Hydrolysis of L-a-Amino-ε-caprolactam by Yeasts

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Cells of Cryptococcus laurentii, which had been isolated from soil as microorganisms utilizing and hydrolyzing L-aminolactam,** catalyzed the asymmetrical and quantitative hydrolysis reaction of DL-aminolactam, used as a 10% aqueous solution at pH 9.0, producing L-lysine. A cell-free extract of the yeast cells was fractionated by ammonium sulfate and the active fraction was also used for the hydrolysis of L-aminolactam under various conditions.

Yeasts which are able to hydrolyze L-aminolactam have been obtained by screening both organisms from soil and authentic strains from the Institute for Fermentation, Osaka (IFO).1,2) The L-aminolactam-hydrolyzing yeasts so screened belong to three species, namely, Cryptococcus laurentii, Candida humicola and Trichosporon cutaneum.1,2) Of the three species, C. laurentii was the most suitable for the L-aminolactam-hydrolyzing catalyst.1,2)

This paper describes hydrolysis of L- and DL-aminolactam by intact and dried cells of two strains of C. laurentii and by a crude enzyme preparation from an extract of the cells.

MATERIALS AND METHODS

Chemicals. DL-, L- and D-Aminolactam were prepared by the methods described previously.1)

Determination of aminolactam and lysine. These compounds in the reaction mixture were determined by paper chromatography as described previously1) and by use of an amino acid analyzer (Nihondenshi Co.).

Enzymatic Conversion of DL-a-Amino-ε-caprolactam into L-Lysine. Part II. (Part I, see Reference 1).

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** a-Amino-ε-caprolactam is abbreviated to aminolactam.

Cultured medium.1,2) To the basal medium composed of 0.1% of KH₂PO₄, 0.05% of MgSO₄·7H₂O, 0.002% of MnCl₂·5H₂O, 0.05% of yeast extract and tap water were added glucose and aminolactam·HCl as indicated in each experiment, and the pH was adjusted to 7.0 with dil. KOH. For the agar culture, 2% Bacto agar was added. Sterilization was done at 120°C for 15 min.

Microorganisms. Two strains identified as C. laurentii, TORAY 2001 and 2100,1,2) were used.

Acetone-dried and lyophilized cells. Acetone-dried and lyophilized cells of the yeasts were prepared by the usual methods.

Measurement of the enzyme activity. The reaction mixture, composed of 1 ml of 10% L-aminolactam·HCl (pH 9.0, adjusted by KOH) and 1 ml of suspension of acetone-dried cells (10 mg/ml) or enzyme solution, was incubated at 43°C for 1 hr with gentle shaking. Reaction was stopped by heating at 100°C for 5 min.

Cell-free extract. C. laurentii TORAY 2001 was cultivated in 1 liter of the basal medium containing 0.5% glucose and 2% DL-aminolactam·HCl at 30°C for 20 hr. From the culture broth 180 g of wet cells were obtained by centrifugation. Six grams of the cell paste were dried by acetone, and the specific activity was 6.6 μmol/hr/mg of dried cells. The remaining 174 g of the paste was dispersed in 300 ml of 0.01 M phosphate buffer (pH 7.0) and subjected to destruction by a French press. The suspension was centrifuged and 300 ml of cell-free extract, with activity of 300 μmol/hr/ml, was obtained.

Ammonium sulfate fractionation. The extract prepared above was fractionated by adding ammonium sulfate to increase the percentage saturation by 10% with each addition. The enzyme activity was con-
centrated in the fraction of 30~40% saturation of ammonium sulfate and its activity was 700 μmol/hr/mg protein. Protein was determined by the method of Lowry et al. This fraction was used as crude enzyme preparation in the experiments.

Measurement of optical rotation. The optical rotation of lysine and aminolactam was measured by an automatic polarimeter (Yanagimoto OR-50). Pure L-lysine·HCl was found to be [α]₀⁺ 21.10° (c=5, 6N-HCl) and pure L-aminolactam·HCl to be [α]₀⁻ 27.95° (c=5, H₂O).

Paper chromatography for identification of L-lysine produced. Paper chromatography was performed using two kinds of solvents: (1) N-BuOH, CH₃COOH, H₂O=4:1:2, (2) phenol, H₂O=7:3 and two kinds of location reagents: (1) 0.2% ninhydrin in 75% ethanol, (2) CS₂, N-BuOH=1:1 and 1% AgNO₃/N-HNO₃. Elemental analysis. Elemental analysis of the reaction products was performed by the analysis group in the Basic Research Laboratory, Toray Industries, Inc.

RESULTS

1. L-Aminolactam hydrolysis under various conditions
   a. Effect of pH. Figure 1 shows the pH-activity profiles of hydrolysis of L-

   ![Fig. 1. Effect of pH on Hydrolysis of L-Aminolactam.](image)

   Reaction mixtures were composed of 2 ml of 300 mM L-aminolactam·HCl (pH was adjusted with KOH as indicated in the figure) and 0.1 ml of the cell suspension (8.8 mg of acetone-dried cells) or 0.1 ml of the enzyme solution in total volume of 2.1 ml. The reaction was carried out at 43°C for 45 min.

   ![Fig. 2. Effect of Temperature on Hydrolysis of L-Aminolactam.](image)

   The composition of the reaction mixtures was the same as in Fig. 1, except that the pH was adjusted to 9.0 with KOH. The reaction was carried out at various temperatures as indicated in the figure for 20 min.

   ![Fig. 3. Progress of Hydrolysis of L-Aminolactam by Acetone-dried Cells.](image)

   Reaction mixtures were composed of 5 ml of 10% L-aminolactam·HCl (pH 9.0, adjusted by KOH) and 5 mg of the acetone-dried cells. The reaction was carried out at 40°C and 60°C, respectively.

   ![Fig. 2. Effect of Temperature on Hydrolysis of L-Aminolactam.](image)

   The composition of the reaction mixtures was the same as in Fig. 1, except that the pH was adjusted to 9.0 with KOH. The reaction was carried out at various temperatures as indicated in the figure for 20 min.

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   ![Fig. 1. Effect of pH on Hydrolysis of L-Aminolactam.](image)

   Reaction mixtures were composed of 2 ml of 300 mM L-aminolactam·HCl (pH was adjusted with KOH as indicated in the figure) and 0.1 ml of the cell suspension (8.8 mg of acetone-dried cells) or 0.1 ml of the enzyme solution in total volume of 2.1 ml. The reaction was carried out at 43°C for 45 min.
activity was shown at temperatures of around 70°C when assayed after 20 min incubation.

**c. Progress of hydrolysis of L-aminolactam by acetone-dried cells.** Figure 3 shows the progress curve of the hydrolysis of L-aminolactam, used as 10% solution, by the acetone-dried cells of *C. laurentii* TORAY 2001. The hydrolysis at 60°C continued until 80 hr, whereas the reaction at 40°C was still proceeding linearly over 100 hr.

2. **Complete hydrolysis of L-aminolactam**

   **a.** A typical time course of complete hydrolysis of L-aminolactam by the acetone-dried cells of *C. laurentii* TORAY 2001 is shown in Fig. 4. As shown in the figure, the reaction was completed in 12 hr. L-Lysine formed was isolated by the author's method2) from the reaction mixture as a monohydrochloride in a yield of 98%. Its optical rotation was $[\alpha]_D^{26} +21.0^\circ$ ($c=5$, 6 N HCl), so that the optical purity was 99.5%.

   **b.** Figure 5 shows a typical time course of asymmetrical hydrolysis of DL-aminolactam by the lyophilized cells of *C. laurentii* TORAY 2100. The reaction was completed in 7 hr. L-Lysine and D-aminolactam were separately isolated as crystals of monohydrochlorides from the reaction mixture by the author's method2) in a yield of 97% and 96%, respectively. The optical rotation of L-lysine·HCl isolated was $[\alpha]_D^{25} +20.95^\circ$ ($c=5$, 6 N HCl) and that of D-aminolactam isolated was $[\alpha]_D^{25} +27.67^\circ$ ($c=5$, H$_2$O), so that the optical purity of them was calculated to be 99.3% and 99.0%, respectively.

   **c.** The identification of the reaction products obtained in the above experiments a and b with authentic L-lysine was carried out by the following methods: (1) paper chromatography, (2) amino acid analysis by an automatic analyzer, (3) IR spectra and (4) elemental analysis. The result of the elemental analysis is: Calcd. for C$_6$H$_{15}$N$_2$O$_2$Cl: C, 39.45; H, 8.29; N, 15.34; Cl, 19.40%. Found: Product a, C, 39.37; H, 8.59; N, 15.54; Cl, 19.82; Product b, C, 39.29; H, 8.51; N, 15.51; Cl, 19.73%.

3. **Induction of the enzyme activity in growing cells**

   *C. laurentii* TORAY 2001 was cultured in a bouillon medium with or without 1% L-aminolactam·HCl for 18 hr. The cells were harvested, washed and dried with acetone, and the L-aminolactam-hydrolyzing activity was
Table I. Induction of L-Aminolactam-Hydrolyzing Activity in Growing Cells

Cells were cultured in Bouillon medium with or without L-aminolactam for 18 hr, harvested and dried by acetone. Reaction mixtures were composed of 10% L-aminolactam·HCl (pH 9.0 adjusted by KOH) and 1 ml of suspensions of the dried cells. The reaction was carried out at 50°C for 1 hr.

<table>
<thead>
<tr>
<th>Cells cultured</th>
<th>Specific activity&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without L-aminolactam</td>
<td>3.6</td>
</tr>
<tr>
<td>With L-aminolactam</td>
<td>18.2</td>
</tr>
</tbody>
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<sup>a</sup> μmole of L-lysine produced/hr/mg of cells.

examined. As shown in Table I, the cell preparation obtained from medium without L-aminolactam showed a very low activity, whereas the activity of the cell preparation obtained from the medium with L-aminolactam was five times as high as that of the former cells. This fact indicates that the L-aminolactam-hydrolyzing enzyme is formed adaptively on L-aminolactam in the growing cells.

**DISCUSSION**

The present paper seems to be the first report on the asymmetrical and quantitative hydrolysis of aminolactam, though a patent of Seto<sup>5</sup> describing the formation of lysine from aminolactam using *Aspergillus ustus* has been made.

The stereospecificity of the hydrolysis of aminolactam by *C. laurentii* was absolutely strict. The conversion rate of L-aminolactam into L-lysine by the yeast was almost 100%.

Racemization of neither aminolactam nor lysine took place. This may be due to the absence of enzymes responsible for these racemizations in the yeasts. Furthermore, all the L-lysine produced was accumulated in the reaction mixture, so long as the initial concentration of aminolactam was higher than about 3%. Accordingly, it is supposed that metabolic degradation of L-lysine by the yeasts under such conditions can not occur.

These results suggest the possibility of industrial utilization of the yeast cells as a catalyst for the selective hydrolysis of L-aminolactam to produce L-lysine. Thus, the author’s speculation on the enzymatic process for L-lysine production described in the previous paper<sup>1</sup> has been partially realized.

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**REFERENCES**