Chem. Pharm. Bull. 16(6)1023—1029(1968)

UDC 577.155.3.072 : 576.851.1.093

Studies on Acylase Activity and Micro-organisms. XXV.[1] Purification and Properties of Benzoylamino-acid Amidohydrolase in KT 801 (*Pseudomonas* sp.)

YUKIO KAMEDA, TAIJYU KURAMOTO, KATSUHIKO MATSUI, and TAKAKO EBARA

Faculty of Pharmaceutical Sciences, Kanazawa University[2]

(Received August 15, 1967)

In order to study the enzymatic properties of α-amino acylase of KT 801 (*Pseudomonas* sp.), which has δ-ornithine acylase, purification of the α-amino acylase have been attempted. α-Amino acylase of KT 801 was extracted by means of spheroplast formation and purified by fractionation with ammonium sulfate, acrinol treatment, and CM Sephadex chromatography. The specific activity of the purified enzyme toward N-benzoyl-L-alanine was 2500 μM/hr/mg and it was represented about 70 fold purification over the original cell suspension. This enzyme has a pH optimum around 8.0 toward benzoyl-L-alanine. It has an optical specificity and hydrolyzes benzoyl-L-amino acids. Also, it hydrolyzes α-N-benzoyl-L-amino acids but not phenylacetyl amino acids. The ratio of the activities toward α-N-benzoyl amino acids remained constant all through the purification steps except for 2-N-benzoyl-L-ornithine. These results suggest that one enzyme hydrolyze these α-N-benzoyl amino acids. Then, the α-amino acylase of KT 801 was provisionally named "benzoylamino acid amidohydrolase."

In previous papers,[3-5] the authors reported that KT 801 (*Pseudomonas* sp.), which was isolated from soil, has extremely high δ-ornithine acylase activity, which can hydrolyze 5-N-acyl-ornithine, and purified δ-ornithine acylase preparation has not α-amino acylase activity, that is, hydrolytic activity toward α-acylamino acids. It is well known that α-amino acylases are widely distributed on plants, animals, and micro-organisms.[6,7] Then, the authors investigated the occurrence of α-amino acylase activity in KT 801.

KT 801 was grown in bouillon medium and the cell was harvested by centrifugation. α-Amino acylase activity was measured by Moore and Stein's ninhydrin method[8] after incubation of α-benzoylamino acids with the cell suspension. It was observed that KT 801 has α-amino acylase activity besides δ-ornithine acylase activity. In a previous paper,[4] it was reported that an amount of δ-ornithine acylase in this micro-organisms is greatly affected by constituents of medium. Then, various culture media for the production of α-amino acylase in KT 801 have been investigated and KT 801 has been grown in the best medium. Since it was observed that α-amino acylase is released when EDTA-lysozyme spheroplasts are made, α-amino acylase has been extracted in cell free state from cells by this method. Then, it was purified by ammonium sulfate fractionation, acrinol treatment, and CM-Sephadex chromatography. Subsequently, the authors investigated some properties such as pH optima, stability, and substrate specificity of this enzyme.

---

1) Part XXIV: K. Matsui, *Chem. Pharm. Bull.* (Tokyo), **15**, 1586 (1967).
2) Location: *Takara-machi, Kanazawa.*
3) Y. Kameda and K. Matsui, *Chem. Pharm. Bull.* (Tokyo), **15**, 1573 (1967).
4) K. Matsui, M. Kumagai, and Y. Kameda, *Chem. Pharm. Bull.* (Tokyo), **15**, 1578 (1967).
5) K. Matsui, *Chem. Pharm. Bull.* (Tokyo), **15**, 1586 (1967).
6) J.P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," **2**, John Wiley & Sons, Inc., 1961, p. 1778.
7) I. Chibata and T. Tosa, *Bull. Agr. Chem. Soc. Japan*, **23**, 370 (1959).
8) S. Moore and W.H. Stein, *J. Biol. Chem.*, **176**, 367 (1948).

NII-Electronic Library Service

Materials and Methods

Preparation of Substrate——Substrates used for the present work were prepared by established methods, characterized by their melting points and other properties, and all of which were in good agreement with those of literature.

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 inoculated with 1 ml of seed culture of KT 801, which was prepared by stationary culture of the organism at 27° for 2 days in the same medium. The cultivation was conducted at 27° by reciprocal shaking (108 c/min, stroke 7 cm) for an appropriate period.

Enzyme Solution——Enzyme solutions used in the studies for culture medium were obtained as follows. 100 ml of culture medium after 1 or 2 days were centrifuged (3000 rpm, 15 min) and the precipitate was washed once with H_2O. The obtained cells were suspended in 50—200 ml of H_2O and were used as enzyme solution.

Enzyme Reaction——(1) Standard Enzyme Reaction: The reaction mixture, containing 1 ml of 0.01 M N-benzoyl-L-alanine and 0.5 ml of 0.1 M Tris buffer (pH 8.0) was preincubated for 5 min at 37°, after which 0.5 ml of appropriately diluted enzyme solution was added and incubated at 37°. The liberated amino acid was measured by Moore and Stein's colorimetric ninhydrin method.[8] After 0, 10, and 20 min, 0.2 ml of incubated mixture were added to a series of test tubes which was preheated in boiling water and held at this temperature for 2 min. To each of the test tubes, 1 ml of ninhydrin reagent[8] (Dissolve 0.08 g of $SnCl_2 \cdot 2H_2O$ in 50 ml of 0.1 M citrate buffer, pH 5.0. Add this solution to 2 g of ninhydrin dissolved in 50 ml of methyl cellosolve) was added and the mixture was immersed in boiling water for 20 min. The solution was diluted with 5 ml of 60% EtOH, and the absorption intensity was measured at 570 mμ, giving a measure of the free amino acid produced by the enzymatic hydrolysis. The enzyme activity is expressed in terms of micromoles of liberated alanine per hr per mg of protein under the above condition. Protein content of the enzyme solution was determined by the method of Lowry, *et al.*[9] with crystalline bovine serum albumin as a standard.

(2) Estimation of Hydrolytic Activity toward α-N-Acyl Amino Acid: The method of estimation of hydrolytic activity toward α-N-acyl amino acids was entirely the same with the above described standard enzyme reaction.

(3) Estimation of Hydrolytic Activity toward 2-N-Benzoyl-L-ornithine or 5-N-Benzoyl-L-ornithine: The method of estimation of hydrolytic activity toward 2-N-benzoyl or 5-N-benzoyl-L-ornithine was described in previous paper.[4] The reaction mixture containing 1 ml of 0.01 M 2-N-benzoyl-L-ornithine (or 5-N-benzoyl-L-ornithine) and 0.5 ml of 0.1 M Tris buffer (pH 8.0) was preincubated at 37° for 5 min, after which 0.5 ml of appropriately diluted enzyme solution was added and incubated at 37°. After 0, 10, and 20 min, 0.4 ml of the incubated mixture was added to a solution of 1.6 ml of AcOH and 1 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.

Enzyme Source for the Purification of α-Amino Acylase (Benzoylaminoacid Amidohydrolase)——One liter of the medium containing 0.1% benzoylglycine, 1% peptone, 0.1% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 1 ml/liter of 1% $CaCl_2$, and 0.5 ml/liter of 1% $FeCl_3$ (pH 7.2) were distributed in 3 liter 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 conducted at 27° by reciprocal shaking (108 c/min, stroke 7 cm) for 24 hr. The cells were harvested by centrifugation (15000 rpm, 15 min) and used as enzyme source.

Extraction of α-Amino Acylase from Cells——6 g of wet cells obtained from 1 liter of the above culture fluid were routinely suspended in 450 ml of sucrose-Tris medium (the mixture of 300 ml of 20% sucrose and 150 ml of 0.1 M Tris buffer, pH 8.0) at 25°. The suspension was supplemented with EDTA to a concentration of 0.001 M, followed immediately by sufficient 1% lysozyme to give 10 μg per ml. Timing of the reaction began at this point. The mixture was gently agitated on a reciprocal shaker at 25°, and the extent of spheroplast formation was followed by observing an optical density at 660 mμ after 10 fold dilution of the suspension in H_2O. After 20—30 min, the suspension were centrifuged (8500 rpm, 10 min) and the resulting supernatant was used cell free extract.

Purification of α-Amino Acylase——All purification steps reported in the following paragraphs were carried out at 4°.

Ammonium Sulfate Fractionation——137 g (0.4 saturation) of solid ammonium sulfate was added to 450 ml of the above cell free 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 (10000 rpm, 15 min). 52.5 g (0.6 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

9) O.H. Lowry, N.J. Rosenbrough, A.L. Forr, and R.T. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

NII-Electronic Library Service

precipitate was collected by centrifuation (10000 rpm, 15 min). The precipitate was dissolved in a small quantity of 0.01 M Tris buffer (pH 8.0), and dialyzed against 2 liter of 0.01 M Tris buffer overnight.

Acrinol Treatment——To 100 ml of the above dialyzed solution, 30 ml of 1% acrinol solution was added and the resulting precipitate was removed by centrifugation (15000 rpm, 5 min). To remove excess acrinol, this enzyme solution was passed through a column of Sephadex G–50 (3.4×43 cm) bufferized with 0.01 M Tris buffer at pH 8.0, eluted with 0.01 M Tris buffer and fractions were collected in 20 g portions. Enzymes were eluted in the fraction between tubes 7 and 15.

Chromatography on CM Sephadex C–50——2 g of CM Sephadex C–50 was allowed to swell in an excess of H_2O, the fine particles were removed by decantation, and the CM Sephadex was washed successively with appropriate volume of 0.5 N NaOH, H_2O, 0.5 N HCl, and H_2O until the washings became at nearly neutral pH. Prewashed CM Sephadex C–50 was packed in a column (1×30 cm) and equilibrated by washing with 0.01 M Tris buffer (pH 8.0). 100 ml of the enzyme solution from the previous step was passed into the column and washed in with 50 ml of 0.01 M Tris buffer. After washing, a linear gradient elution was applied with 200 ml of 0.01 M Tris buffer in the mixing vessel and 200 ml of 0.1 M sodium chloride in 0.01 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. The protein peak containing active enzyme usually appeared at fraction 21—25, and these fractions were combined and used in all experiments for the examination of enzymatic properties.

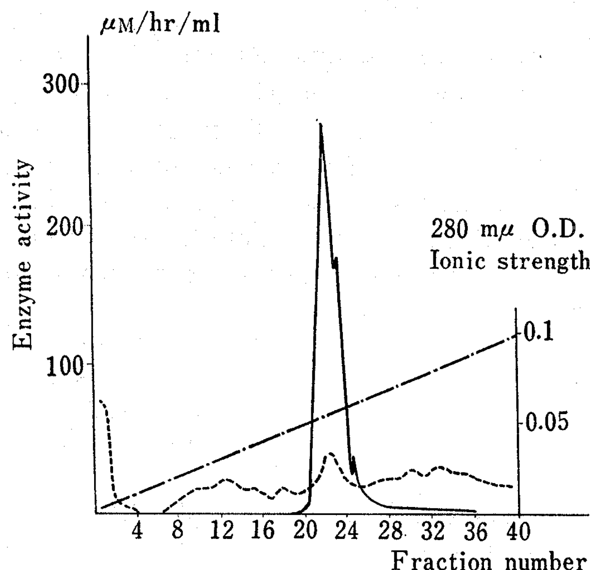

Fig. 1. Chromatography on CM Sephadex C–50

Enzyme activity is indicated by (————). Protein concentration measured at 280 mμ is indicated by (-----------). Ionic strength gradient is indicated by (—·—·—). Assays of the enzyme were done according to the standard method described in "Method."

Results and Discussion

Effect of Culture Medium on the Production of α-Amino Acylase

Since it has been a common experience that an amount of enzyme activity in micro-organisms is induced by the presence of a specific substance, usually a substrate, in the environment, the authors investigated whether benzoyl glycine affect the production of α–amino acylase in KT 801 (*Pseudomonas* sp.). As shown in Table I, α–amino acylase was produced in either presence or absence of benzoyl glycine in bouillon, but 1.5 times more enzyme was produced in the case of presence. Furthermore, about 3 times more enzyme

Table I. Effect of Culture Medium on the Production of α–Amino Acylase

Media	Incubation (days)	Total Protein (mg/100 ml)	Enzyme Activity (μM/hr/100 ml)
Bz–Gly + Inorganic Salts	1	28	330
Bz–Gly + Inorganic Salts	2	24.2	390
Bz–Gly + Peptone + Inorganic Salts	1	72	720
Bz–Gly + Peptone + Inorganic Salts	2	65.5	540
Bz–Gly + Boulilon	1	120	330
Bz–Gly + Bouillon	2	110	235
Bouillon	1	130	230

Benzoyl glycine was added at 0.1% concentration. Inorganic salts consist 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% $FeCl_3$.
Digest consisting 1 ml of 0.01 M N-benzoylglycine, 0.5 ml of 0.1 M Tris buffer (pH 8.0), and 0.5 ml of enzyme solution is incubated at 37°. Activity is measured by the procedure described in "Method." Enzyme activity is expressed in terms of μM of liberated glycine per hr per 100 ml of medium.

NII-Electronic Library Service

production was observed when KT 801 was grown in benzoylglycine–peptone medium. Thus, the medium containing 0.1% benzoylglycine, 1% peptone, 0.1% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 1 ml/liter of 1% $CaCl_2$, and 0.5 ml/liter of 1% $FeCl_3$ (pH 7.2) was selected for the production of α–amino acylase.

Purification of α-Amino Acylase of KT 801

As shown in Table II, α–amino acylase of KT 801 had been extracted in cell free state with a yield of 73%. Then, it was purified by ammonium sulfate fractionation, acrinol treatment and CM Sephadex chromatography. The elution pattern of CM Sephadex chromatography was shown in Fig. 1. α–Amino acylase activiy in the fractions 21 to 25 were 76% of the added acivity. These fractions were combined and used as "purified enzyme" for the following studies. The specific activity of this enzyme solution was 2534 μM/hr/mg and

Table II. Purification of α–Amino Acylase of KT 801

	Volume (ml)	Protein (mg/ml)	Total Protein (mg)	Activity (μM/hr/ml)	Specific Activity (μM/hr/mg)	Total Activity (μM/hr)	Recovery (%)	Purification
Cell Suspension	450	1.24	560	46	37	20900	100	1
Cell Free Extract	450	—	—	33.8	—	15200	73	—
$(NH_4)_2SO_4$ Fraction	67	3.6	241	188	52.3	12596	60.3	1.4
Acrinol Treatment Solution	100	0.23	23	73	317	7300	35	8.6
Purified Enzyme	50	0.044	2.2	111.5	2534	5570	26.6	68.5

Table III. Effect of pH on the Stability of α–Amino Acylase

pH and Buffer	Relative Activity		
	0 hr	24 hr	48 hr
6.0 Phosphate	100	23	0
7.0 Phosphate	100	67	37
8.0 Tris	100	75	57
9.0 Tris	100	66	35
10.0 Carbonate	100	57	19
8.0 Tris + 10^{-3}M Mercaptoethanol	100	83	71

The activity toward N-benzoyl-L-alanine is 2100 μM/hr/mg at 0 hr and this is taken 100.
Mixture of 1 ml of enzyme solution and 1 ml of 0.05 M each buffer are allowed to stand at 4°. After indicated hours, 0.5 ml of the mixture are taken out, diluted with 0.05 M Tris buffer (pH 8.0) appropriately and measured the enzyme activities under standard conditions.

represented about 70 fold purification over the original cell suspension. As shown in Table III, this α–amino acylase is labile, and the purification procedure was carried out as rapidly as possible, in order to mininize the loss of activity.

Effects of pH on Activity and Stability

The effect of pH on the α–amino acylase activity against N–benzoyl–L–alanine was determined over a pH rang of 5.0 to 10.0 with citrate, phosphate, Tris, and carbonate buffers. As shown in Fig. 2, optimal pH is found at the neighborhood of 8.0 and the activity is considerably low below pH 7.0 or above pH 9.0. Then, the effect of pH on the stability of the α–

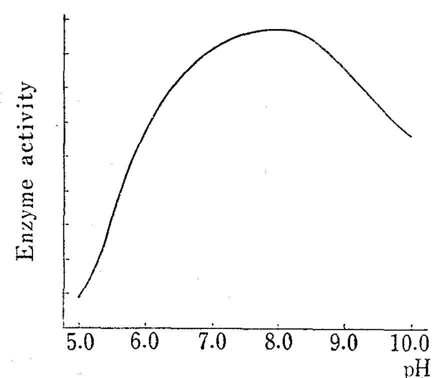

Fig. 2. Effect of pH on the α–Amino Acylase Activity
Standard assay conditions were used.

NII-Electronic Library Service

amino acylase was studied. Mixture of 1 ml of the enzyme solution and 1 ml of 0.05 M each buffer were allowed to stand at 4° for 1 or 2 days and the enzyme activities were measured at pH 8.0. As shown in Table III, this enzyme was unstable, but the activity loss was relative low at pH 8.0. Furthermore, it was more stable in the presence of 10^{-3} M mercaptoethanol.

Effect of Metal Ions, Chelating Agents and Other Compounds

It has been well known that various α-amino acylases are specifically activated by Co^{2+} or Zn^{2+} [10–13]. In order to study the influence of metal ions on the α-amino acylase activity, various metal ions were added to the enzyme assay. As shown in Table IV, Mg^{2+} has a slight stimulatory effect on the α-amino acylase activity at 10^{-3} M, but Co^{2+}, Zn^{2+}, Cu^{2+}, and Hg^{2+} have considerably inhibitory effect. Then the effect of chelating agents and other compounds on the activity was investigated. EDTA has not inhibitory effect at 10^{-3} M, but *o*-phenanthroline have completly inhibited at 10^{-3} M. The sulfhydryl reagents such as *p*-chloromercuribenzoate has slight inhibitory effect at 10^{-3} M. H_2O_2 has completly inhibited at 10^{-3} M and mercaptoethanol had not inhibitory effect.

TABLE IV. Effect of Metal Iones, Chelating Agents, and Other Compounds

Compounds	Relative Activity[a] 10^{-3}M[b]	Compounds	Relative Activity[a] 10^{-3}M[b]
Ca^{2+}	100	Hg^{2+}	0
Mg^{2+}	120	EDTA	100
Mn^{2+}	98	*o*-Phenanthroline	0
Ni^{2+}	90	Ascorbic Acid	100
Co^{2+}	65	Mercaptoethanol	100
Cu^{2+}	0	H_2O_2	0
Fe^{2+}	90	*p*-Chloromercuribenzoate	66
Zn^{2+}	50		

a) The activity under no addition is taken as control (100).
b) Final concentration in reaction mixture.
The reaction mixture contains 1 ml of 0.01 M benzoyl-L-alanine, 0.5 ml of appropriately diluted enzyme solution, and 0.5 ml of 0.1 M Tris buffer (pH 8.0), which contains the indicated test compounds. The activity was measured under standard conditions.

Effect of Substrate Concentration

The effect of substrate concentration on the α-amino acylase activity was investigated, and the results obtained have been plotted by the method of Lineweaver and Burk[14] for the estimation of Km. As shown in Fig. 3, Km was calculated to be 6×10^{-3} M for N-benzoyl-L-alanine.

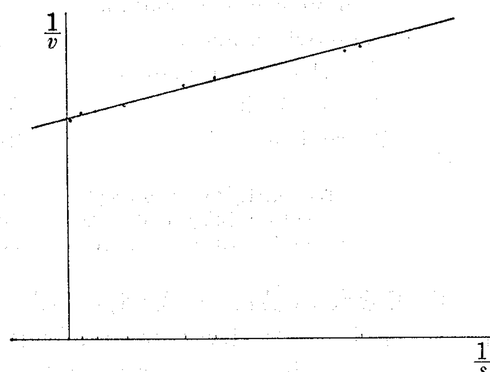

Fig. 3. Effect of Substrate Concentration

The activity is measured under standard conditions except substrate concentration

Substrate Specificity

α-Amino acylases are widely distributed in animals, plants, and microorganisms[6,7] and they have different substrate specificities. For example, hog kidney amino acylase I acts more readily on N-acylated aliphatic amino acids,

10) R. Marshall, S.M. Birnbaum, and J.P. Greenstein, *J. Am. Chem. Soc.*, **78**, 4636 (1956).
11) R. Shirasaka, *Ann. Takamine Lab.*, **10**, 74 (1958).
12) B.L. Vallee and H. Neurath, *J. Am. Chem. Soc.*, **76**, 5006 (1954); *J. Biol. Chem.*, **217**, 253 (1955).
13) S. Wada, *J. Biochem.*, **46**, 445, 1541 (1959).
14) H. Lineweaver and D. Burk, *J. Am. Chem. Soc.*, **56**, 658 (1934).

NII-Electronic Library Service

whereas pancreatic carboxypeptidase acts more readily on N-acylated amino acids containing aromatic substituents on the β-carbon atom. In order to clarify the substrate specificity of the α-amino acylase, susceptibility of the various acyl derivatives of amino acids toward the enzyme was investigated.

1) Optical Specificity——As shown in Table V, α-amino acylase of KT 801 could hydrolyze benzoyl-L-alanine and benzoyl-L-methionine but could not hydrolyze benzoyl-D-alanine and benzoyl-D-methionine at a measurable rate. That is, this α-amino acylase has an optical specificity and can hydrolyze only acyl-L-amino acids.

Table V. Optical Specificity of α-Amino Acylase of KT 801

Substrate	Relative Activity	Substrate	Relative Activity
Benzoyl-L-alanine	100	Benzoyl-L-methionine	95
Benzoyl-D-alanine	0	Benzoyl-D-methionine	0

The activity toward benzoyl-L-alanine is 2000 μM/hr/mg and this is taken 100.
Digest consisting 1 ml of 0.01 M substrate, 0.5 ml of 0.1 M Tirs buffer (pH 8.0), and 0.5 ml of the enzyme solution is incubated at 37°. Activities are measured by the procedure described in "Method."

2) Effect of Acyl Group——In order to test the effect of acyl groups toward α-amino acylase activity, a number of acyl-DL-alanine, indicated in Table VI, were prepared and the susceptibilities to the enzyme were investigated at pH 8.0. As shown in Table VI, benzoyl-DL-alanine is the most susceptible in tested compounds and dichloroacetyl-DL-alanine and octanoyl-DL-alanine hydrolyzed by the enzyme at a rate of about one thirtieth and one fiftieth of the benzoyl derivative respectively. On the other hand, acetyl-DL-alanine and phenylacetyl-DL-alanine could not hydrolyze at a measurable rate. From these results, it is revealed that the susceptibility of substrate are considerably affected by acyl groups.

Table VI. Substrate Specificity of α-Amino Acylase of KT 801

Substrate	Relative Activity	Substrate	Relative Activity
Benzoyl-DL-alanine	100	Benzoyl-DL-leucine	67
Acetyl-DL-alanine	0	Benzoyl-DL-methionine	95
Dichloroacetyl-DL-alanine	2.9	Benzoyl-DL-threonine	1
Octanoyl-DL-alanine	2	Benzoyl-DL-aspartic Acid	8.5
Phenylacetyl-DL-alanine	0	Benzoyl-DL-glutamic Acid	8
Benzoylglycine	56	Benzoyl-DL-phenylalanine	13
Benzoyl-DL-valine	27	Benzoyl-DL-tryptophan	5

The activity toward benzoyl-DL-alanine is 2000 μM/hr/mg and this is taken 100.
Digest consisting 1 ml of 0.02 M substrate, 0.5 ml of 0.1 M Tris buffer (pH 8.0), and 0.5 ml of the enzyme solution is incubated at 37°. Activities are measured by the procedure described in "Method."

3) Effect of Amino Acids——As shown in Table VI, benzoyl-DL-alanine is the most susceptible in the tested compounds and the susceptibility followed to benzoyl-DL-methionine, benzoyl-DL-leucine, benzoylglycine, benzoyl-DL-valine, benzoyl-DL-phenylalanine, benzoyl-DL-aspartic acid, benzoyl-DL-glutamic acid, benzoyl-DL-tryptophan, benzoyl-DL-threonine. As shown in Table VII, the ratio of the activities toward these benzoyl amino acids remained constant all through the purification steps. These results strongly suggest that one enzyme hydrolyzes these benzoyl amino acids. On the other hand, the activity toward 5-N-benzoyl-L-ornithine (δ-ornithine acylase) and 2-N-benzoyl-L-ornithine decreased through these purification steps. That is, this α-amino acylase is clearly different from δ-ornithine acylase. As shown in Table VI, this α-amino acylase could not hydrolyze phenylacetyl amino acids. That is, this α-amino acylase has a specificity toward benzoyl-L-amino acids. The authors

NII-Electronic Library Service

previously reported that an α–amino acylase of *E. coli* K–12 recombinante has a specificity toward phenylacetyl–L–amino acids.[15] From these results, it is obvious that the specificity of α–amino acylase present not only amino acid part, but acyl part. Then, the α–amino acylase of KT 801 was provisionally named "benzoylamino acid amidohydrolase."

Table VII. Ratio of Activities in Purification Steps

Substrate	Relative Activity		
	Cell Suspension	Acrinol Treatment	Purified Enzyme
Benzoyl–DL–alanine	100	100	100
Benzoyl–glycine	65	61	56
Benzoyl–DL–valine	20	23	27
Benzoyl–DL–leucine	63	75	67
Benzoyl–DL–methionine	98	96	95
Benzoyl–DL–thereonine	0.5	1	1
Benzoyl–DL–aspartic Acid	7	6	8.5
Benzoyl–DL–glutamic Aicd	6	6	8
Benzoyl–DL–phenylalanine	12	17	13
Benzoyl–DL–tryptophan	3.2	5	5
2–N–Benzoyl–L–ornithine	55	49	11
5–N–Benzoyl–L–ornithine	320	167	10
Phenylacetyl–DL–alanine	0	0	0
Phenylacetyl–DL–leucine	0	0	0
Phenylacetyl–DL–methionine	0	0	0
Phenylacetyl–DL–aspartic Aicd	0	0	0
Phenylacetyl–DL–phenylalanine	0	0	0

The activities toward benzoyl–DL–alanine are 35 μM/hr/mg (cell suspension), 300 μM/hr/mg (acrinol treatment solution), and 2000 μM/hr/mg (purified enzyme), and these are respectively taken as standard (100). Activities are measured by the procedure described in "Method."

Acknowledgement The research was supported in part by a grant from the Ministry of Education to which the authors' grateful acknowledgements are made.

15) Y. Kameda and T. Iwahi, presented at the 24th Meeting of the Pharmaceutical Society of Japan, Kyoto. April 8, 1967.

NII-Electronic Library Service