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Studies on Kallikreins. V.[1] Purification and Characterization of Rat Intestinal Kallikrein

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Kallikrein [EC 3.4.21.8] was extracted from rat small intestine and purified by acetone fractionation, DEAE-Sephadex A-50 chromatography, Sephadex G-100 gel filtration, hydroxylapatite chromatography, Sephadex G-50 gel filtration and Ampholine isoelectric focusing. Rat intestinal kallikrein (RIK) was separated into 4 or 5 active components and the isoelectric points of the two main fractions were 4.18 (RIK-A) and 4.03 (RIK-B).

The final preparations of RIK-A and -B were homogeneous in disc electrophoresis in polyacrylamide gel and their vasodilator activities were 160 and 90 KU per A_{280}, respectively. The molecular weights of RIK-A and -B were estimated by Sephadex G-100 gel filtration to be 33000 and 35000, respectively, while the values obtained by SDS-polyacrylamide gel electrophoresis were both 32000. The amino acid compositions of the two RIK's were similar.

The K_m values of RIK-A against BAEE and TAME were 0.11 and 0.09 mM, and those of RIK-B were 0.08 and 0.11 mM, respectively. For both RIK-A and -B the optimal pH for hydrolysis of BAEE was 9.2, and both enzymes were stable at pH 7—9.

The substrate specificities of RIK-A and -B were similar to that of rat pancreatic kallikrein. Both RIK's were inhibited by aprotinin and leupeptin, but not by SBTI, LBTI, antipain, pepstatin, or chymostatin.

Keywords——intestinal kallikrein; pancreatic kallikrein; rat; multiple forms; molecular weight; amino acid composition; substrate specificity; proteinase inhibitors

Kallikrein [EC 3.4.21.8], one of the serine proteinases, is widely distributed in various organs.[3] Werle *et al.*[4] reported that the small and large intestine of mammals contained trypsin-activatable hypotensive activity, and their findings were later confirmed by other investigators.[5,6] Erdös *et al.*[7] reported that a kallikrein extracted from the colon was similar to the plasma kallikrein, but the enzymic properties of the intestinal enzyme have not yet been studied in detail, as it has not yet been isolated in a pure form.

Meanwhile, we had found that pancreatic kallikrein was transported to the mesenteric vascular system from the intestinal lumen.[8] We had also found that *in vitro* intestinal transport of valine from the mucosal to the serosal side was enhanced by the addition of pancreatic kallikrein to the mucosal side or of bradykinin to the serosal side.[1] Hog pancreatic kallikrein was used in these investigations. However, since the intestine contains an endogeneous kallikrein,[4] it is of interest to study whether the intestinal kallikrein itself affects the transport of various nutrients. This paper deals with the purification and characterization of kallikrein

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from rat small intestine. The enzymic properties of the purified intestinal kallikrein were compared with those of rat pancreatic kallikrein (RPK).

Experimental

Materials——N^{α}-Benzoyl-L-arginine ethylester (BAEE), N^{α}-*p*-toluenesulfonyl-L-arginine methylester (TAME), acetyl-L-arginine methylester (AcAME), N^{α}-*p*-toluenesulfonyl-L-lysine methylester (TLME), acetyl-L-lysine methylester (AcLME), acetyl-L-glycyl-lysine methylester (AcGLME), L-arginine methylester (AME), L-histidine methylester (HME), leupeptin, antipain, chymostatin, and pepstatin were obtained from the Protein Research Foundation (Osaka, Japan). N^{α}-Benzoyl-DL-arginine-*p*-nitroanilide (BA*p*NA), soybean trypsin inhibitor (SBTI), lima bean trypsin inhibitor (LBTI), and trypsin [EC 3.4.21.4] were obtained from Sigma Chem. Co. (U.S.A.), and N^{α}-benzoyl-L-tyrosine ethylester (BTEE) was from Calbiochem. Corp. (U.S.A.). Hydroxylapatite was from Seikagaku Kogyo Co. (Tokyo). DEAE-Sephadex A-50 (3.5 ± 0.5 meq/g), Sephadex G-100 and Sephadex G-50 were from Pharmacia Fine Chemicals (Sweden). Aprotinin (Bayer AG), carrier ampholytes (pH 3.5—5, LKB-Produkter AB) and plasmin [EC 3.4.21.7] (Green Cross Corporation, Japan) were also used. Marker proteins for the estimation of molecular weights by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) were supplied by Daiichi Chemical Co. Rat plasma kallikrein was partially purified by acetone fractionation, DEAE-cellulose adsorption and elution, and CM-Sephadex C-50 chromatography. Other chemicals used were of guaranteed grade.

Assays of Kallikrein——The vasodilator activity of kallikrein was determined as described previously by measuring the increase of arterial blood flow in dogs.[9] The activity was expressed in terms of the kallikrein unit (KU). Esterolytic activities against BAEE,[10] BTEE,[11] TAME,[11] and BA*p*NA[12] were measured by spectrophotometric methods at 25°, pH 8.0. Esterolysis of TAME[9] and other synthetic methylester derivatives at 30°, pH 8.0, were measured by a colorimetric method with chromotropic acid. One unit of the esterolytic activity (EU) was defined as that amount of activity which hydrolyzed 1 μmol of BAEE in 1 min.

Estimation of Protein Concentration——Protein concentration was estimated by measuring the absorbance at 280 nm with a quartz cell of 1 cm light path.

Disc Gel Electrophoresis——This was carried out in 7% (w/v) polyacrylamide cylindrical gel (0.5×8.0 cm) with the Tris-glycine buffer (pH 8.9) system according to the procedure of Davis.[13] Gels were run at 2 mA per gel for 1.5 hr at room temperature. Protein was stained with Coomassie brilliant blue R-250 dissolved in 10% (w/v) trichloroacetic acid.

Isoelectric Focusing——Isoelectric focusing was performed according to the method of Vesterberg and Svensson[14] with an LKB Ampholine column (110 ml). Ampholytes with a pH range of 3.5—5 was used at 1% final concentration and electrophoresis was carried out for 40 hr at a constant voltage of 500 V at 4°. After electrophoresis, the column content was fractionated into 1.5 ml fractions and the pH of each fraction was measured at 4°. Next, 0.5 ml of 0.5 M Tris-HCl buffer, pH 8.0, was added to each fraction, and the absorbance at 280 nm and esterolytic activity against BAEE were measured.

Molecular Weight Estimation——The molecular weights of kallikreins were estimated by Sephadex G-100 gel filtration.[15] The standard proteins were cytochrome c (M.W., 13000), myoglobin (M.W., 17000), SBTI (M.W., 21000), ovalbumin (M.W., 45000), and bovine serum albumin (M.W., 67000), and blue dextran was used to determine the void volume. Molecular weights were also estimated by SDS-PAGE in 7.5% total polyacrylamide containing 1% SDS, run for 4 hr at 5 mA per tube (constant) according to the method of Fairbanks *et al.*[16] The electrode buffer was 0.1 M Tris-EDTA acetate buffer containing 0.1% SDS (pH 7.4). After electrophoresis, the gel was stained for protein with 0.1% Coomassie brilliant blue R-250 in 10% trichloroacetic acid. Standard protein was dissolved at 1 mg/ml in 0.02 M Tris-EDTA HCl buffer containing 2% SDS and 8% 2-mercaptoethanol, and a calibration curve in the molecular weight range from 14300 to 71500 was made to estimate the molecular weight of the purified intestinal kallikrein.

Amino Acid Analyses——RIK-A (1.89 mg) and RIK-B (1.18 mg) were hydrolyzed in evacuated and sealed tubes for 24 hr at 110° with redistilled 6 N HCl. The amino acid analyses were carried out with a

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JEOL-5AH amino acid analyzer (Nihon-Denshi, Japan) according to the method of Spackman *et al.*[17] Tryptophan was determined with a Hitachi 835-50 amino acid analyzer (Hitachi Ltd, Japan) by the method of Hugli and Moore.[18]

Results

Distribution of Vasodilator Activity in Rat Small Intestine

Wistar albino rats, weighing 150 to 350 g, were sacrificed and the whole small intestine was immediately excised. After removal of the intestinal contents by washing with cold saline, the intestine was everted and blotted with filter paper to remove excess liquid. The intestine was stored at −20° until use. Frozen rat intestines were thawed, and three different materials prepared as follows: 1) the mucosal layer of the intestine, which was scraped off with the fingers; 2) the mucosal epithelial cell layer, which was separated by Stern's method,[19] (an everted intestine was placed in 2 vol of 5.6 mM citrated-phosphate buffer, pH 7.4, at 37°, and the epithelial cells were separated by gentle shaking for 1 hr at 37°); and 3) the intestine, which was minced with an MS 12B-4 meat chopper (Nanjo Tekko Co.). The vasodilator activity was extracted from these materials with deionized water (1 ml per 1 g of original wet weight of intestine). After extraction by gentle stirring for 6—10 hr at room temperature, the suspension was centrifuged to remove insoluble material and the supernatant was assayed for vasodilator activity. The results are shown in Table I. Kallikrein activity was found to be present in all three different supernatants, but the minced intestine contained the most potent vasodilator activity. Hence, the minced intestine was used for the following RIK purification.

TABLE I. Kallikrein Contents in Rat Small Intestine

	Original intestine (g wet weight)	Total KU	KU/g
Mucosal layer	340[a]	80.4	0.24
Epithelial cells	337[a]	42.8	0.13
Minced intestine	395	428	1.08

a) Mucosal layer and epithelial cells were obtained from the indicated amount of intestine.

Purification of Rat Intestinal Kallikrein

The following purification steps were carried out at 4° unless otherwise stated. Kallikrein activity was measured against BAEE during the purification procedures.

Step 1. Extraction——Minced intestine (376 g) was suspended in 1130 ml of deionized water and stirred gently for 6 hr at room temperature. The insoluble material was removed by centrifugation (7000 rpm for 30 min) and fatty substances were eliminated from the supernatant by filtration through gauze.

Step 2. The First Acetone Fractionation——Ice-cold acetone was added dropwise to the supernatant from step 1 to a final concentration of 40% (v/v), and the precipitated formed was removed by centrifugation at 7000 rpm for 20 min. Cold acetone was further added to the supernatant to a concentration of 70% (v/v). The precipitate was collected by centrifugation and dissolved in 200 ml of 0.01 M phosphate buffer (pH 6.8).

Step 3. The Second Acetone Fractionation——The above solution was again fractionated with acetone. The 45—67% (v/v) acetone precipitate was dissolved in 100 ml of 0.1 M Tris–

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HCl buffer (pH 8.0) containing 0.1 M NaCl, and dialyzed against the same buffer (2 liters × 3) for 15 hr.

Step 4. DEAE-Sephadex A-50 Chromatography——The dialyzed material was applied to a DEAE–Sephadex A-50 column (2.0 × 70 cm) equilibrated with 0.1 M Tris–HCl buffer (pH 8.0) containing 0.1 M NaCl. The esterolytic activity was eluted with a linear gradient of 0.1 to 0.5 M NaCl in the above buffer (1 liter in each reservoir), and the active fractions were pooled (140 ml) (Fig. 1).

Step 5. Gel Chromatography on Sephadex G-100——The active fraction was concentrated to 7 ml in an Amicon ultrafiltration cell (model 402) with a UM-10 membrane in an ice bath, and applied to a Sephadex G-100 column (1.5 × 90 cm) equilibrated with 0.01 M phosphate buffer, pH 6.8. The active fractions were combined (30 ml), and dialyzed against the above buffer (1 liter × 2).

Step 6. Hydroxylapatite Chromatography——The sample from step 5 was loaded on a hydroxylapatite column (1.4 × 25 cm) equilibrated with 0.01 M phosphate buffer, pH 6.8. The column was washed with the same buffer until less than 0.01 A_{280} per ml of the eluate was obtained, then RIK was eluted with 0.1 M phosphate buffer, pH 6.8, and the active fractions were pooled (10 ml).

Step 7. Gel filtration on Sephadex G-50——The pool from step 6 was filtered through a Sephadex G-50 column (2.5 × 90 cm) equilibrated with 0.01 M $HCOONH_4$ (pH 8.0). The active fractions (on the basis of BAEE esterolytic activities) were combined (15 ml) and lyophilized.

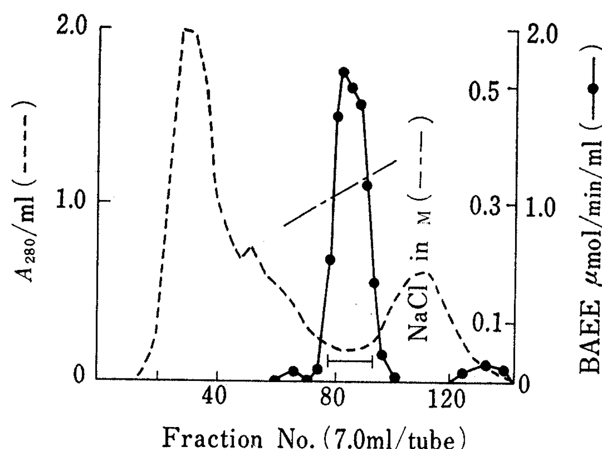

Fig. 1. DEAE–Sephadex A-50 Chromatography of Rat Intestinal Kallikrein

Bracketed fractions were pooled.

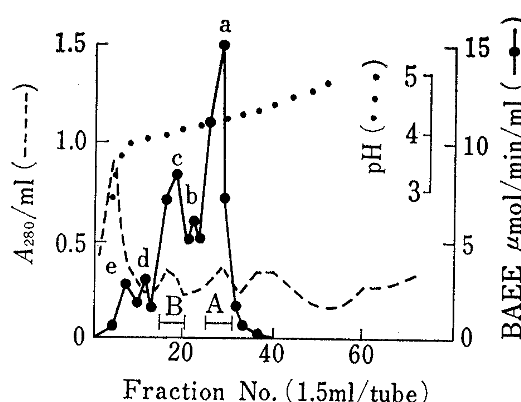

Fig. 2. Ampholine Isoelectric Focusing of Rat Intestinal Kallikrein

Bracketed fractions were pooled.
a: p*I* 4.18 (RIK-A), b: p*I* 4.11, c: p*I* 4.03 (RIK-B), d: p*I* 3.87, e: p*I* 3.72

The overall results of these purification steps are summarized in Table II. The purified RIK preparation had a vasodilator activity of 114 KU per A_{280}, and gave a single major band with a very faint sub-band in disc gel electrophoresis.

Isoelectric Focusing

The lyophilized material (total 284 EU) was further purified by isoelectric focusing, and five active components with p*I*'s 3.27, 3.87, 4.03, 4.11 and 4.18 were separated by this procedure (Fig. 2). Two major enzyme peaks, namely RIK-A (p*I* 4.18) and RIK-B (p*I* 4.03), were separately pooled, and applied to a column of Sephadex G-50 (1.5 × 90 cm) equilibrated with 0.01 M $HCOONH_4$, pH 8.0, to remove carrier ampholyte and sucrose. The results are summarized in Table III. The two final preparations of RIK's gave a single band in disc gel electrophoresis; they were indistinguishable when the two preparations were run together (Fig. 3).

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TABLE II. Summary of the Purification of Rat Intestinal Kallikrein

Procedure	Total A_{280}	Vasodilator act.		Esterolytic act.	
		Total KU	KU/A_{280}	Total EU[a]	EU/A_{280}
Extraction	65400	454	0.007	398	0.006
Acetone (40—70%)	3530	369	0.105	292	0.083
Acetone (45—67%)	601	286	0.476	253	0.421
DEAE-Sephadex A-50	27.1	276	10.2	217	8.00
Sephadex G-100	18.7	213	11.4	196	10.5
Hydroxylapatite	2.4	209	87.1	130	54.2
Sephadex G-50	1.8	206	114	98.0	54.4

a) EU: BAEE μmol/min.

TABLE III. Isoelectric Focusing of Rat Intestinal Kallikrein

Kallikrein	Total A_{280}	Vasodilator act.		Esterolytic act.		KU/EU
		Total KU	KU/A_{280}	Total EU[a]	EU/A_{280}	
RIK	9.73	625	64.2	284	29.2	2.20
RIK-A	0.61	97.8	160.0	47.6	78.0	2.05
RIK-B	0.58	52.2	90.0	33.8	58.3	1.54

a) EU: BAEE μmol/min.

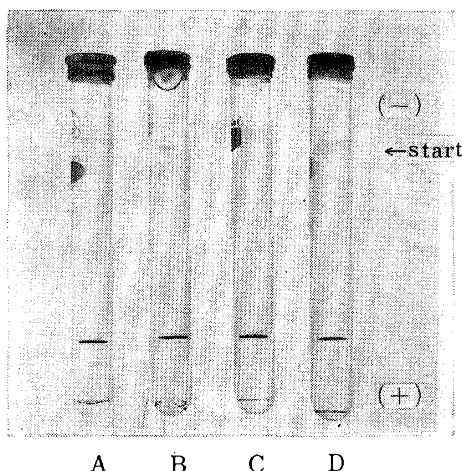

Fig. 3. Disc Gel Electrophoresis of RIK-A, RIK-B and PPK

A: RIK-A (0.03 A_{280}), B: RIK-B (0.03 A_{280}), C: RIK-A (0.015 A_{280})+RIK-B (0.015 A_{280}), D: RPK (0.03 A_{280}).

Molecular Weights and Amino Acid Compositions

The molecular weights of RIK-A and RIK-B were estimated by Sephadex G-100 gel filtration to be 33000 and 35000, respectively, but the molecular weights of both RIK's were found to be 32000 by SDS-PAGE.

The amino acid compositions of RIK-A and -B were very similar to each other, except for the amounts of lysine and a few other amino acids (Table IV).

Substrate Specificities

The specific activities of RIK-A and -B against various substrates are summarized in Table V. Hydrolysis of BTEE and BA*p*NA by both enzymes was negligible.

The K_m values of RIK-A, -B, and RPK against BAEE and TAME were determined from Lineweaver-Burk plots. The K_m values are similar for all these kallikreins (Table V). Substrate inhibition was observed in this experiment, and the velocities of BAEE and TAME hydrolysis by both RIK's decreased slightly at a substrate concentration of 1 mM.

The substrate specificities of RIK-A, -B, and RPK were also studied with various derivatives of arginine and lysine methylester (Table VI). Among them, AcAME was most readily hydrolyzed by these enzymes. Although both intestinal kallikreins showed similar specific activities with these substrates, both were different from rat plasma kallikrein, trypsin, and plasmin as regards substrate specificity.

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Table IV. Amino Acid Compositions of RIK-A, RIK-B and RPK

Amino acid	% of amino acid residues in 100 residues		
	RIK-A	RIK-B	RPK[a]
Lysine	3.95	2.15	4.76
Histidine	2.47	2.26	2.20
Arginine	2.39	2.56	0.73
Aspartic acid	11.4	12.4	12.8
Threonine	5.96	5.38	5.13
Serine	5.89	5.90	4.40
Glutamic acid	12.3	13.6	10.2
Proline	8.28	7.21	8.79
Glycine	11.3	12.7	9.16
Alanine	5.38	5.14	3.66
Cysteine	3.68	5.03	2.93
Valine	6.73	6.00	6.23
Methionine	1.57	1.38	2.20
Isoleucine	3.63	3.64	4.76
Leucine	8.17	6.96	11.7
Tyrosine	3.07	3.33	3.30
Phenylalanine	2.68	2.93	2.20
Tryptophan	1.13	1.43	4.40

a) Cited from ref. 20.

Table V. Substrate Specificities of RIK-A, RIK-B and RPK

Kallikrein	KU/A_{280}	Units/A_{280}					K_m (mM)[c]	
		BAEE	TAME[a]	TAME[b]	BTEE	BA*p*NA	BAEE	TAME[a]
RIK-A	160	78.0	10.1	14.8	N.D.[d]	N.D.	0.11	0.09
RIK-B	90.0	58.3	5.28	10.4	N.D.	N.D.	0.08	0.11
RPK	143	67.7	12.2	13.6	N.D.	N.D.	0.07	0.06

a) Determined spectrophotometrically.
b) Determined colorimetrically.
c) Determined from Lineweaver–Burk plots.
d) N.D.: not detectable.

Table VI. Substrate Specificities of RIK-A, RIK-B, RPK and other enzymes

Substrate	RIK-A	RIK-B	RPK	Trypsin	Plasmin	Rat plasma K.
TAME	100	100	100	100	100	100
AcAME	870	862	724	35	112	39
AME	25	27	23	17	11	177
TLME	18	21	21	55	186	165
AcGLME	97	97	137	15	625	42
AcLME	49	59	36	—	—	—
LME	3.8	4.4	1.9	—	—	—
HME	2.3	2.9	0.1	—	—	—

Esterolytic activities of the enzymes were measured by the colorimetric method with chromotropic acid.[9] The activity was expressed relative to TAME-hydrolyzing activity taken as 100.

Optimum pH

The effects of pH on BAEE hydrolysis by RIK-A, -B, and RPK were examined in the Britton-Robinson wide-range buffer, and the optimal pH's for these enzymes were found to be around 9.2.

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Heat and pH Stabilities

RIK-A, -B, and RPK were incubated at 40, 50, 60, and 70° for 10 and 30 min in 0.05 M Tris-HCl buffer (pH 8.0), and the BAEE esterolytic activities were determined. The initial activities of RIK-A, -B, and RPK were 0.44, 0.40, and 0.46 EU/ml, respectively. All of these enzymes were stable to these treatments, more than 90% of the activity being retained after incubation at 50° for 30 min and 60% after 30 min at 60°.

RIK-A (0.2 EU/0.2 ml), RIK-B (0.22 EU/0.2 ml), and RPK (0.22 EU/0.2 ml) were incubated with 1.0 ml of 0.08 M Britton-Robinson wide-range buffer between pH 5—11 at 4° for 36 hr, and the residual activities determined at pH 8.0. More than 90% of the initial activity was retained in the pH range from 7 to 10, and 70% at pH 5.

Effects of Various Proteinase Inhibitors on RIK-A, -B, and RPK

Equal volumes of the kallikrein and inhibitor solution (the final concentrations are shown in Table VII) were mixed and incubated for 5 min at 25°, and the residual EU was measured. As shown in Table VII, RIK-A, -B, and RPK were inhibited by aprotinin and leupeptin, but were insensitive to SBTI, LBTI, antipain, pepstatin, chymostatin, TLCK, and TPCK. Like other kallikreins from rat and dog pancreas,[20] RIK-A and -B were inhibited by DFP, indicating that they belong to the group of serine proteinases.

TABLE VII. Inhibitory Effects of Various Proteinase Inhibitors on RIK-A, RIK-B and RPK

Inhibitor	Inhibitor amount	Inhibition %		
		RIK-A (0.87 EU)	RIK-B (0.86 EU)	RPK (0.90 EU)
Aprotinin	10 KIU	20	14	18
	20	52	44	50
	30	86	89	87
	40	90	90	90
SBTI	1.0 mg	10	15	16
	5.0	13	17	18
LBTI	1.0 mg	N.D.	N.D.	12
Leupeptin	0.1 mg	28	27	45
	0.2	40	38	50
	0.3	50	45	54
	0.5	60	55	61
Antipain	0.5 mg	19	16	18
Chymostatin	0.5 mg	N.D.	N.D.	15
Pepstatin	0.5 mg	N.D.	N.D.	11
DFP	1.0 mM	N.D.	N.D.	N.D.
	10	54	58	40
TPCK	1.0 mM	N.D.	N.D.	N.D.
TLCK	1.0 mM	N.D.	N.D.	N.D.
L-Cysteine	1.0 mM	N.D.	N.D.	N.D.
Thioglycol	1.0 mM	6	8	10
EDTA·2Na	1.0 mM	N.D.	N.D.	N.D.
8-Hydroxylquinoline-5-sulfonate	1.0 mM	28	25	25

Final concentrations (per ml) of kallikreins and inhibitors are given in parenthesis.
N.D.: not detectable.

Discussion

In 1960, Werle *et al.*[4] reported that the levels of prekallikrein and kallikrein in rat small intestine were 0—0.2, and 0.8—1.2 KU per g wet weight, respectively. In our investigation,

20) Y. Hojima, M. Yamashita, N. Ochi, C. Moriwaki, and H. Moriya, *J. Biochem.*, **81**, 599 (1977).

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the vasodilator activity of the extract from minced intestine was approximately 1 KU per g wet weight. This value is in good agreement with the results of Werle.[4] Zeitlin *et al.*[21] found that kallikrein and prekallikrein were present in the mucosal layer of the colon but not in the muscle layer. However, our data suggest that kallikrein is present in not only the mucosal layer but also other parts of the small intestine (Table I).

RIK was separated into five active fractions with different p*I*'s by isoelectric focusing. Among them, RIK-A and -B were homogeneous in disc electrophoresis (Fig. 3). The existence of multiple forms of kallikreins has been reported by many investigators in rat urine,[22-24] human urine,[25,26] dog pancreas,[20] hog pancreas,[27-29] and rat submaxillary gland.[30] Moriya *et al.*[31] reported that the multiple forms of hog pancreatic kallikrein were characterized by different amounts of neutral hexose and glucosamine, and the microheterogeneity of hog pancreatic kallikrein-B was attributed to differences in sialic acid content. Therefore, there may be differences in carbohydrate composition, especially sialic acid, between RIK-A and -B although no evidence has yet been obtained. The amino acid compositions of RIK-A and -B were almost identical, though there were some differences in the amounts of lysine and a few other amino acids. However, the difference between the amino acid compositions of RIK's and RPK was more significant than that between RIK-A and -B. The differences in the amounts of arginine, glutamic acid, leucine, cysteine, tryptophan, *etc.*, were marked. Hojima *et al.*[20] reported that RPK was not separated into multiple forms by isoelectric focusing, but stated that the p*I* of RPK was approximately 4.1, which is very close to those of RIK's. The apparent molecular weights of RIK's were slightly larger than that of RPK; the molecular weight of RPK was reported to be about 30000 by Hojima *et al.*,[21] but those of RIK-A and -B were estimated to be 33000 and 35000 by Sephadex G-100 gel filtration. These values are in good agreement with the results previously reported by Zeitlin.[32] We suggest that the intestinal kallikrein is not derived from RPK, but is endogeneous to the small intestine.

Zeitlin[33] reported that a rat intestinal homogenate had two pH optima against BAEE at pH's 6.5 and 8.5, but only one pH optimum at 8.5 on kininliberation. The purified RIK-A and -B had only one optimum pH at 9.2 in BAEE hydrolysis. If another BAEE esterase was present in rat intestine as described by Zeitlin,[33] it might have been eliminated during the present purification procedures.

Sulfhydryl compounds or chelating agents did not inhibit RIK-A and -B. Both enzymes were insensitive to pepstatin, antipain, chymostatin, SBTI, TLCK, and TPCK. Therefore, RIK's are neither thiol- nor metal-enzymes, nor trypsin-, chymotrypsin-, pepsin- or plasma kallikrein-like enzymes. RIK-A, -B, and RPK were inhibited by aprotinin, leupeptin, and DFP. Zeitlin[6] reported that the kinin-forming activity of the rat intestinal homogenate was not inhibited by TLCK or SBTI, but was inhibited by aprotinin. More recently, Zimmermann *et al.*[34] described a kallikrein from human large intestine which was inhibited by

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aprotinin, but not by SBTI, whereas Seki *et al.*[7] reported that human colon kallikrein was sensitive to both inhibitors. In view of the actions of proteinase inhibitors on both human kallikreins, the former is thought to resemble the glandular kallikreins while the latter is similar to plasma kallikrein. Our results with RIK-A and -B have shown that both RIK's belong to the class of serine proteinases and have the properties of glandular kallikreins.

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