Note

Mycelial β -N-Acetylglucosaminidase of *Streptomyces* sp. Having β -N-Acetylgalactosaminidase ActivityTooru IWAMOTO, Megumu INAOKA
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β -N-Acetylglucosaminidase (EC 3.2.1.30) is widely distributed in animal tissues, higher plants and microorganisms. Among various glycosidases β -N-acetylglucosaminidase has been recommended as a useful enzyme for studies on the structure of the carbohydrate moieties in glycoprotein and glycolipid molecules. For the study of these enzymes β -N-acetylglucosaminidases from fungi^{1~3)} and bacteria^{4~6)} appear to be the most suitable because the microbial enzymes, unlike those from animal tissues,^{7~9)} seem to be devoid of isozymic forms and therefore an investigation on these enzymes is supposed not to be complicated. It has been shown that most of the enzymes from microorganisms except *Bacillus subtilis*⁵⁾ and *Escherichia coli*⁶⁾ have the activities of both β -N-acetylglucosaminidase and β -N-acetylgalactosaminidase and that these activities are due to the same enzyme protein.^{1~4,10,11)}

We found that some strains of *Streptomyces* produced β -N-acetylglucosaminidase and that the greater part of it was retained on the mycelial surface.¹²⁾ There have been very few reports about this enzyme from *Streptomyces* because this enzyme may be associated on the mycelia, although most other microbial enzymes were found to be released in the growth medium^{1~4,10,11)} except for those of *B. subtilis*⁵⁾ and *E. coli*.⁶⁾ The mycelia were found to exhibit β -N-acetylgalactosaminidase as well as β -N-acetylglucosaminidase activities. This report deals with the question of whether the two activities associated on the mycelia are due to the same protein as in other sources, or not.

Streptomyces albus IFO 3710 and the strain of *Streptomyces* described previously, named L-13,¹²⁾ were used in this study. These organisms were grown on a medium composed of 0.5% glucose, 0.2% yeast extract, 0.2% K₂HPO₄ and 0.1% MgSO₄·7H₂O, pH 7.0. Ten ml of seed broth cultivated at 37°C for 1 day was transferred to a 1 liter volume conical flask containing 200 ml of the medium and cultivation was carried out for 2 days with

shaking at 37°C. The mycelia collected by centrifugation were washed with deionized water and lyophilized.

β -N-Acetylglucosaminidase and β -N-acetylgalactosaminidase activities were assayed by use of *p*-nitrophenyl β -N-acetylglucosaminide and *p*-nitrophenyl β -N-acetylgalactosaminide as substrates, respectively. The reaction mixture contained 1.0 ml of 2 mM substrate solution, 1.0 ml of 20 mM phosphate buffer, pH 6.5, and 2 or 5 mg lyophilized mycelia. After incubation at 37°C for 10 min, 4.0 ml of 0.1 M sodium carbonate solution was added to stop the reaction and the mixture was filtered through Toyo No. 5C filter paper. The liberated *p*-nitrophenol was determined by reading the absorbance at 430 nm. One unit of enzyme activity was defined as the amount of enzyme contained in 1 mg mycelia which released 1 nmol of *p*-nitrophenol per min.

p-Nitrophenyl β -N-acetylglucosaminide and *p*-nitrophenyl β -N-acetylgalactosaminide were purchased from Koch-Light Laboratories. Phenyl β -N-acetylglucosaminide and phenyl β -N-acetylgalactosaminide were from Nakarai Chemical, Ltd.

S. albus and the L-13 strain were grown for 3 days. At appropriate intervals, their mycelia were taken and subjected to the enzyme assays. As Fig. 1 shows, the highest activities of β -N-acetylglucosaminidase and β -N-acetylgalactosaminidase were obtained on 48 hr cultivation of the two organisms.

The effects of *N*-acetylglucosamine and *N*-acetylgalactosamine, the respective end products of the actions of β -N-acetylglucosaminidase and β -N-acetylgalactosaminidase, on both enzyme activities are summarized in Table I. β -N-Acetylglucosaminidase action was found to be inhibited by *N*-acetylgalactosamine as well as by *N*-acetylglucosamine. Similarly, β -N-acetylgalactosaminidase action was found to be inhibited by the two compounds. Both enzyme actions were inhibited more powerfully by *N*-acetylgalactosamine than by *N*-acetylglucosamine. This type of cross inhibition has been described with *Paecilomyces persicinus*, the activities of the two enzyme of which were shown to be due to the same protein.³⁾

The effect of phenyl β -N-acetylgalactosaminide, a substrate of β -N-acetylgalactosaminidase, on the hydrolysis of *p*-nitrophenyl β -N-acetylglucosaminide by β -N-acetylglucosaminidase was investigated. Similarly, the effect of phenyl β -N-acetylglucosaminide on β -N-acetylgalactosaminidase activity was examined. After the reaction mixture containing 1 ml of 2 mM *p*-nitrophenyl β -N-acetylglucosaminide solution and 1 ml of phenyl- β -N-acetylgalactosaminide solution of varying concentrations was incubated with mycelia at 37°C for 10 min, *p*-nitrophenol liberated was measured. The effect of phenyl β -N-acetylgalactosaminide was investigated by the same procedure. As Fig. 2 shows, the hydrolysis of *p*-nitrophenyl β -N-acetylglucosaminide was found to be inhibited by phenyl β -N-acetylgalactosaminide and the hydrolysis of *p*-nitrophenyl β -N-acetylgalactosaminide

FIG. 1. Time Course of Production of β -N-Acetylglucosaminidase and β -N-Acetylgalactosaminidase by *S. albus* and the L-13 Strain.

Enzyme activities of β -N-acetylglucosaminidase (○) and β -N-acetylgalactosaminidase (●) produced by *S. albus* (left) and the L-13 strain (right) were assayed.

TABLE I. EFFECT OF N-ACETYLGLUCOSAMINE AND N-ACETYL GALACTOSAMINE ON β -N-ACETYLGLUCOSAMINIDASE AND β -N-ACETYL GALACTOSAMINIDASE ACTIVITIES

Enzyme activities were expressed as a percentage of the activity level in the absence of compounds.

Compound	<i>S. albus</i>		L-13	
	β GlcNAcase	β GalNAcase	β GlcNAcase	β GalNAcase
None	100.0	100.0	100.0	100.0
GlcNAc	58.7	64.8	61.0	75.0
GalNAc	47.0	50.2	47.6	55.2

β GlcNAcase, β -N-acetylglucosaminidase activity; β GalNAcase, β -N-acetylgalactosaminidase activity; GlcNAc, N-acetylglucosamine; GalNAc, N-acetylgalactosamine.

FIG. 2. Cross Inhibition of β -N-Acetylglucosaminidase and β -N-Acetylgalactosaminidase by Phenyl Compounds of N-Acetylgalactosamine and N-Acetylglucosamine.

β -N-Acetylglucosaminidase (○) and β -N-acetylgalactosaminidase (●) activities were measured in the presence of phenyl β -N-acetylgalactosaminide and phenyl β -N-acetylglucosaminide, respectively.

Left, *S. albus*; right, L-13 strain.

was inhibited by phenyl β -N-acetylglucosaminide. The phenyl compound of N-acetylgalactosamine inhibited greater than that of N-acetylglucosamine just like the inhibition by the end product. If the activities of β -N-acetylglucosaminidase and β -N-acetylgalactosaminidase are due to the same protein, action of one enzyme toward the substrate should be inhibited by the substrate of the other enzyme.

Therefore, these types of inhibitions of β -N-acetylglucosaminidase and β -N-acetylgalactosaminidase by the end product and the substrate suggest the possibility that the two enzyme activities in the mycelia of *S. albus* and the L-13 strain are due to the same enzyme protein as found with the enzymes obtained from many other sources.

REFERENCES

- 1) O. P. Bahl and K. M. L. Agrawal, *J. Biol. Chem.*, **244**, 2970 (1969).
- 2) T. Mega, T. Ikenaka and Y. Matsushima, *J. Biochem.*, **68**, 109 (1970).
- 3) L. A. Eriquez and M. A. Pisano, *J. Bacteriol.*, **137**, 620 (1979).
- 4) T. Kiyohara, T. Terao, K. S. Nakano and T. Osawa, *J. Biochem.*, **80**, 9 (1976).
- 5) J. M. Ortiz, J. B. Gillispie and R. C. Berkeley, *Biochim. Biophys. Acta*, **289**, 174 (1972).
- 6) D. W. Yem and H. C. Wu, *J. Bacteriol.*, **125**, 324 (1976).
- 7) J. C. Caygill, C. P. J. Roston and F. R. Jevons, *Biochem. J.*, **98**, 405 (1966).
- 8) J. Yoshikawa, H. Koide and M. Yamanaka, *Biochem. Biophys. Res. Commun.*, **46**, 11 (1972).
- 9) J. V. Bannister and P. J. R. Phizackerley, *FEBS Letters*, **34**, 120 (1973).
- 10) A. Ohtakara, M. Yoshida, M. Murakami and T. Izumi, *Agric. Biol. Chem.*, **45**, 239 (1981).
- 11) D. Every and J. M. Ashworth, *Biochem. J.*, **143**, 785 (1974).
- 12) T. Iwamoto, M. Kano and M. Inaoka, *J. Ferment. Technol.*, **54**, 316 (1976).