JOURNAL OF BACTERIOLOGY, Jan. 1976, p. 372-373

Vol. 125, No. 1
Printed in U.S.A.

Isolation of *Escherichia coli* K-12 Mutants with Altered Levels of β-*N*-Acetylglucosaminidase

DANIEL W. YEM AND HENRY C. WU*

Department of Microbiology, University of Connecticut Health Center, Farmington, Connecticut 06032

Received for publication 15 September 1975

Eight mutants with less than 25% of the wild-type level of β-*N*-acetylglucosaminidase activity have been isolated from *Escherichia coli* K-12. Studies on these mutants suggest that less than 1% of the wild-type level of this enzyme may be adequate for the normal growth and division of *E. coli* cells.

In the preceding paper, we reported the purification and characterization of β-*N*-acetylglucosaminidase from *Escherichia coli* K-12 (3). To gain further insight into the physiological function of this autolytic enzyme in the normal growth or division of *E. coli* cells, we have isolated mutants of *E. coli* with altered levels of this enzyme.

Because of the extremely low activity of β-*N*-acetylglucosaminidase in *E. coli* K-12 cells, it was not possible to determine the presence or absence of this enzyme in a bacterial colony either by spraying toluene-treated colonies with a solution containing the substrate (*p*-nitrophenyl - β - D - 2 - acetamido - 2 - deoxyglucopyranoside) or by plating bacterial cells on plates containing this compound. Therefore, we had to resort to the assay of the apparent activity of this enzyme in whole cell suspensions.

The time course of this in vivo assay of β-*N*-acetylglucosaminidase activity in *E. coli* cells is shown in Fig. 1. Addition of chloramphenicol at a final concentration of 200 μg/ml to the incubation mixture has no effect on enzyme activity. Nor did treatments of the cell suspensions with any of the following surface-active chemicals, including toluene (0.2%), sodium deoxycholate (0.2%), Triton X-100 (0.2%), sodium dodecyl sulfate (0.2%), or ethylenediaminetetraacetate (5 mM), increase the activity of this enzyme in the whole cell suspension. Thus, it appears that, at least in the case of the wild-type cells, the rate-limiting step in the hydrolysis of *p*-nitrophenyl-β-D-2-acetamido-2-deoxyglucopyranoside by the whole cells is not the entry of the substrate into the cells, but instead lies in the enzymatic reaction itself.

An overnight culture of the wild type, strain AB1157, in L broth (3) was diluted 20-fold into fresh medium. Cells in the early exponential growth were treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine at 100 μg/ml in tris(hydroxymethyl)aminomethane - maleate buffer (pH 6.0) for 20 min at 37 C (1). The mutagenized cells were centrifuged at 7,000 × *g* for 10 min and washed twice with saline. The cells were resuspended in L broth and were grown overnight at 30 C. The overnight culture was diluted and plated on LC plates (3) to obtain single colonies.

After mutagenesis and phenotypic expression, a large number of bacterial cultures each of which was derived from a single colony was screened for the activity of β-*N*-acetylglucosa-

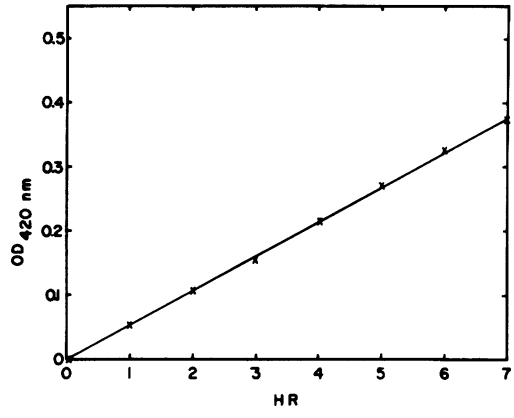

FIG. 1. *Time course for the in vivo assay of β-N-acetylglucosaminidase. AB1157 cells were grown to exponential phase in nutrient broth, washed once with normal saline, and resuspended in a reaction mixture containing 50 mM tris(hydroxymethyl)aminomethane-maleate buffer (pH 6.8) and 4 mM p-nitrophenyl-β-D-2-acetamido-2-deoxyglucopyranoside. The suspension was incubated at 37 C and aliquots were taken at various times. The reaction in the aliquots was terminated with the addition of K_2CO_3 at a final concentration of 0.4 M. The cells were centrifuged and the absorbance of the supernatant fluid at 420 nm was determined.*

minidase by the in vivo assay. From over 2,000 colonies, we isolated eight independent mutants with reduced levels of this enzyme and one mutant with elevated level, as compared to that in the parental strain. The specific activities of this enzyme in the crude extracts of these mutants were measured, and the results (Table 1) were in good agreement with the in vivo assays using whole cell suspensions. Mixed incubation of crude extract from each of the eight β-*N*-acetylglucosaminidase-negative mutants with that from the wild-type strain showed no evidence for the presence of a diffusible inhibitor of this enzyme in any of these mutants (data not shown).

Strain E593, which showed less than 0.2% of the activity of this enzyme found in the wild-type strain, nevertheless grew and divided normally in L broth, proteose peptone beef extract broth, nutrient broth, and M9 minimal glucose medium at both 30 and 42 C. Cellular morphology of this mutant was also normal under these conditions. It appears, therefore, that more than 99% of the activity of this enzyme in the wild-type strain is dispensable for the normal growth of *E. coli*. Likewise, strain E581 appeared nearly normal in growth and morphology, despite a two- to fourfold increase in the specific activity of this enzyme as compared to the wild-type strain.

These mutants deficient in the activity of β-*N*-acetylglucosaminidase were as sensitive to bactericidal action of ampicillin as the parental strain.

The specific activity of this enzyme in the wild-type strain is extremely low so that the rate-limiting step in the in vivo hydrolysis of *p* - nitrophenyl - β - D - 2 - acetamido - 2 - deoxyglucopyranoside by whole cell suspensions is the enzymatic hydrolysis rather than the transport of the substrate across the cell envelope. The level of this enzyme could not be induced by the presence of glycopeptide in the growth media nor did it vary with the nature of the growth medium and the carbon sources. Therefore, it is unlikely that the primary physiological function of this enzyme is a catabolic enzyme for scavenging *N*-acetylglucosamine from the growth media.

TABLE 1. *Level of β-N-acetylglucosaminidase in parent and mutant strains*

Strain	Specific activity of β-*N*-acetylglucosaminidase (nmol/h per mg)	Activity (%)
Wild type		
AB1157	49.9	100
Mutants		
E578	3.2	6
E580	1.3	3
E592	0.9	2
E593	<0.1	<0.2
E594	1.5	3
E595	<0.1	<0.2
E596	11.6	23
E603	3.6	7
E581	101.1	202

This enzyme, though active on peptidoglycan in vitro (3), may be of secondary importance in the total degradation of peptidoglycan, rather than being the enzyme involved in the initial breakage of this covalently closed sacculus. This conclusion would be consistent with the genetic evidence that most of the activity of this enzyme do not appear to be essential for the growth and division of *E. coli* cells. Similar conclusions have been reached in the case of an extracellular exo-β-*N*-acetylglucosaminidase of *Bacillus subtilis* by Ortiz (2).

This work was supported by a U.S. Public Health Service Grant (CA-11371) from the National Cancer Institute and by a grant (BC-148A) from the American Cancer Society.

LITERATURE CITED

1. Adelberg, E. A., M. Mandel, and G. C. C. Chen. 1965. Optimal conditions for mutagenesis by N-methyl-N'-nitro-N-nitrosoguanidine in *Escherichia coli* K-12. Biochem. Biophys. Res. Commun. **18**:788–795.
2. Ortiz, J. M. 1974. Mutant of *Bacillus subtilis* lacking exo-β-*N*-acetylglucosaminidase activity. J. Bacteriol. **117**:909–910.
3. Yem, D. W., and H. C. Wu. 1976. Purification and properties of β-*N*-acetylglucosaminidase from *Escherichia coli*. J. Bacteriol. **125**:324–331.