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202. Katsuhiko Matsui, Michihiko Kumagai, and Yukio Kameda*1 : Studies on Acylase Activity and Microorganisms. XXIII.*2 Purification of δ-Ornithine Acylase (5-N-Acylornithine Amidohydrolase).*3

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Since KT 801 (*Pseudomonas* sp.) isolated from soil showed mardedly high δ-ornithine acylase (5-N-acylornithine amidohydrolase) activity, purification of the enzyme have been attempted. In the first place, cultural conditions for the production of this enzyme were investigated. As a result, it was confirmed that a medium containing 0.1% 5-N-benzoyl-L-ornithine, 1% peptone and some inorganic salts is most suitable for the enzyme production and that more δ-ornithine acylase is produced by shaken culture or submerged culture in jar fermentor than by stationary culture.

Then, δ-ornithine acylase was extracted by means of freezing and thawing method from KT 801 cells. By this method, 60~70% of the enzyme activity estimated in cell suspension was extracted, although only 5~8% of cellular proteins was extracted. Subsequently, δ-ornithine acylase was purified by fractionation with ammonium sulfate and chromatography on DEAE cellulose and DEAE Sephadex A-50. The specific activity of the purified enzyme preparation was 41,280 μ*M*/hr./mg. and it was represented abut 300 fold purification over the original cell suspention. This enzyme preparation gave single peak in Tiselius electrophoresis or analytical ultracentrifuge.

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In a previous paper,[1] the authors reported on the distribution of δ-ornithine acylase activity, that is hydrolytic activity toward 5-N-acylornithine in microorganisms and that KT 801 (*Pseudomonas* sp.) has the highest activity among the tested organisms. The purpose of this report is to describe in detail the purification of this new enzyme.

It is well known that an amount of enzyme activity in microorganisms is greatly affected by cultural condition and constituents of medium in many cases. Then, various cultural conditions for the production of δ-ornithine acylase in KT 801 have been investigated and KT 801 has been grown in a large scale under the best condition. Subsequently, δ-ornithine acylase has been extracted in cell free state from the cells by means of freezing and thawing method and purified by ammonium sulfate fractionation and chromatography on DEAE cellulose etc. The purified δ-ornithine acylase gave a single peak in Tiselius electrophoresis or analytical ultracentrifuge.

Materials and Methods

Compounds used

The preparations of 5-N-benzoyl-L-ornithine and 6-N-benzoyl-L-lysine were the same as previously described.[1] Benzoylglycine,[2] 3-benzamidopropionic acid,[3] and 6-benzamidocaproic acid[4] were prepared by established methods.

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*2 Part XXII. Y. Kameda, K. Matsui : This Bulletin, **15**, 1573 (1967).

*3 This work was presented at the Meeting of the Kanto Division of the Pharmaceutical Society of Japan, Tokyo. December 24, 1966. This work is a part of the Ph. D. thesis of K. Matsui, University of Tokyo, March 1967.

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4) J.C. Eck, C.S. Marvel : Org. Synth., **19**, 21 (1939).

Cultivations

Unless otherwise noted cultivation was carried out by the following manner. To 500 ml. shaking flasks, 100 ml. of respective media (pH 7.2) were distributed, sterilized in an autoclave under 1.0 kg./cm^2 pressure for 20 min., and in oculated with 1 ml. of seed culture of KT 801, which was prepared by stationary culture of the organism at 27° for 2 days in 5-N-benzoyl-L-ornithine and inorganic salts medium. The cultivation was conducted at 27° by reciprocal shaking (108 c/min., stroke 7 cm.) for an appropriate period.

In stationary culture, the organism was incubated at 27° using the same flasks used for shaken culture.

In the case of jar fermentor, 6 L. of a medium in 15 L. jar was inoculated with 200 ml. of seed culture prepared by the above shaken culture for 1 day. Cultivation in jar was carried out at 27° with aeration (5 or 10 L./min./6 L. medium) and agitation (a small amount of octanol was used for antifoaming agent).

Enzyme Solution

Enzyme solutions used in the studies for cultural conditions were obtained as follows. Five ml. of culture broth including cells were taken out under as homogeneous as possible suspended condition after 1, 2, and 3 days, and were separated to cells and supernatants by centrifugation (3,000 r.p.m., 15 min.). The cells were suspended in 1~5 ml. of H_2O, and were used as enzyme solution I (in cell). On the other hand, supernatant solutions obtained by the centrifugation were used as enzyme solution II (in broth).

Enzyme Source for the Purification of δ-Ornithine Acylase

One L. of the medium containing 0.1% of 5-N-benzoyl-L-ornithine, 1% of peptone, 0.1% of K_2HPO_4, 0.05% of $MgSO_4 \cdot 7H_2O$, 1 ml./L. of 1% $CaCl_2$, and 0.5 ml./L. of 1% $FeCl_3$ (pH 7.2) were distributed in 3 L. shaking flasks, sterilized in an autoclave under 1.0 kg./cm^2 pressure for 20 min., and inoculated with 50 ml. of seed culture of KT 801, which was prepared by shaken culture at 27° for 1 day in the same medium. The cultivation was conducted at 27° by reciprocal shaking (108 c/min., stroke 7 cm.) for 40 hr. The cells were harvested by centrifugation (15,000 r.p.m., 15 min.) and used as enzyme source.

Standard Enzyme Reaction

1) Standard enzyme reaction used in the studies for cultural conditions was carried out as follows. A mixture of 1 ml. of 0.01*M* 5-N-benzoyl-L-ornithine, 0.5 ml. of 0.1*M* phosphate buffer (pH 7.0), and 0.5 ml. of enzyme solution was incubated at 37° and after 10 and 20 min., the liberated ornithine was measured by Chinard's acidic colorimetric ninhydrin method[5] with a minor modification.[1] That is, 0.4 ml. of the incubated solution was addded to 1.6 ml. of AcOH and 1.0 ml. of a 2% solution of ninhydrin in AcOH, and the mixture was immersed in boiling water for 30 min. The solution was diluted with 7.0 ml. of 50% EtOH, and the absorption intensity after 5 fold dilution with 50% EtOH was measured at 515 mμ, giving a measure of the free ornithine produced by the enzymatic hydrolysis. The enzyme activity is expressed in terms of micromoles of liberated ornithine per hr. per cells or supernatant broth in a unit ml. of media. Growth was measured by the optical density at 660 mμ.

2) Standard enzyme reaction used in the studies for purification of δ-ornithine acylase was carried out as follows. The reaction mixture, containing 1 ml. of 0.01*M* 5-N-benzoyl-L-ornithine and 0.5 ml. of 0.1*M* Tris buffer (pH 8.2) was preincubated for 5 min. at 37°, after which 0.5 ml. of appropriately diluted enzyme solution was added and incubated at 37°. After 5 and 10 min., the liberated ornithine was measured by above mentioned acidic colorimetric ninhydrin method. The enzyme activity is expressed in terms of micromoles of liberated ornithine per hr. per mg. of protein under the above conditions. Protein content of the enzyme solution was determined by the method of Lowry, *et al.*[6] with crystalline bovine serum albumin as a standard.

Extraction of δ-Ornithine Acylase from Cells

1) Alumina Grinding——Eight g. of wet cells obtained from 1 L. of the above culture fluid were ground with 12 g. of alumina (Wako Pure Chemical Industries, Ltd., W-800) and extracted with 80 ml. of 0.01*M* Tris buffer (pH 8.2). The supernatant solution obtained by centrifugation (15,000 r.p.m., 15 min.) was used as an enzyme extract. The extraction of the precipitate was repeated once more with 30 ml. of 0.01*M* Tris buffer and the supernatant by centrifugation was added to above extract.

2) French Press——To 8 g. of wet cells, 30 ml. of 0.01*M* Tris buffer was added and the cell suspension was passed through a French pressure cell (400 kg./cm^2, Ohtake Company, Ltd.) for disruption of cells. The cell debris was removed by centrifugation (15,000 r.p.m., 15 min.) and the supernatant solution was used as enzyme extract.

3) Autolysis with Toluene——To 8 g. of wet cells, 100 ml. of 0.01*M* Tris buffer was added. After homogenization, 10 ml. of toluene was added and the mixture was allowed to stand at 27° for 24 hr. The supernatant solution obtained by centrifugation (15,000 r.p.m., 15 min.) was used as enzyme extract.

4) Freezing and Thawing——To 8 g. of wet cells, 80 ml. of 0.01*M* Tris buffer was added and homogenized. The cell suspension was freezed in deep freezer and next day, it was thawed in incubator at 37°. The supernatant solution obtained by centrifugation was used as enzyme extract.

5) F.P. Chinard: J. Biol. Chem., **199**, 91 (1952).
6) O.H. Lowry, N.J. Rosenbrough, A.L. Forr, R.T. Randall: *Ibid.*, **193**, 265 (1951).

Results and Discussion

Effect of Substrate

Since it has been a common experience that an amonnt of enzyme activity in microorganisms is induced by the presence of a specific substance, usually a substrate, in the environment, the authors investigated whether 5-N-benzoyl-L-ornithine affect the production of δ-ornithine acylase in KT 801 (*Pseudomonas* sp.). As shown in Table I, δ-ornithine acylase was produced in either presence or absence of 5-N-benzoyl-L-ornithine in bouillon, but about 3 times more enzyme was produced in the case of presence.

Table I. Effect of 5-N-Benzoyl-L-ornithine on the Production of δ-Ornithine Acylase

Media	5-N-Bz-L-Orn. (m*M*)	Enzyme Activity μ*M*/hr./ml.		
		in Cell	in Broth	Total
Bouillon	0	27.8	1.2	29.0
Bouillon	10	63.4	45.6	109.0

Enzyme activity is expressed in terms of μ*M* of liberated ornithine per hr. per ml. of medium.
Digest consisting 1 ml. of 0.01*M* 5-N-benzoyl-L-ornithine, 0.5 ml. of 0.1*M* phosphate buffer (pH 7.0), and 0.5 ml. of enzyme solution is incubated at 37°. Enzyme solution was prepared from the 2 days shaken culture. Activity is measured by the procedure described in "Method."

Effect of Carbon Sources

The basal medium employed in this experiments contained 0.1% NH_4Cl, 0.1% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 2 drops/100 ml. of 1% $CaCl_2$, and 1 drop/100 ml. of 1% $FeCl_3$ (pH 7.2). To the basal medium, respective carbon sources shown in Table II were added at 0.2% concentration. The shaken culture was carried out at 27° in 500 ml. shaking flasks, which contain 100 ml. of respective media. Appropriate aliquots of the culture fluid were taken out at the indicated times and centrifuged at 3000 r.p.m. for 15 min. The cell pellets were suspended in 0.1*M* phosphate buffer (pH 7.0) and used as the enzyme source. Enzyme assay was carried out under standard conditions with 5-N-benzoyl-L-ornithine as the substrate. As shown in Table II, it is obvious that 5-N-benzoyl-L-ornithine is the best carbon source for the enzyme production. In the case of ornithine, the enzyme production decreased to about one third of that with 5-N-benzoyl-L-ornithine, but the growth of organism indicated by optical density was also poor. The media containing other carbon sources result in much lesser enzyme production. Particularly in the case of acetate, lactate, and aspartate, the enzyme activity was hardly detectable.

Effect of Nitrogen Sources

To the basal medium containing 0.2% 5-N-benzoyl-L-ornithine, 0.1% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 2 drops/100 ml. of 1% $CaCl_2$, and 1 drop/100 ml. of 1% $FeCl_3$ (pH 7.2), the nitrogen sources were added at 0.1 or 0.2% concentration. The condition of cultivation and enzyme assay are the same as described in above. The results are shown in Table III. Of the tested sources, peptone was most excellent and NH_4Cl followed.

Effect of Concentration of 5-N-Benzoyl-L-ornithine and Peptone

As mentioned above, 5-N-benzoyl-L-ornithine and peptone were found to be advantageous as carbon and nitrogen sources for the production of δ-ornithine acylase. Therefore, further experiments were carried out at the varied concentration of 5-N-benzoyl-L-ornithine (0.05~0.4%) and peptone (0.2~2%) using the previous basal medium. The condi-

Table II. Effect of Carbon Sources on the Production of δ-Ornithine Acylase

Carbon Sources	Incubation (days)	Growth 660 mμ O. D.	Enzyme Activity μM/hr./ml. in Cell	in Broth	Total
5-N-Bz-L-Orn.	1	0.64	26.0	3.0	29.0
	2	0.84	25.0	6.0	31.0
	3	0.59	16.0	14.0	30.0
6-N-Bz-L-Lys.	1	0.49	4.0	4.0	8.0
	2	0.56	6.0	4.0	10.0
	3	0.39	6.0	5.0	11.0
Bz-Gly.	1	0.25	2.1	0.6	2.7
	2	0.72	4.2	1.1	5.3
	3	0.95	3.3	3.2	6.5
Bz-β-Ala.	1	0.17	1.2	0.4	1.6
	2	0.68	3.6	1.1	4.7
	3	0.90	6.0	4.2	10.2
6-Benzamido caproic acid	1	0.19	1.2	0.8	2.0
	2	0.62	3.0	1.0	4.0
	3	0.56	4.0	2.4	6.4
Bz-OH	1	0.0	0	0	0
	2	0.17	2.0	1.0	3.0
	3	0.25	3.0	2.0	5.0
L-Orn.	1	0.34	9.7	3.0	12.7
	2	0.46	9.8	5.0	14.8
L-Glu.	1	0.61	2.0	0.2	2.2
	2	0.81	2.3	0.2	2.5
L-Asp.	1	0.62	0.3	0	0.3
	2	0.80	0.4	0	0.4
Acetate	1	0.60	0.1	0	0.1
	2	0.87	0.1	0	0.1
Succinate	1	0.60	1.2	0	1.2
	2	0.83	1.2	0	1.2
Lactate	1	0.56	0.1	0	0.1
	2	0.83	0.1	0	0.1
Bouillon	1	1.2	23.0	2.0	25.0
	2	1.5	24.0	5.0	29.0
	3	1.5	17.2	10.2	27.4

The conditions and assay method are the same as those in Table I.

Table III. Effect of Nitrogen Sources on the Production of δ-Ornithine Acylase

Nitrogen Sources	Incubation (days)	Growth 660 mμ O. D.	Enzyme Activity μM/hr./ml. in Cell	in Broth	Total
NH_4Cl	1	0.64	26.0	3.0	29.0
	2	0.84	25.0	6.0	31.0
	3	0.59	16.0	14.0	30.0
Urea	1	0.60	20.0	1.1	21.1
	2	0.65	19.0	1.6	20.6
	3	0.77	15.0	3.2	18.2
Peptone	1	0.70	33.0	7.2	40.2
	2	0.95	30.0	11.6	41.6
	3	0.90	20.0	21.9	41.9

The conditions and assay method are the same as those in Table I. Enzyme activity is expressed in terms of μM of liberated ornithine per hr. per ml. of medium.

tions of cultivation and enzyme assay are the same as described above. As shown in Table Ⅳ, the enzyme production was inferior at the peptone concentrations below 0.5% to those with above 1% concentrations. The enzyme production was more affected by the concentration of 5-N-benzoyl-L-ornithine. At the 5-N-benzoyl-L-ornithine concentrations below 0.05% or above 0.2%, the enzyme production was inferior to that with 0.1% concentration. Particularly, the enzyme production was significantly inhibited in the case of 0.4%. Thus, the medium containing 0.1% 5-N-benzoyl-L-ornithine, 1% peptone, 0.1% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 2 drops/100 ml. of 1% $CaCl_2$, and 1 drop/100 ml. of 1% $FeCl_3$ (pH 7.2) was selected as the best medium for the production of δ-ornithine acylase.

Table Ⅳ. Effect of Concentration of 5-N-Benzoyl-L-ornithine and Peptone on the Production of δ-Ornithine Acylase

5-N-Bz-L-Orn. Concentration (%)	Peptone Concentration (%)	Incubation (days)	Growth 660 mμ O.D.	Enzyme Activity μ*M*/hr./ml. in Cell	in Broth	Total
0.2	0.2	1	0.70	33.0	7.2	40.2
0.2	0.2	2	0.95	30.0	11.6	41.6
0.2	0.5	1	1.0	48.0	17.0	65.0
0.2	0.5	2	1.2	40.0	41.0	81.0
0.2	1.0	1	1.3	56.0	17.0	73.0
0.2	1.0	2	1.3	43.0	46.0	89.0
0.4	0.5	1	1.2	13.5	4.8	18.3
0.4	0.5	2	1.2	10.0	7.6	17.6
0.4	1.0	1	1.3	27.6	6.4	34.0
0.4	1.0	2	1.3	18.0	16.2	34.2
0.05	1.0	1	1.0	41.0	7.0	48.0
0.1	1.0	1	1.2	80.0	14.0	94.0
0.2	2.0	1	1.5	94.0	25.0	119.0

The conditions and assay method are the same as those in Table I. Enzyme activity is expressed in terms of μ*M* of liberated ornithine per hr. per ml. of medium.

Effect of Cultural Methods for the Production of δ-Ornithine Acylase

Effect of cultural methods such as stationary culture, shaken culture, and jar fermentor culture employing above mentioned the best medium were examined as the preliminary test for the large scale production of the enzyme. As shown in Table Ⅴ, growth was less and enzyme production was markedly inferior in stationary culture. In shaken culture, an amount of medium contained in 500 ml. shaking flasks affected to

Table Ⅴ. Effect of Cultural Methods for the Production of δ-Ornithine Acylase

Cultural Method	Incubation (days)	Growth 660 mμ O.D.	Enzyme Activity μ*M*/hr./L. in Cell	in Broth	Total
Stationary Culture	1	0.83	24600	10800	35400
Stationary Culture	2	0.92	43400	14800	58200
Shaken Culture (100 ml./500 ml.)	1	1.0	72700	13800	86500
Shaken Culture (200 ml./500 ml.)	1	1.1	69500	13800	83300
Shaken Culture (300 ml./500 ml.)	1	0.82	29000	5000	34000
Jar fermentor (10 L./min./6 L.)	1	0.95	71000	13000	84000
Jar fermentor (5 L./min./6 L.)	1	0.75	33000	6500	39500

In shaken culture, an amount of medium contained in 500 ml. shaking flasks were varied 100 ml. to 300 ml. In the experiments in jar fermentor, oxygen supply was varied by air flow (5 or 10 L./min./6 L. medium). The conditions and assay method are the same as those in Table I. Enzyme activity is expressed in terms of μ*M* of liberated ornithine per hr. per L. of medium.

the enzyme production. Particulary, the enzyme production was markedly inferior in the case of 300 ml. of medium contains in 500 ml. shaking flasks. In the experiments in jar fermentor, oxygen supply was varied by air flow (5 or 10 L./min./6 L. medium). As shown in Table V, the enzyme production was markedly inferior in the case of 5 L./min. air flow. The enzyme activity produced by jar fermentor culture (10 L./min./6 L. medium air flow) was nearly equal to that in shaken culture (100 ml. or 200 ml. of medium contain in 500 ml. shaking flasks). That is, the shaken culture or the jar fermentor culture was selected as the best cultural method for the production of δ-ornithine acylase.

Extraction of δ-Ornithine Acylase from KT 801 Cells

In order to find out the most effective extraction method for the δ-ornithine acylase, alumina grinding, French press, autolysis with toluene, and freezing and thawing procedures were tested. Enzyme activities and protein contents of cell free extract by these procedures were assayed and the results were shown in Table VI. As indicated in the Table, alumina grinding, French press, and freezing and thawing were found to be effective extraction procedures. Especially, in the freezing and thawing method, protein content of the extract was markedly poor. That is, 60~70% of the enzyme activity estimated in cell suspension was extracted by freezing and thawing, but only 5~8% of the cellular proteins was extracted by it. This method is easy to operate and most suitable for the subsequent purification procedures.

Table VI. Extraction of δ-Ornithine Acylase from KT 801 Cells

Procedure	Volume (ml.)	Protein (mg./ml.)	Total Protein (mg.)	Activity (μ*M*/hr./ml.)	Specific Activity (μ*M*/hr./mg.)	Total Activity (μ*M*/hr.)
Alumina Grinding	97	2.4	233	594	248	57600
French Press	25	17	425	2940	173	73800
Autolysis	128	0.375	48	294	785	37600
Freezing and Thawing	106	0.44	46.6	618	1400	65500

Purification of δ-Ornithine Acylase

Preparation of Cell Free Extract

45 g. of wet cells (obtained from 6 L. of culture medium) were suspended in 450 ml. of 0.01*M* Tris buffer (pH 8.2) and freezed in deepfreezer for over nitht. It was then thawed in incubator at 37° and centrifuged at 15000 r.p.m. for 15 min. The supernatant was made up to a final volume of 500 ml. with the buffer.

Ammonium Sulfate Fractionation

152 g. (0.4 saturation) of solid ammonium sulfate was added to 500 ml. of the crude extract with stirring. The solution was kept at pH 7.5~8.5 by occasional addition of 5% ammonium hydroxide. The resulting precipitate was removed by centrifugation (15000 r.p.m., 15 min.). 131 (0.8 saturation) of solid ammonium sulfate was added to the supernatant under the conditions similar to those described above. The solution was allowed to stand for 1 hr. in refrigerator and the resulting precipitate was collected by centrifugation (15000 r.p.m., 15 min.). The precipitate was dissolved in a small quantity of 0.01*M* Tris buffer (pH 8.2), and dialyzed against 2 L. of the same buffer in a refrigerator.

Chromatography on DEAE Cellulose

DEAE cellulose was washed succesively with appropriate volume of 0.5*N* HCl, H_2O, 0.5*N* NaOH, and H_2O until the washings became at nearly neutral pH. A column of

DEAE cellulose (2×40 cm.) was prepared and equilibrated by washing with 2 L. of 0.01*M* Tris buffer (pH 8.2). 59 ml. of the enzyme solution from the previous step was layered on the column, and adsorbent was washed with 200 ml. of 0.01*M* Tris buffer (pH 8.2). A linear gradient elution of sodium chloride was then applied with 500 ml. of above buffer in the mixing vessel and 500 ml. of 0.3*M* sodium chloride in above buffer in the second container. The fractions were collected in 20 g. portions at a flow rate of 35 ml./hr. δ-Ornithine acylase activity in the fractions 16 to 19 were 70% of the added activity. These fractions were combined, concentrated by lyophilization, and dialyzed for 24 hr. against 100 volumes of 0.02*M* Tris buffer (pH 8.2) (the buffer was changed twice).

Chromatography on DEAE Sephadex A-50

2 g. of DEAE Sephadex A-50 was allowed to swell in an excess of H_2O, the fine particles were removed by decantation, and the DEAE Sephadex was washed successively with appropriate volume of 0.5*N* HCl, H_2O, 0.5*N* NaOH, and H_2O until the washings became at nearly neutral pH. Prewashed DEAE Sephadex A-50 was packed in a column (1×20 cm.) and equilibrated by washing with 0.02*M* Tris buffer (pH 8.2). 22 ml. of the enzyme solution from the previous step was passed into the column and washed in with 150 ml. of 0.02*M* Tris buffer. After washing, a linear gradient elution was applied with 200 ml. of 0.02*M* Tris buffer in the mixing vessel and 200 ml. of 0.2*M* sodium chloride in 0.02*M* Tris buffer in the second container. The fractions were collected in 10 g. portions at a flow rate of 15 ml./hr. The elution pattern was shown in Fig. 1. δ-Ornithine acylase activity in the fractions 19 to 22 were 72% of the added aitivity. These fractions were combined and used in all experiments for the examination of enzymatic properties. The results of the enzyme purification are summarized in Table Ⅶ. The specific activity of this enzyme solution was 41280 μ*M*/hr./mg. and represented about 27.5 fold purification over the

Table Ⅶ. Purification of δ-Ornithine Acylase

Fraction	Volume (ml.)	Protein (mg./ml.)	Total Protein (mg.)	Activity (μ*M*/hr./ml.)	Specific Activity (μ*M*/hr./mg.)	Total Activity (μ*M*/hr.)	Recovery (%)	Purification
Cell Free Extract	500	0.52	260	780	1500	390000	100	1
$(NH_4)_2SO_4$ Fraction	63	0.90	55.7	5130	5700	318000	81.5	3.8
DEAE Cellulose Fraction	84	0.071	5.96	2610	36980	219600	56.4	24.5
DEAE Sephadex Fraction	44.3	0.080	3.55	3300	41280	146400	37.6	27.5

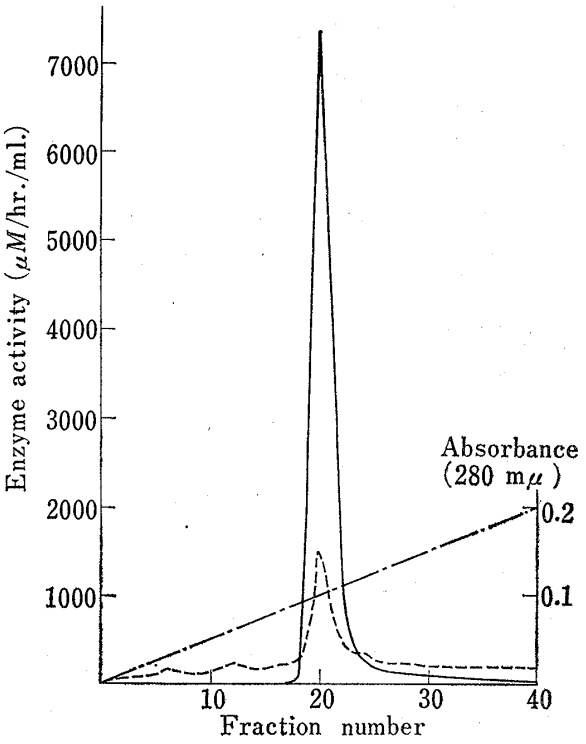

Fig. 1. Chromatography on DEAE Sephadex A-50

(————) indicates enzyme activity.
(- - - - - - -) indicates protein concentration measured as absorbance at 280 mμ.
(— — — —) indicates ionic strength gradient.
Assays of the enzyme were done according to the standard method described in the text.

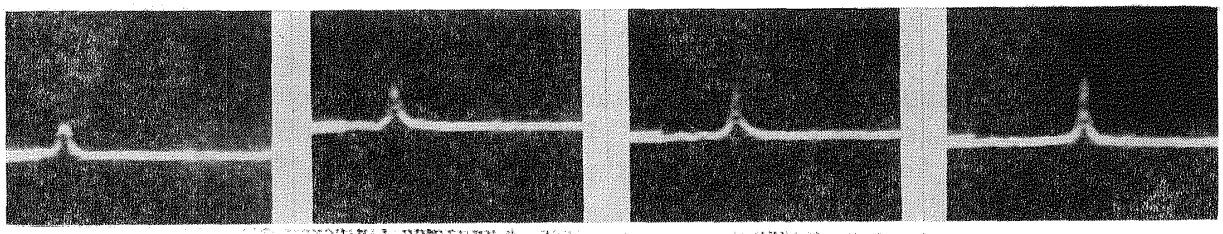

Fig. 2. Electrophoretic Pattern of Purified δ-Ornithine Acylase

Phosphate buffer, pH 7.8, ionic strength 0.2, 60V, 9 mA.

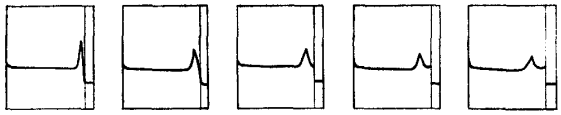

Fig. 3. Ultracentrifugal Pattern of Purified δ-Ornithine Acylase

Tris buffer, 0.01*M*, pH 8.2, ionic strength 0.15, temp. 18.4°. Photographs are taken at intervals of 8 min. at a rotor speed of 996 r.p.s. from left to right.

original crude extract. This enzyme preparation gave a single peak in Tiselius electrophoresis or analytical ultracentrifuge (Fig. 2 and 3). As shown in Table Ⅶ, δ-ornithine acylase was only 27.5 fold purified by these purification steps. But, it was purified about 10 fold in the step of extraction by freezing and thawing. Then, it was purified about 300 fold from the state of cell suspension.

Homogeneity of Purified Enzyme Preparation

Tiselius Electrophoresis

Purified enzyme preparation was concentrated by lyophilization. The powder obtained was dissolved in phosphate buffer (ionic strength 0.2, pH 7.8) and dialyzed against same buffer for 24 hr. Tiselius electrophoresis of the purified preparation was carried out in the phosphate buffer at 60 V, 9 mA for 2.5 hr. Photographs (Fig. 2) were taken at interval of 30 min. A single peak is observed in Fig. 2.

Analytical Ultracentrifuge

Purified enzyme preparation was concentrated by lyophilization. The powder obtained was dissolved in 0.01*M* Tris buffer (pH 8.2) which contains 0.15*M* sodium chloride and dialyzed against same buffer for 24 hr. The concentration of the protein was 3 mg./ml. This enzyme preparation gave a single peek (Fig. 3) in the analytical ultracentrifuge at 19.4°. Photographs were taken at intervals of 8 min. at a rotor speed of 996 r.p.s. from left to right.

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