were precipitated by dropwise addition of one-eighth volume of 2% protamine sulfate. The protamine sulfate supernatant was obtained by centrifugation at 54,000 × *g* for 30 min.

(iii) Hydroxyapatite chromatography. The protamine sulfate supernatant was applied to a hydroxyapatite column (5.5 by 55 cm) that had been previously equilibrated with 1 mM sodium phosphate buffer (pH 6.8). The column was washed with the equilibrating buffer until the eluate was transparent at 280 nm. Then the column was eluted with a linear gradient made up of 1 liter each of the equilibrating buffer and an identical buffer with sodium phosphate at 250 mM. The fractions containing the enzyme were pooled, concentrated by ultrafiltration with an Amicon cell (filter PM10), and dialyzed against the equilibrating buffer of the next column. The degree of equilibration was checked by conductivity measurements. Recovery from this column was 100% of total activity. Fifteen percent of the total activity in fractions with relatively lower specific activities was discarded. No activity was lost during ultrafiltration when pressure was applied at 15 lb/in^2. When higher pressure (25 lb/in^2) was used, recovery of activity was about 50%.

(iv) First DEAE-cellulose chromatography (pH 6.8). The equilibrated sample was centrifuged at 40,000 × *g* for 20 min and the supernatant was applied to a diethylaminoethyl (DEAE)-cellulose column (5.5 by 25 cm) that had been previously equilibrated with 10 mM Tris-maleate buffer (pH 6.8). The column was washed and then eluted with a linear gradient made up of 750 ml each of the equilibrating buffer and an identical buffer containing 0.24 M sodium chloride. Recovery was 95% of total activity. Only fractions with highest specific

TABLE 1. *Localization of β-N-acetylglucosaminidase in E. coli cells*

Experiment	Fraction	Total activity (%)		
		β-*N*-acetylglucosaminidase	Phosphoglucoisomerase	Alkaline phosphatase
1. Osmotic shock treatment	Shock fluid	3	1	60
	Sonified pellet	97	99	40
2. EDTA[a]-lysozyme treatment	Supernatant	33	36	92
	Sonified spheroplasts	67	64	8
3. Sonified cell extract	100,000 × *g* supernatant	87	88	ND[b]
	100,000 × *g* pellet	13	12	ND

[a] EDTA, Ethylenediaminetetraacetate.
[b] ND, Not determined.

activities were pooled, concentrated by ultrafiltration, and dialyzed to equilibrium with the equilibrating buffer of the following column.

(v) DEAE-cellulose chromatography (pH 8.0). The equilibrated sample was applied to a DEAE-cellulose column (5.5 by 18 cm) that had been previously equilibrated with 10 mM Tris-HCl buffer (pH 8.0). The column was washed initially with the equilibrating buffer and then with an identical buffer containing 100 mM sodium chloride. Finally, the column was eluted with a linear gradient consisting of 500 ml each of the equilibrating buffer containing 100 mM sodium chloride and an identical buffer containing 300 mM sodium chloride. Recovery was 91% of total activity. Only fractions with highest specific activities were pooled and concentrated.

(vi) Sephadex G-100 gel filtration. The concentrated enzyme was further purified on a Sephadex G-100 column (5 by 70 cm) that had been previously equilibrated with 10 mM Tris-hydrochloride buffer (pH 6.8). Recovery was 100% of total activity. Active fractions were pooled and applied onto the next column.

(vii) Second DEAE-cellulose chromatography (pH 6.8). It was found that, when the enzyme aged, it became more anionic and a second DEAE-cellulose chromatography (pH 6.8) was found to give good separation of the enzyme from the other proteins. The column (2.5 by 40 cm) was washed with the equilibrating buffer which contained 10 mM Tris-maleate buffer (pH 6.8) and was eluted with a linear gradient made up of 350 ml each of the equilibrating buffer and an identical buffer containing 0.24 M sodium chloride. All active fractions were pooled, concentrated, and dialyzed against the equilibrating buffer of the final column.

(viii) DEAE-cellulose chromatography (pH 5.7). Preliminary experiments had shown that this column gave excellent purification of the enzyme; however, the enzyme activity was unstable at pH 5.7. Therefore this purification step was performed as a last resort and with as much speed as possible. The equilibrated sample was applied to the column (2.5 by 20 cm) that was previously equilibrated with 10 mM Tris-maleate buffer (pH 5.7). The column was washed with the equilibrating buffer and was eluted with a linear gradient made up of 150 ml each of the equilibrating buffer and an identical buffer containing 0.08 M sodium chloride. Active fractions were pooled, the pH was adjusted to 6.8, and the fractions were concentrated. Recovery was 50% of total activity.

A summary of the purification of this enzyme is presented in Table 2.

Criteria of purity and molecular weight of purified β-N-acetylglucosaminidase. Polyacrylamide gel electrophoresis of the purified enzyme in 0.5% sodium dodecyl sulfate (Fig. 1A) and in alkaline-urea (Fig. 1B) indicated the presence of a single polypeptide band. Molecular weight of the purified enzyme was determined by two methods. Sephadex G-100 gel filtration revealed an active enzyme with an apparent molecular weight of 33,500 (Fig. 2A), whereas sodium dodecyl sulfate gel electrophoresis showed a single band with a molecular weight of 38,000 (Fig. 2B). It appears most likely that this enzyme in its active form consists of a single polypeptide with an approximate molecular weight of 36,000. The reduction of the apparent molecular weight of this enzyme as determined by Sephadex G-100 gel filtration most likely results from an interaction of glycosidase with the dextran gel.

Stability of the purified enzyme. Similar to the enzyme in *E. coli* B (17), the enzyme from *E. coli* K-12 is also extremely unstable. However, the activity of this enzyme can be stabilized by the addition of glycerol. In the presence of 20% glycerol the activity of the enzyme is stable when stored at −70 C for at least 3 months or at −20 C for 2 months. The enzyme

TABLE 2. *Purification of β-N-acetylglucosaminidase from E. coli K-12*

Fraction	Total protein (mg)	Sp act (μmol/h per mg)	Yield (%)	Purification (fold)
1. Crude Extract	25,000	0.15	100	1
2. Protamine sulfate supernatant	25,200	0.15	105	1
3. Hydroxyapatite	13,400	0.23	85	2
4. DEAE-cellulose (pH 6.8)	2,336	0.90	56	6
5. DEAE-cellulose (pH 8.0)	94	10.87	28	73
6. Sephadex G-100	49	18.60	25	126
7. DEAE-cellulose (pH 6.8)	9	101.30	25	684
8. DEAE-cellulose (pH 5.7)	3	162.30	13	1,094

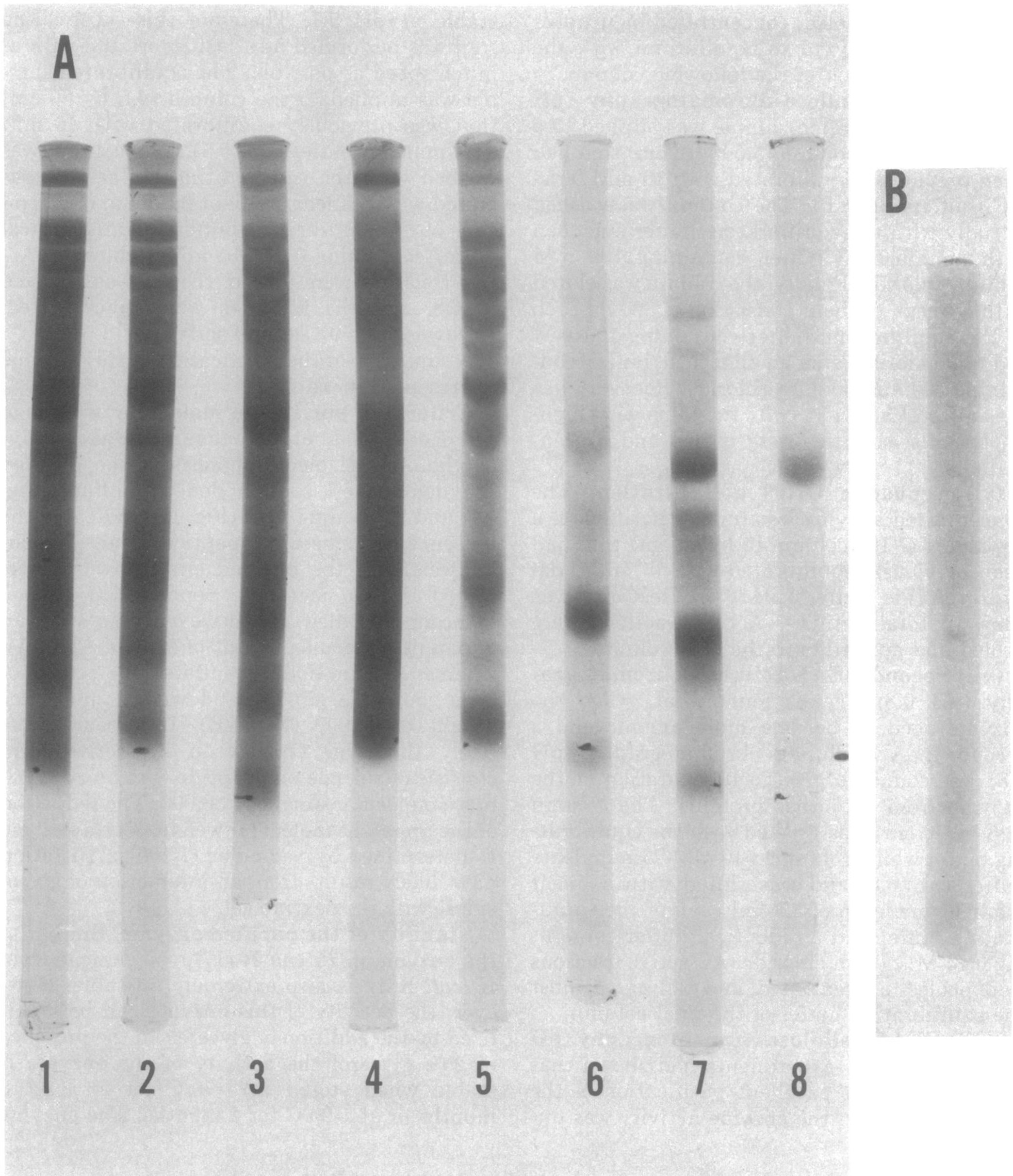

FIG. 1. (*A*) *Sodium dodecyl sulfate* (*SDS*) *polyacrylamide gel electrophoresis of β-N-acetylglucosaminidase at various stages of purification.* (*1*) *Crude extract;* (*2*) *protamine sulfate supernatant;* (*3*) *after hydroxyapatite column chromatography;* (*4*) *after first DEAE-cellulose column chromatography at pH 6.8;* (*5*) *after DEAE-cellulose column chromatography at pH 8.0;* (*6*) *after Sephadex G-100 gel filtration;* (*7*) *after second DEAE-cellulose column chromatography at pH 6.8;* (*8*) *after DEAE-cellulose column chromatography at pH 5.7. 20 to 200 μg of protein was applied to each gel. Samples were incubated at 70 C for 20 min in 1% SDS, 1% 2-mercaptoethanol, and 10% glycerol, buffered at pH 7.1 with 0.01 M sodium phosphate. Electrophoresis in 7.5% polyacrylamide gel containing 0.5% SDS was carried out at room temperature with a constant current of 3 mA per gel. The gels were stained with Coomassie brilliant blue.* (*B*) *Alkaline urea gel electrophoresis of purified β-N-acetylglucosaminidase. 15 μg of purified enzyme was applied to a 7.5% polyacrylamide gel containing 8 M urea and 0.12 M Tris-hydrochloride buffer* (*pH 8.5*)*. Electrophoresis was carried out with 25 mM Tris-glycine* (*pH 9.1*) *as the running buffer at a constant current of 4 mA per gel at room temperature. Gels were stained with Coomassie brilliant blue.*

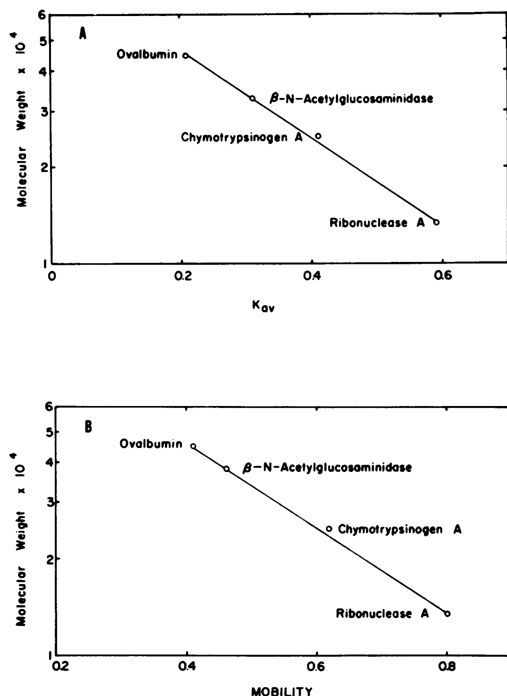

FIG. 2. *Determinations of molecular weight of β-N-acetylglucosaminidase. (A) By Sephadex G-100 chromatography: A Sephadex G-100 column (1.6 by 85 cm) was equilibrated with 0.01 M Tris-maleate buffer (pH 6.8) containing 10% glycerol and 0.02% NaN_3. The void volume of the column was determined with 1 ml of blue dextran-2000 (1 mg/ml) and the total elution volume was determined using 1 ml of 2 M NaCl. 2 mg each of the protein standards was used to establish the calibration curve. 50 µg of the purified β-N-acetylglucosaminidase was applied to the column together with the protein standards. The elution volumes of the protein standards were determined by measuring absorbance at 280 nm, whereas that for the β-N-acetylglucosaminidase was monitored by assaying for the enzyme activity. (B) By sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis: 20 µg each of the protein standards and the purified enzyme was applied to each gel. The conditions for SDS polyacrylamide gel electrophoresis were identical to those described in the legend to Fig. 1A. A plot of the logarithm of molecular weight of the marker protein versus mobility in the gel provided the standard curve.*

is even more stable in 50% glycerol but it is impractical to use this high concentration of glycerol for column fractionation.

pH and ionic strength optima. The pH optimum of the purified enzyme was 7.7 in Tris-maleate buffer. However, the same activity in the crude enzyme preparation showed a pH optimum of 6.8. The optimum concentration of Tris-maleate buffer (pH 7.7) was 50 mM.

Substrate specificity. (i) Artificial substrates. As indicated by the results shown in Table 3, the purified enzyme is specific for the β-linkage. The α-linked compound was not hydrolyzed. The 2-acetamido group of the substrate is essential since the glucose derivative remained inactive, nor is the enzyme active towards β-*N*-acetyl galactosaminide. The K_m for *p*-nitrophenyl-β-D-2-acetamido-2-deoxyglucopyranoside was 0.43 mM (Fig. 3).

(ii) Natural substrates. The K_m for glycopeptide monomer was 2.58 mM (Fig. 4). Many attempts were made to determine the K_m for glycopeptide dimer but none of the results was statistically significant as analyzed by the computer program of Cleland for linear lines (3). Glycopeptide dimer was found to stimulate the V_{max} of the reaction utilizing *p*-nitrophenol-β-D-2-acetamido-2-deoxyglucopyranoside as substrate. Radioactivity was released from [^{3}H]glucosamine-labeled murein-lipoprotein complex by the purified enzyme. This labeled radioactive product migrated with the same R_f as *N*-acetylglucosamine in two descending chromatographic systems (isobutyric acid-1 M NH_4OH, 5:3; and *n*-butanol-pyridine-water, 6:4:3). When this radioactive product was eluted from the paper chromatogram and hydrolyzed in 4 N hydrochloric acid for 10 h at 100 C, the resulting radioactive product migrated with the same R_f as glucosamine in the same two chromatographic systems. These results indicate that the purified enzyme releases radioactive *N*-acetylglucosamine from [^{3}H]-glucosamine-labeled murein-lipoprotein complex. Similarly, one of the products of the reaction of the enzyme with glycopeptide monomer or dimer was identified as *N*-acetylglucosamine. All attempts to demonstrate any transglucosaminidase activity of this enzyme were unsuccessful.

Effectors. The activity of the enzyme was slightly inhibited by *N*-acetylglucosamine and

TABLE 3. *Substrate specificity of β-N-acetylglucosaminidase of E. coli K-12*

Substrate	Hydrolysis
p-Nitrophenyl-β-D-2-acetamido-2-deoxyglucopyranoside	+
p-Nitrophenyl-α-D-2-acetamido-2-deoxyglucopyranoside	−
p-Nitrophenyl-β-D-2-acetamido-2-deoxygalactopyranoside	−
p-Nitrophenyl-β-D-glucopyranoside	−
Murein-lipoprotein complex	+
Glycopeptide monomer (C_6)	+
Glycopeptide dimer (C_3)	+

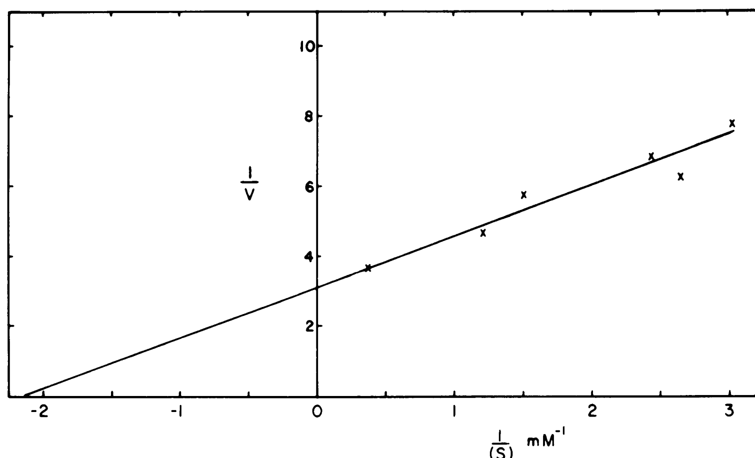

FIG. 3. *Lineweaver-Burk plot of velocity versus substrate concentration for β-N-acetylglucosaminidase. p-Nitrophenyl-β-D-2-acetamido-2-deoxyglucopyranoside was used as the substrate. Velocity of the enzyme reaction was expressed as absorbance at 420 nm after 30 min of incubation at 37 C.*

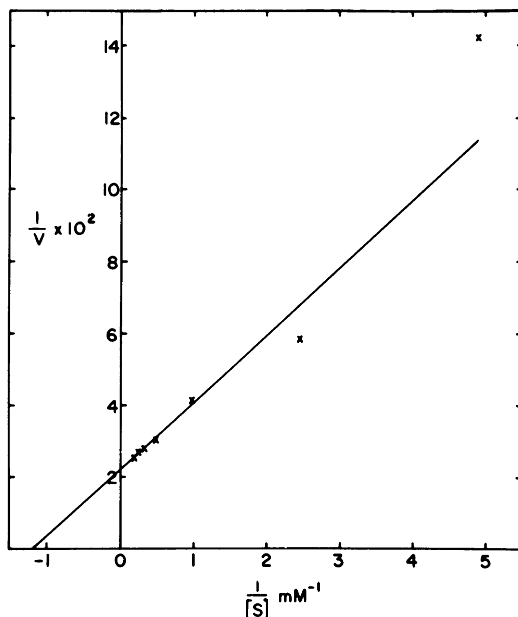

FIG. 4. *Determination of K_m of the purified β-N-acetylglucosaminidase for glycopeptide monomer (C_6). Velocity of the enzyme reaction was expressed as nanomoles of N-acetylglucosamine formed after 1 h of incubation at 37 C.*

N-acetylmuramic acid and was strongly inhibited by *N*-acetylglucosaminolactone (Table 4). Ethylenediaminetetraacetate, monothioglycerol, and phenyl-methyl sulfonyl fluoride (up to 400 μM) did not affect the activity of the purified enzyme.

TABLE 4. *Inhibition of β-N-acetylglucosaminidase*

Compound added	Concn[a] (mM)	Total activity[b] (%)
None		100
Glucosamine	7.3	81
Muramic acid	6.6	94
N-acetylglucosamine	7.3	44
N-acetylmuramic acid	6.6	46
N-acetylglucosaminolactone	7.0	11
	0.7	17
	0.07	29

[a] The compounds were added to give the various final concentrations in the reaction mixtures.

[b] Enzyme activity was determined using *p*-nitrophenyl-β-D-2-acetamido-2-deoxyglucopyranoside as substrate. A crude extract was used.

DISCUSSION

β-*N*-acetylglucosaminidase has been purified from *E. coli* K-12. The salient features of this enzyme that one should take into consideration in assigning its physiological functions are summarized below.

It is a soluble cytoplasmic enzyme.

The activity of this enzyme does not vary significantly with respect to the growth media (minimal medium versus enriched medium), carbon source (glucose versus succinate), or phase of growth (exponential versus stationary). Nor is its synthesis subject to repression by *N*-acetylglucosamine or glucosamine (data not shown).

This enzyme is an exo-*N*-acetylglucosaminidase. It releases *N*-acetylglucosamine from mu-

rein-lipoprotein complex, from glycopeptide monomer (C6), and from glycopeptide dimer (C3) obtained by lysozyme digestion of murein.

Although N-acetylglucosamine and N-acetylmuramic acid do show some inhibition of the activity of this enzyme at fairly high concentrations of these two compounds, the most potent inhibitor is N-acetylglucosaminolactone, as previously found for many similar enzymes. This observation is more relevant to the mechanism of the reaction than to any potential implication of an in vivo regulation of the activity of this enzyme.

Attempts to demonstrate any transglucosaminidase activity of this enzyme were unsuccessful.

The physiological function of this enzyme for the growth and division of *E. coli* cells will be assessed in the accompanying note by considering the physiological consequence (or lack of it) of mutations affecting its activity.

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