Short Communication

A New Enzyme "Nitrile Hydratase" which Degrades Acetonitrile in Combination with Amidase

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Enzymic hydrolysis of nitrile to carboxylic acid has been studied with barley leaves and microbial cells.¹⁻³⁾ Nitrilase (EC 3.5.5.1) catalyzes the hydrolysis of nitrile to the corresponding carboxylic acid and ammonia without forming amide as an intermediate and amide does not act as the substrate.

In the course of investigation on microbial degradation of nitrile compounds, we found that *Arthrobacter* sp. J-1⁴⁾ formed acetamide in the culture broth when grown on acetonitrile. Although a nitrilase activity was detected in the cell-free extract, the activity was lost as it was highly purified.

This paper describes how that the nitrilase activity of *Arthrobacter* sp. J-1 is shown by the combination of nitrile hydratase and amidase.

Arthrobacter sp. J-1 was grown on the basal medium⁵⁾ containing 0.4% (w/v) acetonitrile. The same amount of acetonitrile was added at 24-hr cultivation time. The cultivation was carried out aerobically at 28°C for 48 hr. Washed cells from 30 liters of the culture broth were suspended in 0.1 M buffer and disrupted by an ultrasonic oscillator below 10°C. The buffer used in this work was potassium phosphate buffer, pH 7. As the cell-free extract showed both nitrilase and amidase activities, the following fractionation was carried out detecting ammonia formation from acetonitrile and acetamide as the substrate. To the cell-free extract, ammonium sulfate was added to 30~60% saturation. The resultant precipitate was collected by centrifugation and dissolved

FIG. 1. Separation of Nitrilase and Amidase by Hydroxyapatite Column Chromatography.

The column used was 2.9×3.5 cm. Five milliliters fractions were collected. Nitrilase activity was assayed at 30°C by measuring the formation of ammonia. The reaction mixture contained 100 μmol buffer, 10 μmol acetonitrile and enzyme solution in total volume of 0.5 ml. Formed ammonia was determined by the method of Harper.³⁾ Amidase activity was assayed similarly, with 10 μmol acetamide as the substrate in place of acetonitrile. One unit of the activity is defined as the amount of enzyme which catalyzes the formation of 1 μmol of ammonia per min under the above conditions., absorbance at 280 nm; ●, nitrilase activity; △, amidase activity; ○, ammonia forming activity from acetonitrile with an addition of 0.06 U amidase.

in 0.01 M buffer. The enzyme solution was dialyzed and applied to DEAE-cellulose column chromatography under stepwise elution. Active fractions eluted with 0.05 M buffer containing 0.4 M NaCl were collected and again precipitated with ammonium sulfate (80% saturation). Nitrilase and amidase activities could not be separated up to this stage. The active precipitate formed was dissolved, dialyzed and then applied to hydroxyapatite column chromatography. The enzyme was eluted with a linear concentration of buffer from 0.01 M to 0.06 M. As shown in Fig. 1, nitrilase and amidase activities were separated. When 0.06 U of amidase prepared by this procedure was added to the nitrilase fraction, ammonia formation from acetonitrile increased significantly. The nitrilase fraction was concentrated by ultrafiltration. The enzyme solution was then applied to a Sephadex G-150 column and Sephadex G-100 column which

FIG. 2. Gel Filtration of Ammonia Forming Activity from Acetonitrile on Sephadex G-100.

The column used was 0.9×115 cm. Fractions (0.6 ml) were collected. The enzyme activity was determined as described in Fig. 1. ·····, absorbance at 280 nm; ●, nitrilase activity; ○, ammonia forming activity from acetonitrile with an addition of 0.06 U amidase.

were equilibrated with 0.05 M buffer containing 0.1 M NaCl. The nitrilase activity was completely lost in this stage. Instead, the activity which formed ammonia with the addition of amidase was eluted (Fig. 2). The active fractions were pooled and concentrated by ultrafiltration. The enzyme fraction formed stoichiometrically acetamide, which was determined by gas-liquid chromatography, from acetonitrile. Similarly, the amidase separated by hydroxyapatite column chromatography

catalyzed the hydrolysis of acetamide to form a stoichiometric amount of acetic acid and ammonia. Thus, the nitrilase activity found in the cell-free extract of *Arthrobacter* sp. J-1 was proved to be a combination of nitrile hydratase and amidase.

Jallageas *et al.*⁶⁾ have suggested that acetonitrilase and acetamidase function in the degradation of acetonitrile. However, no clear enzymological investigation has been made. We have been the first to prove the presence of acetonitrile hydratase which participates in the degradation of aliphatic nitriles. Further purification and characterization of nitrile hydratase and amidase of *Arthrobacter* sp. J-1 are under investigation.

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REFERENCES

- 1) K. V. Thimann and S. Mahadevan, *Arch. Biochem. Biophys.*, **105**, 133 (1964).
- 2) R. H. Hook and W. G. Robinson, *J. Biol. Chem.*, **239**, 4263 (1964).
- 3) D. B. Harper, *Biochem. J.*, **165**, 309 (1977).
- 4) H. Yamada, Y. Asano, T. Hino and Y. Tani, *J. Ferment. Technol.*, **57**, 8 (1979).
- 5) H. Yamada, Y. Asano and Y. Tani, *ibid.*, in press.
- 6) J. C. Jallageas, A. Arnaud and P. Galzy, *Anal. Biochem.*, **95**, 436 (1979).