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Studies on Acidic Arginine Esterase Excreted in Urine. I. Purification and Characterization of Dog Urinary Arginine Esterase[1)]

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A preliminary experiment employing the technique of isoelectric focusing on an Ampholine column revealed the presence of three arginine ester hydrolyzing activities (using *N*-α-benzoyl-L-arginine ethyl ester (Bz–Arg–Et) as substrate) which show isoelectric points (pI) of 4.2, 4.5 and 4.7. The first fraction (pI 4.2), as expected from its elution position, was confirmed to be urinary kallikrein based on vasodilation activity measurement. The second (pI 4.5) and third (pI 4.7) fractions were hitherto unidentified arginine esterases, and we tentatively designated them as DUAE (dog urinary arginine esterase)-1 and -2, respectively.

DUAE-2 was purified to homogeneity mainly by chromatographic methods (about 140-fold purification from dialyzed dog urine), and it represented about 7% of the initial *N*-α-tosyl-L-arginine methyl ester (Tos–Arg–Me) hydrolyzing activity. The specific activity of the finally purified DUAE-2 was 12.4 μmol/min/A_{280} of Tos–Arg–Me esterolytic activity and this enzyme had neither plasmin nor plasminogen activator activity. The molecular weight of this enzyme was estimated to be 3.0×10^4 daltons by Sephadex G-100 gel filtration and vertical plate polyacrylamide gel electrophoresis with sodium dodecyl sulfate (SDS). The optimum pH for Tos–Arg–Me esterolytic activity of DUAE-2 was found to be 9.0 and this enzyme was fairly heat stable. Soybean trypsin inhibitor strongly suppressed the Tos–Arg–Me esterolytic activity of DUAE-2, while aprotinin and ovomucoid trypsin inhibitor were less effective. The esterolytic and amidolytic activities of this enzyme showed broad profiles against arginine and lysine derivatives as substrates.

Keywords——dog urine; arginine ester hydrolyzing enzyme; enzyme purification; substrate specificity; isoelectric focusing

Our previous paper[2)] reported that two arginine ester hydrolyzing enzymes exist in dog urine, and one of them was identified as urinary kallikrein. The other one, having no vasodilator activity, seemed to be a hitherto unidentified arginine ester hydrolyzing enzyme. In 1974, Nustad and Pierce separated a new arginine ester hydrolyzing enzyme called "Esterase A" from rat urine,[3)] and we reported that a non-kallikrein arginine ester hydrolyzing enzyme existed in human urine.[4)] Some of the properties of this non-kallikrein arginine esterase in rat urine were reported.[5)] We recently described the purification and characterization of kallikreins from dog urinary organs.[2,6)] However, the enzymatic relationship between these kallikreins and the new arginine ester hydrolyzing enzyme in dog urine is unclear. In the present paper, we report on the purification and characterization of dog urinary arginine esterase (DUAE).

Materials and Methods

Dog Urine——This was directly collected from the bladder by catheterization of healthy adult dogs of either sex,

and the collected urine was stored frozen until use.

Materials——*N*-α-Tosyl-L-arginine methyl ester (Tos–Arg–Me), L-arginine methyl ester (H–Arg–Me), *N*-α-tosyl-L-lysine methyl ester (Tos–Lys–Me), *N*-α-acetyl-L-lysine methyl ester (Ac–Lys–Me), *N*-α-benzoyl-L-arginine ethyl ester (Bz–Arg–Et), *N*-α-benzoyl-L-tyrosine ethyl ester (Bz–Tyr–Et), *N*-α-benzoyl-DL-arginine-*p*-nitroanilide (Bz–DL-Arg–*p*NA), and L-leucine-*p*-nitroanilide (H–Leu–*p*NA) were supplied by the Peptide Institute Inc., Osaka, Japan. Acetyl-L-alanyl-L-alanyl-L-alanine methyl ester (Ac–Ala–Ala–Ala–Me), *N*-α-benzoyl-L-arginine-*p*-nitroanilide (Bz–Arg–*p*NA), carbobenzoxy-L-alanyl-L-valine methyl ester (CBZ–Ala–Val–Me), myoglobin, soybean trypsin inhibitor, ovomucoid trypsin inhibitor, and cytochrome c were obtained from Sigma Chemical Co., U.S.A. *N*-α-Benzoyl-L-arginine methyl ester (Bz–Arg–Me), *N*-α-benzoyl-DL-arginine-β-naphthylamide (Bz–DL-Arg–β-NFA), and carbobenzoxy-glycyl-glycyl-L-arginine-β-naphthylamide (CBZ–Gly–Gly–Arg–β-NFA) were from Serva Chemical Co., West Germany. Bovine serum albumin, bovine fibrinogen, and egg white albumin were obtained from Daiichi Chemical Co., Tokyo, Japan. Bovine thrombin, bovine plasminogen, and human urokinase were kindly supplied by Green Cross Drug Mfg. Co., Osaka, Japan. D-Valyl-L-leucyl-L-arginine-*p*-nitroanilide (H–Val–Leu–Arg–*p*NA) was supplied by AB Kabi Diagnostica, Sweden. All other chemicals used were of analytical reagent grade from commercial sources.

Assay of Enzyme——Esterolytic activities were measured both by the colorimetric method (methyl ester substrates)[7] and by spectrophotometric methods (Bz–Arg–Et and Tos–Arg–Me)[8] at pH 8.0 and 30 °C. Amidolytic activities were assayed by modifications of the methods reported by Amundsen (*p*-nitroanilide substrates)[9] and Hitomi (β-naphthylamide substrates)[10] at pH 8.0 and 30 °C. All esterolytic and amidolytic activities were expressed in terms of μ mol of substrate hydrolyzed per min. Vasodilator activity was measured by the method of Moriya[11] and was expressed in terms of the kallikrein unit (KU). Fibrinolytic and urokinase activities were determined by the method of Mullertz[12] and commercially available fibrinogen was purified by affinity chromatography using lysine Sepharose. Kinin-releasing and kininase activities were assayed by the method of Moriya's group[13] and partially purified bovine kininogen was prepared in our laboratory.

Protein Concentration——The protein concentration was estimated by measuring the absorbance at 280 nm in a 1 cm width cuvette.

Electrophoresis——Disc electrophoresis on a polyacrylamide gel column was carried out based on the technique of Davis[14] with 7% gel and 0.04 M Tris-glycinate buffer, pH 8.6. Vertical plate polyacrylamide gel electrophoresis employing sodium dodecyl sulfate (SDS) was carried out by modification of the method of Weintraub[15] in 7.5% gel with 0.08 M Tris-EDTA-borate buffer, pH 9.2. The gel was stained for protein with 0.2% Coomassie Brilliant Blue.

Isoelectric Focusing——This was done using the Ampholine system as developed by Vesterberg and Svensson.[16] The pH range of 3.5 to 5.0 of carrier ampholyte was used, and electrophoresis was performed for 40 h at 500 V constant voltage with a cooling system set at 4 °C.

Results

Preliminary Isoelectric Focusing Experiment

The dialyzed dog urine (4900 ml) was adsorbed on DEAE-cellulose (50 g) by a batch method. After 2 h of adsorption, the DEAE-cellulose was packed in a column (3 × 50 cm) and the column was eluted with 0.05 M Tris-HCl buffer containing 0.5 M NaCl at pH 7.5. The eluted solution was dialyzed against deionized water and the dialyzate was applied to an Ampholine column. After electrophoresis, fractions of 1 ml were collected and the pH, vasodilator activity, and esterolytic activity of the fractions were determined. The elution profile is shown in Fig. 1. The Bz–Arg–Et hydrolyzing activity was separated into three peaks at isoelectric point (pI) values of 4.2, 4.5, and 4.7.

The first eluted peak (pI 4.2) had both esterolytic and vasodilator activities, and was concluded to be dog urinary kallikrein. The second and third peaks had only esterolytic activity, but not vasodilator activity. We tentatively called these two peaks dog urinary arginine esterase-1 (DUAE-1, pI 4.5) and -2 (DUAE-2, pI 4.7). We were not able to purify DUAE-1 because of its instability, but we achieved the purification of DUAE-2.

Purification of DUAE-2

All steps were carried out at room temperature.

Step 1. DEAE-Cellulose Adsorption and Elution——The dog urine (7000 ml) was dialyzed against tap water. The dialyzed dog urine (7500 ml) had 0.09 μmol/min/A_{280} of Tos–Arg–Me esterolytic activity. The procedure was performed according to step 1 of the dog

urinary kallikrein purification procedure described in the previous paper.[2] Total absorbance at 280 nm and esterolytic activity toward Tos–Arg–Me in the non-kallikrein arginine esterase fraction recovered in this step were 1200 and 770 μmol/min, respectively, and the elution profile is shown in Fig. 2A.

Step 2. DEAE-Cellulose Chromatography——The active preparation from step 1 (210 ml) was dialyzed against 0.05 M Tris-HCl buffer at pH 7.5 and applied to a DEAE-cellulose column (2.5 × 90 cm) pre-equilibrated with the same buffer. The column was eluted with a gradient elution system using the same buffer containing 0.05 to 0.5 M NaCl, and the chromatogram of this step is shown in Fig. 2B. The presence of two active peaks of Tos–Arg–Me esterolytic activity were eluted, and the former active peak was confirmed to be DUAE-2. The fractions of DUAE-2 collected and pooled (130 ml). Total absorbance at 280 nm and specific activity of Tos–Arg–Me esterolysis were 404 and 1.2 μmol/min/A_{280}, respectively.

Steps 3 to 5. Sephadex G-100 Gel Filtration, DEAE-Sephadex A-50 Chromatography, and Sephadex G-75 Gel Filtration——These purification procedures were the same as those used for purification of dog urinary kallikrein.[2] The consecutive recoveries of protein (A_{280}) and esterolytic activity (Tos–Arg–Me hydrolysis) are given in Table I.

Step 6. Lysine Sepharose Adsorption and Elution——The pooled active preparation from step 5 (17 ml) in 0.05 M Tris-HCl buffer at pH 7.5 was applied to a lysine Sepharose column (2 × 20 cm) pre-equilibrated with the same buffer. The initial eluate using the same buffer contained most of the Tos–Arg–Me esterolytic activity and the eluate with *trans*-4-(aminomethyl) cyclohexane carboxylic acid (0.05 M) had minor activity (Fig. 2C). The first active fractions were collected and pooled (24 ml); the total absorbance at 280 nm of the pooled fraction was 9. The total activity and specific activity of this preparation were 113 μmol/min and 12.4 μmol/min/A_{280}, respectively.

A summary of the overall results of purification of DUAE-2 is shown in Table I. The specific activity, measured in terms of esterolysis of Tos–Arg–Me, of purified DUAE-2 was 139-times as much as that of the initial dialyzed dog urine, and the final preparation contained 7.1% of the esterolytic activity calculated at the first step. The final preparation was homogeneous on disc gel column electrophoresis as shown in Fig. 3.

Isoelectric Point

Measurement of the isoelectric point of DUAE-2 by using isoelectric focusing gave only

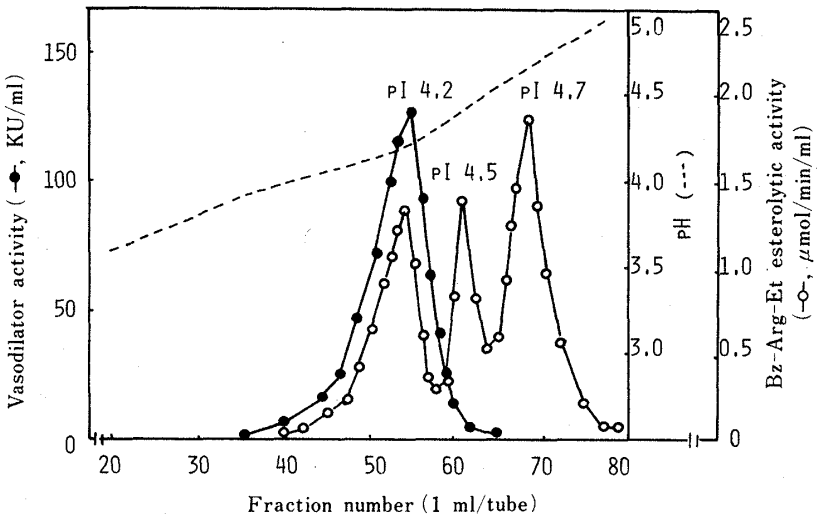

Fig. 1. Isoelectric Focusing of DEAE-Cellulose Adsorbed Dog Urine

Carrier ampholyte was used in the pH range of 3.5 to 5.0 —●—, vasodilator activity; —○—, esterolytic activity; ----, pH.

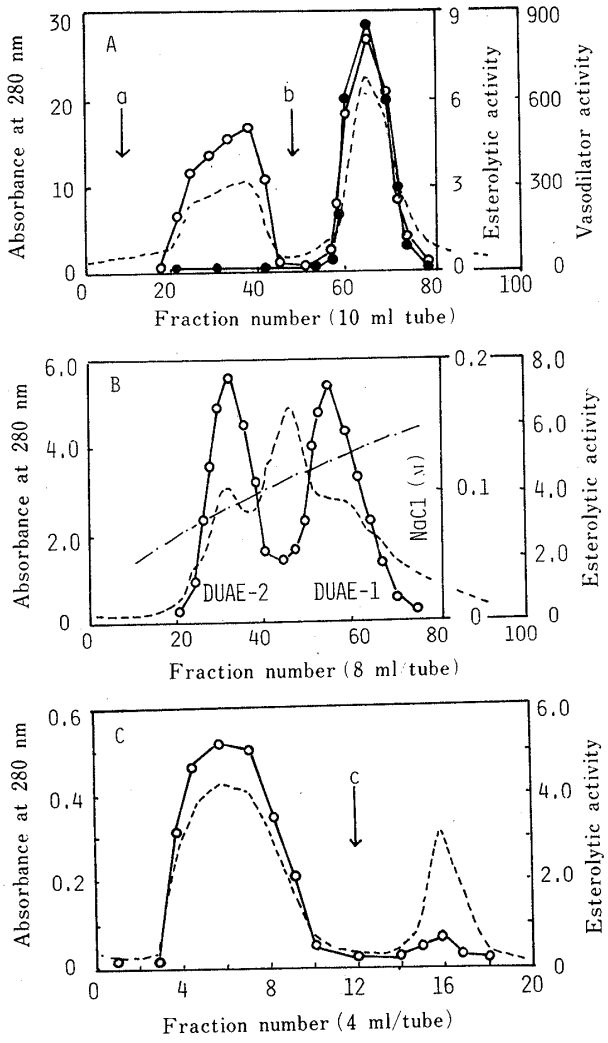

Fig. 2. Elution Profiles of DUAE-2 on DEAE-Cellulose Adsorption and Elution (A), DEAE-Cellulose Chromatography (B), and Lysine Sepharose Adsorption and Elution (C)

(A): a and b, elution positions with 0.05 M Tris-HCl buffer containing 0.15 and 0.5 M NaCl at pH 7.0, respectively.

(C): c, elution position with 0.05 M Tris-HCl buffer containing 0.05 M *trans*-4-(aminomethyl)-cyclohexane carboxylic acid (*trans*-AMCA) at pH 7.0.

—○—, Tos–Arg–Me esterolytic activity; ----, concentration of protein (A_{280}/ml); - - - -, concentration of NaCl; —●—, vasodilator activity.

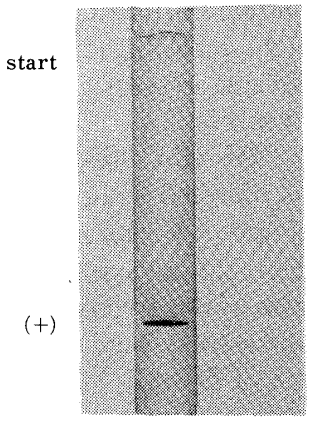

Fig. 3. Disc Gel Electrophoresis of DUAE-2

TABLE I. Summary of the Purification of DUAE-2

Step	Procedure	Protein	Esterolytic activity		
		Total protein (A_{280})	Total activity (μmol/min)	Specific activity (μmol/min/A_{280})	Purification factor
	Dialyzed dog urine (7000 ml)	18500	1600	0.09	1
1	DEAE-cellulose adsorption 0.15 M NaCl elution	1200	770	0.6	7
2	DEAE-cellulose chromatography	404	484	1.2	13
3	Sephadex G-100 gel filtration	128	352	2.7	30
4	DEAE-Sephadex A-50 chromatography	53	279	5.3	59
5	Sephadex G-75 gel filtration	12	138	11.5	128
6	Lysine Sepharose adsorption and elution	9	113	12.4	139

Esterolytic activity was measured by the colorimetric method using Tos–Arg–Me as a substrate at pH 8.0, at 30 °C.[5]

one Bz–Arg–Et esterolytic active peak having a pI value of 4.7 at 20 °C, and this result agreed well with the result of the preliminary experiment (Fig. 1).

Molecular Weight

The approximate molecular weight of purified DUAE-2 was estimated to be 3.0×10^4 by

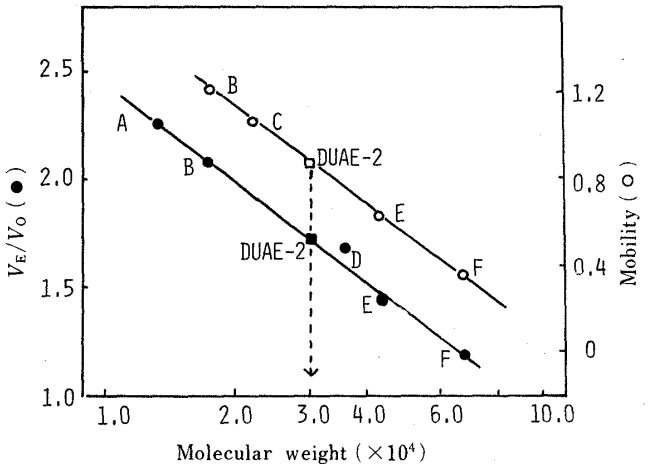

Fig. 4. Estimation of Approximate Molecular Weight of DUAE-2 by Gel Filtration and SDS–Vertical Plate Polyacrylamide Gel Electrophoresis

Authentic proteins were used as markers cytochrome c (A), myoglobin (B), soybean trypsin inhibitor (C), pepsin (D), egg white albumin (E), and bovine serum albumin (F). Gel filtration (●) was performed on a Sephadex G-100 column (1.5 × 80 cm), equilibrated with 0.05 M Tris-HCl buffer containing 0.1 M NaCl at pH 7.0, and blue dextran was also used as a marker for determination of the void volume. SDS (1 w/v%)–vertical plate polyacrylamide (10 w/v%) gel electrophoresis (○) was carried out at a constant voltage of 200 V at 4 °C.

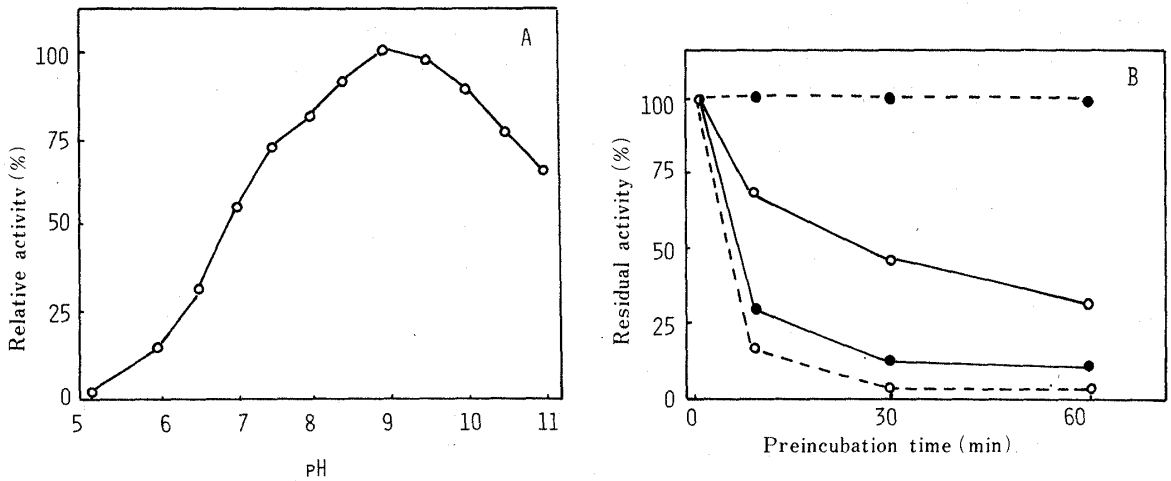

Fig. 5. Optimum pH (A) and Thermal Stability (B) of DUAE-2

Optimum pH and thermal stability were determined by esterolytic assay with chromotropic acid, using Tos–Arg–Me as a substrate. Measurement of optimum pH was done by using 0.08 M modified Britton–Robinson's wide range buffer of various pH values and the activity was expressed as a percentage of that at the optimum pH. For the thermal stability study, purified DUAE-2 was held at the indicated temperatures for 10, 30, and 60 min at pH 8.0, and the residual activity was measured by the esterolytic assay method. The activity was expressed as a percentage of that of the untreated control.
(B): ---●---, 50 °C; —○—, 60 °C; —●—, 75 °C; ---○---, 100 °C.

gel filtration on a Sephadex G-100 column and by SDS–vertical plate polyacrylamide gel electrophoresis, as shown in Fig. 4.

Optimum pH and Thermal Stability

Optimum pH and thermal stability of DUAE-2 were monitored by following its esterolytic activity toward Tos–Arg–Me. Optimum pH was determined with 0.08 M modified Britton–Robinson's wide range buffer from pH 5.0 to 11.0, and the maximum relative activity was found at pH 9.0. The effect of thermal treatment on the esterolytic activity of DUAE-2

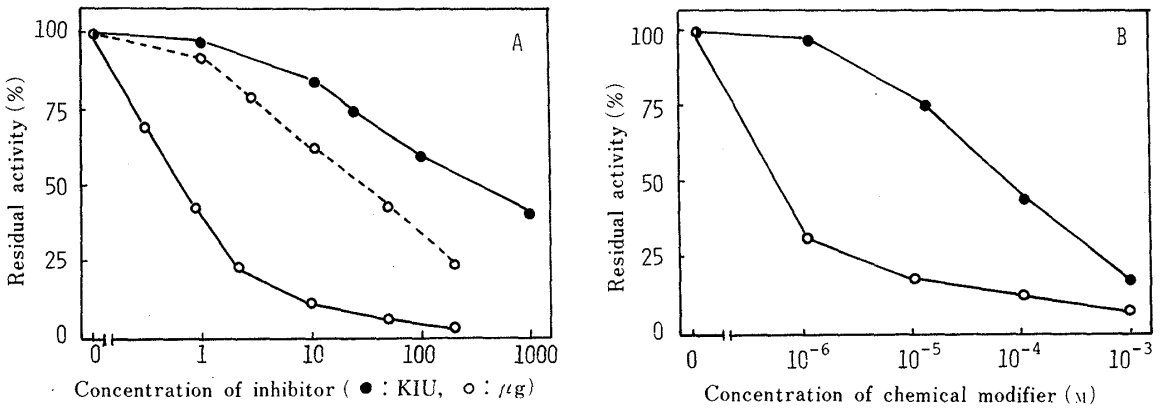

Fig. 6. Effect of Some Inhibitors on the Esterolytic Activity of DUAE-2

A mixture of the designated amounts of inhibitor and enzyme (0.0048 A_{280}) was preincubated at 30 °C for 30 min, pH 8.0, and the remaining activity was measured by the esterolytic assay method with chromotropic acid using Tos–Arg–Me as a substrate. The activity was expressed as a percentage of that of the untreated control.

(A): —●—, aprotinin; ---○---, ovomucoid trypsin inhibitor; —○—, soybean trypsin inhibitor.

(B): —●—, Tos–Lys–CH_2Cl, TLCK; —○—, Tos–Phe–CH_2Cl, TPCK.

was assayed at pH 8.0, and it was found that the DUAE-2 was stable up to 50 °C for 60 min but completely lost its esterolytic activity at 75 °C. The results are summarized in Fig. 5.

Kinin-Releasing, Kininase, Fibrinolytic, and Urokinase Activities

No kinin-releasing and kininase activities (20 ng bradykinin digestion/A_{280} or less) were observed, and the purified DUAE-2 preparation also showed no fibrinolytic or urokinase activity. However, the second peak from step 6 showed weak urokinase activity.

Effect of Inhibitors

The effect of several proteinase inhibitors, such as aprotinin, ovomucoid trypsin inhibitor, and soybean trypsin inhibitor, on the Tos–Arg–Me hydrolyzing activity of purified DUAE-2 was examined after preincubation at 30 °C for 20 min, pH 8.0. As shown in Fig. 5A, DUAE-2 was weakly inhibited by aprotinin and ovomucoid trypsin inhibitor. However, soybean trypsin inhibitor strongly suppressed the esterolytic activity and complete inhibition was caused by 50 μg of inhibitor. This inhibitory action seemed to be stoichiometric, and the molar binding ratio of inhibitor to enzyme was calculated to be 1 : 1.

This enzyme was also clearly inhibited by 10^{-3} M *N*-α-tosyl-L-phenylalanine chloromethylketone (Tos–Phe–CH_2Cl, TPCK) and *N*-α-tosyl-L-lysine chloromethylketone (Tos–Lys–CH_2Cl, TLCK), and the inhibitory effect of TPCK was somewhat stronger than that of TLCK (Fig. 5B).

Substrate Specificity

Esterolytic and amidolytic activities of DUAE-2 toward synthetic arginine, lysine, and other amino acid derivatives as substrates were tested and the results are shown in Table II. Among the esterolytic substrates examined, Tos–Arg–Me, Ac–Lys–Me, Ac–Gly–Lys–Me and especially Tos–Lys–Me were easily hydrolyzed by this enzyme, but Bz–Arg–Me was somewhat less well hydrolyzed.

Amidolytic activity of this enzyme was lower than the esterolytic activity. Of the amidolytic substrates examined, CBZ–Gly–Gly–Arg–β-NFA was the most effective substrate.

Amino Acid Composition of DUAE-2

A protein sample was hydrolyzed with constant-boiling hydrochloric acid at 110 °C in an evacuated sealed tube for 24 h, and amino acids were analyzed with a Hitachi amino acid

TABLE II. Esterolytic and Amidolytic Activities of DUAE-2 toward Synthetic Arginine, Lysine, and Other Amino Acid Derivatives

Substrate	Activity		K_m
	μ mol/min/A_{280}	Ratio	(M)
Tos–Arg–Me	12.4	1.0	6.7×10^{-4}
Bz–Arg–Me	1.65	0.13	1.3×10^{-3}
H–Arg–Me	0.74	0.06	
Tos–Lys–Me	18.1	1.46	
Ac–Lys–Me	9.2	0.74	
Ac–Gly–Lys–Me	12.3	0.99	
Bz–Tyr–Et	0.04	0.003	
CBZ–Ala–Val–Me	N.D.[a]		
Ac–Ala–Ala–Ala–Me	N.D.[a]		
Bz–DL-Arg–*p*NA	0.36	0.03	
Bz–Arg–*p*NA	1.34	0.11	
H–Val–Leu–Arg–*p*NA	5.93	0.48	5.8×10^{-5}
H–Leu–*p*NA	0.001	0.0001	
Bz–DL-Arg–β-NFA	0.48	0.04	
CBZ–Gly–Gly–Arg–β-NFA	11.7	0.94	1.9×10^{-4}

The substrate concentrations of methyl and ethyl esters were 20 mM each, and those of *p*-nitroaniline and β-naphthylamide derivatives were 1 and 2 mM, respectively.
a) N.D.: not detected.

TABLE III. Amino Acid Composition of DUAE-2

Amino acid	Residues per mol	Nearest integer
Asp	26.8	27
Thr	17.0	17
Ser	26.1	26
Glu	32.9	33
Pro	22.8	23
Gly	34.1	34
Ala	26.1	26
Val	13.2	13
Half-Cys	2.1	2
Met	2.0	2
Ile	8.6	9
Leu	17.4	17
Tyr	3.1	3
Phe	7.7	8
Lys	9.0	9
His	4.8	5
Arg	10.1	10

analyzer, model 835. The results are presented in Table III. DUAE-2 was composed of approximately 264 amino acid residues per mol, excluding tryptophan.

Discussion

In the present investigation, a large amount of a new arginine ester hydrolyzing enzyme (0.1 to 0.3 μmol of Tos–Arg–Me hydrolyzed/min/ml) was found in dialyzed dog urine. Calculation based on the result at step 1 and the previous report[2] gave a value of about 55%

for the content of this enzyme in total arginine esterases. This is close to the content of rat urinary arginine esterase A (according to the data in Table I of ref. 3, calculated to be 50%). This non-kallikrein arginine esterase was divided into two active peaks by DEAE-cellulose chromatography (Fig. 2B), and we tentatively designated the first eluted active peak and the later one as dog urinary arginine esterase-2 and -1 (DUAE-2 and -1), respectively. The presence of these two forms of DUAE in the dialyzed dog urine was confirmed by isoelectric focusing (Fig. 1), which gave pI value of 4.5 (DUAE-1) and 4.7 (DUAE-2). We assumed that DUAE-1 and -2 might correspond to rat urinary arginine esterases A-2 and A-1,[5] respectively, based on the elution profile on DEAE-Sephadex A-50 chromatography. It is reasonable that the elution profiles of DUAE-1 and -2 on DEAE-cellulose chromatography (Fig. 2B) and isoelectric focusing (Fig. 1) are reversed. We have not succeeded in the purification of DUAE-1 because of its instability.

The purified DUAE-2 at the final stage was homogeneous in disc gel column electrophoresis. The specific activity toward Tos–Arg–Me of purified DUAE-2 was 12.4 μmol/min/A_{280}, and this value is similar to that of dog urinary kallikrein.[2] The present enzyme had neither kinin-releasing nor kininase activity, and furthermore had neither plasminogen activator nor plasmin activity. These property parallels that of rat urinary arginine esterase A-1,[3,5] except for plasminogen activator and plasmin activities, which were not described in the cited references. Rat urinary arginine esterase A-2 has weak kinin-releasing activity.[5] However, we have not been able to purify DUAE-1, which may correspond to rat urinary arginine esterase A-2.

Based on Sephadex G-100 gel filtration and SDS–vertical plate polyacrylamide gel electrophoresis, the molecular weight of DUAE-2 was estimated to be 3.0×10^4, and this value is in good agreement with those of rat urinary arginine esterase A-1[5] and human urinary arginine esterase (HUAE).[4]

Optimum pH and thermal stability of DUAE-2 were similar to those of dog urinary kallikrein,[2] but the actions of some proteinase inhibitors on the enzyme did not fully coincide with those on dog urinary kallikrein.[2] The Tos–Arg–Me esterolytic activity of DUAE-2 was stoichiometrically suppressed by soybean trypsin inhibitor, and the inhibitory actions of proteinase inhibitors tested against DUAE-2 were rather similar to those on trypsin[17] and dog pancreatic esterase.[18] In the case of kallikrein from dog urinary organs,[6] the vasodilator activity was not inhibited by soybean trypsin inhibitor.

As shown in Table II, purified DUAE-2 seemed to have esterolytic and amidolytic activities towards arginine and lysine derivatives as substrates. In the cases of dog urinary, dog renal and human urinary kallikreins,[19] the amidolytic activities towards arginine and lysine *p*-nitroanilide were lower than the esterolytic activities towards Tos–Arg–Me and Bz–Arg–Me (or Et) as substrates. These results also show that the enzymatic action of DUAE-2, and that of dog urinary and renal kallikreins are clearly different from each other. The K_m value of DUAE-2 operating upon the Tos–Arg–Me substrate was found to be 6.7×10^{-4} M, and this value is about 66-times lower than that of rat urinary arginine esterase A-1.[5] However, the K_m value of dog urinary kallikrein[2] for the Tos–Arg–Me substrate was about 4.5-times higher than that of rat urinary kallikrein.[20] With respect to the behavior with proteinase inhibitors and the substrate specificities, the present enzyme more closely resembles trypsin than kallikrein from urinary organs. Further work is required on the relationship among the enzymatic properties of rat, human and dog urinary arginine esterases, and between those of dog urinary kallikrein and the present esterase.

References and Notes

1) Abbreviations: DUAE, dog urinary arginine esterase; SDS, sodium dodecyl sulfate; Tos–Arg–Me, *N*-α-tosyl-L-

arginine methyl ester; Bz–Arg–Me, *N*-α-benzoyl-L-arginine methyl ester; H–Arg–Me, L-arginine methyl ester; Tos–Lys–Me, *N*-α-tosyl-L-lysine methyl ester; Ac–Lys–Me, *N*-α-acetyl-L-lysine methyl ester; Ac–Gly–Lys–Me, acetyl-glycyl-L-lysine methyl ester; Bz–Tyr–Et, *N*-α-benzoyl-L-tyrosine ethyl ester; Ac–Ala–Ala–Ala–Me, acetyl-L-alanyl-L-alanyl-L-alanine methyl ester; CBZ–Ala–Val–Me, carbobenzoxy-L-alanyl-L-valine methyl ester; Bz–DL-Arg–*p*NA, *N*-α-benzoyl-DL-arginine-*p*-nitroanilide; Bz–Arg–*p*NA, *N*-α-benzoyl-L-arginine-*p*-nitroanilide; H–Val–Leu–Arg–*p*NA, L-valyl-L-leucyl-L-arginine-*p*-nitroanilide; H–Leu–*p*NA, L-leucine-*p*-nitroanilide; Bz–DL-Arg–β-NFA, *N*-α-benzoyl-DL-arginine-β-naphthylamide; CBZ–Gly–Gly–Arg–β-NFA, carbobenzoxy-glycyl-glycyl-L-arginine-β-naphthylamide; Ac–Phe–Arg–Me, acetyl-L-phenylalanyl-L-arginine methyl ester.

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