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HUMAN URINARY KALLIKREIN - BIOCHEMICAL AND PHYSIOLOGICAL ASPECTS

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The history of kallikrein begins with the discovery of urinary kallikrein more than fifty years ago. The renal origin of urinary kallikrein, though not finally proved, is largely accepted. A possible role of kallikrein in the regulation of kidney function and blood pressure has been debated for a long time (Pisano and Auster, 1976). However, neither the question for its origin, nor for its physiological role is finally settled.

Isolation and Characterization

As a first step in the development of a radioimmunoassay and of enzymatic assays for human urinary kallikrein an isolation method for the enzyme was set up (Geiger et al. 1977). The method is summarized in Table 1 (a slightly different method was reported earlier, Mann and Geiger, 1977).

Preparations of human urinary kallikrein obtained from different urine pools were subjected to polyacrylamide gel electrophoresis at pH 6 and 8. Two different patterns of protein bands were obtained. For some preparations only one band was visible after staining, whereas other preparations were resolved into three bands as described also by Matsuda et al. (1976). All bands contained active kallikrein as was demonstrated by active enzyme staining using Z-Ser-Pro-Phe-Arg-MNA and coupling with Fast Blue B Salt (Smith et al. 1975). After incubation with Trasylol, however, only a single band of lower electrophoretic mobility was detected (Fig. 1) for all preparations.

Table 1.

Isolation of human urinary kallikrein

Steps	Purification
Collection of human male urine	-
Dialysis	-
Lyophilization	-
Extraction of the crude urine powder	-
Sephacryl S-200	1
Trasylol Sepharose	89
DEAE-Sepharose	240

Amino acid analyses of the human urinary kallikrein were done by common methods, carbohydrate content was determined as described by Krystal and Graham (1976). The results are shown in Table 2 and 3. According to our preliminary results, the amino acid compositions of human urinary kallikrein is very similar to that of pig pancreatic β-kallikrein. The values for Ser, Gly, Ala, Cys and Met are identical, whereas minor differences exist for the other amino acids. Some differences also exist in the amino acid compositions of preparations isolated from different urine pools.

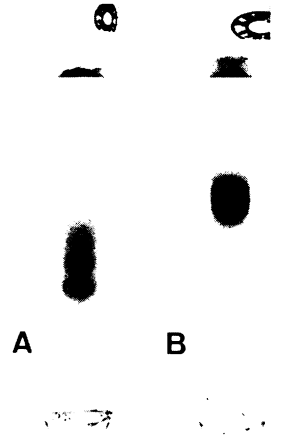

Fig. 1. Acrylamide gel electrophoresis (7.58% gel, pH 8) of human kallikrein.
A: purified enzyme B: purified enzyme complexed with Trasylol

Table 2.

Amino acid composition of kallikreins from human urine and porcine pancreas.

Tryptophan was not determined, cystein after performic acid oxidation (Hirs 1976).

	Human urinary kallikrein	Porcine pancreatic β-kallikrein (Fiedler et al. 1977)
Asp	20-23	28
Thr	14	15
Ser	14	14
Glu	28-30	23
Pro	14	16
Gly	22	22
Ala	12-14	13
Cys	10	10
Val	13-16	10
Met	4	4
Ile	8-9	12
Leu	18	20
Tyr	9	7
Phe	8-12	10
Lys	8-12	10
His	8-9	8
Arg	6	3
Trp	?	7

Table 3.

Carbohydrate content of human urinary kallikrein and porcine pancreatic kallikrein

Kallikrein from	Carbohydrate content g/100 g protein	
Porcine pancreas		(Fiedler et al. 1975)
form A	5.6	
form B.	11.5	
Human urine	10.5 - 15.6	

The differences in both amino acid composition and electrophoretic patterns suggest that our preparations of human urinary kallikrein contain multiple forms as a result of limited proteolysis. Limited proteolytic attack causes cleavages within a protein chain, eventually resulting in the release of single amino acids or peptides. These cleavages and the loss of parts of the protein molecule can give rise to changes in electrophoretic mobility and amino acid composition. Multiple forms caused by limited proteolysis have also been observed for pig pancreatic kallikrein (Fiedler et al. 1977).

Assay Methods

It has been observed in our laboratory that Ac-Phe-ArgOMe is a much better substrate for porcine pancreatic (Fiedler 1976), submandibular and urinary kallikreins (Fritz et al. 1977) than α-N-acetylated arginine esters. The corresponding ethyl ester, Ac-Phe-ArgOEt , is the most rapidly hydrolyzed substrate for human urinary kallikrein described as yet (Fiedler et al. 1978). Therefore, a highly sensitive assay for human urinary kallikrein could be developed employing Ac-Phe-ArgOEt as substrate. The assay (Table 4) is analogous to that with Bz-ArgOEt developed by Trauschold and Werle (1961). The reaction sequence is shown in Fig. 2. The sensitivities of this and other assays for human urinary kallikrein are compiled in Table 5. The sensitivity of the assay allows the convenient measurement of the esterase activity of human urine. Urine samples 20 20 - 100 µl cause a linear absorbance increase of 0.04 to 0.2 per 10 min. Known amounts of human urinary kallikrein added to urine samples raised the esterase activity to the expected extent. If the urine samples contain ethanol it has to be removed by dialysis. Therefore, ethanol intake should be avoided during the urine collection period.

Table 4.

Assay of human urinary kallikrein using the substrate Ac-Phe-ArgOEt

2.00 ml	0.15 M sodium diphosphate buffer, pH 8.7 containing 0.15 M semicarbazidium chloride and 0.0375 M glycine
0.10 ml	0.03 M NAD
0.10 ml	0.015 M AcPheArgOEt acetate
0.02 ml	alcohol dehydrogenase (100 mg/3.4 ml)
(0.28+x) ml	water
	5 min preincubation at 25°C
(0.5-x) ml	enzyme solution
Final volume:	3 ml

The change in absorbance is monitored for 10 min. at 366 nm.

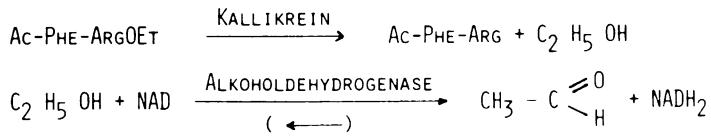

Fig. 2. Reaction scheme of kallikrein assay using Ac-Phe-ArgOEt as substrate.

A number of experiments were undertaken to verify that the esterase activity reflects the kallikrein content of urine. Trasylol completely inhibited the esterase activity. Dialysed urine samples of 10 different persons were assayed. The results were compared with those obtained by the dog blood pressure assay, by a radioimmunoassay for human urinary kallikrein (Mann and Geiger 1977) and by the assay with D-Val-Leu-ArgNHNp as substrate (Fig. 3). Though the correlation coefficient of the results of the Ac-Phe-ArgOEt assay and the dog blood pressure assay (Fig. 3a)

Table 5. Absorbance changes in assays of human urinary kallikrein with various substrates.

(Reaction volume 3 ml, cuvette light path 1 cm)

	Blank ($\Delta A \cdot 10^3 \times min^{-1}$)	Reaction ($\Delta A \cdot 10^3 \times min^{-1} \times U^{-1}$)	Relative Sensitivities
Ac-Phe-ArgOEt	0.6	1 100	46
D-Val-Leu-ArgOEt	0.6	430	20
B_z-ArgOEt	0.5	24	1
Z-TyrONp	15	460	19
D-Val-Leu-Arg-p-nitranilide	0.0	29	1.2
D-Pro-Phe-Arg-p- "		3	
Tos-Gly-Pro-Arg-p- "		1	
Bz-Phe-Val-Arg-p- "		0.3	
Glu-Gly-Arg-p- "		0.3	

is rather close to 1, a considerable scattering of the data is observed. This is not too surprising, since the coefficient of variation of the blood pressure assay for kallikrein amounts to 20% (Arens and Haberland 1973). The correlation between the Ac-Phe-ArgOEt assay and both the radioimmunoassay (Fig. 3b) and the D-Val-Leu-Arg-p-nitroanilide assay is even more satisfactory. The excellent correlation between the two enzymatic assays (Fig. 3c) strongly suggests that in both assays the same enzyme is determined. For a mixture of enzymes a similarly good correlation would only be expected if these enzymes were always excreted in identical ratios.

D-Val-Leu-Arg-p-nitroanilide has been suggested quite recently by KABI (Amundsen et al. 1978) as a substrate for the determination

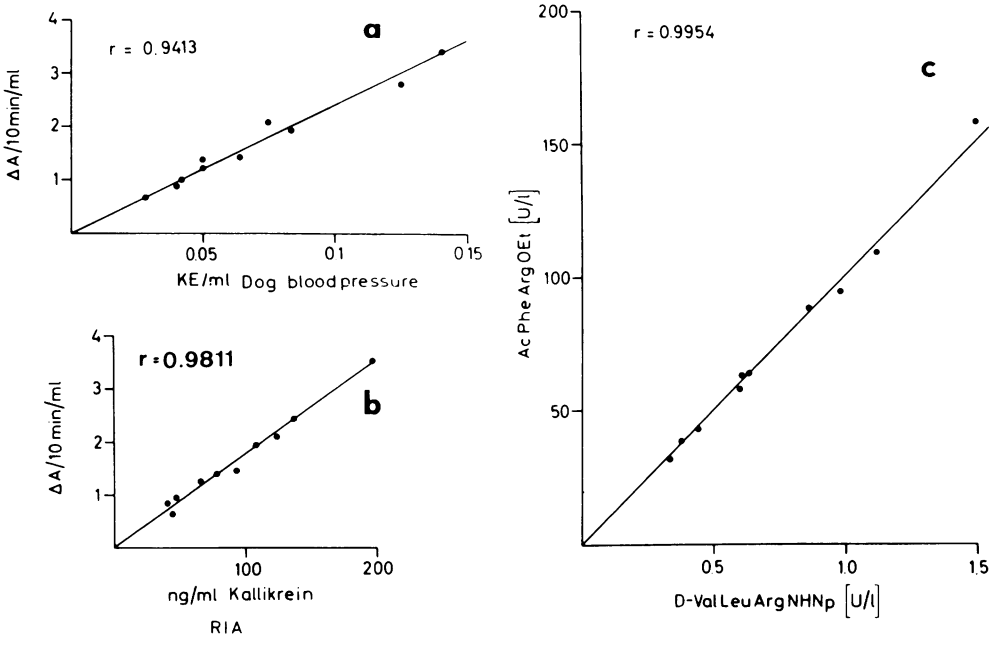

Fig. 3. Comparision of the Ac-Phe-ArgOEt assay for human urinary kallikrein with the blood pressure assay (a), the radioimmunoassay (b) and the D-Val-Leu-ArgNHNp assay (c).

10 samples of human urine were measured.

of human urinary kallikrein. Hydrolysis of p-nitroanilides can be monitored at 405 nm. Negligible spontaneous hydrolysis of these compounds allows working at 37°C. To obtain abosrbance changes of sufficient magnitude, an incubation time of 30 min was necessary for urine samples of 50 to 500 µl. This long incubation time precluded continuous monitoring of the reaction. The results had to be corrected for the inherent absorbance of the urine samples that was determined in parallel (blanks contain water instead of substrate). The reaction conditions are given in Table 6.

Evidently, both Ac-Phe-ArgOEt and D-Val-Leu-ArgNHNp are useful substrates for kallikrein determination in human urine. The advantage of the Ac-Phe-ArgOEt assay is the possibility of continuous monitoring during the test, a drawback is the alcohol sensitivity of the reaction. This is not found in the assay with D-Val-Leu-ArgNHNp, but due to its low sensitivity, this method suffers from the disadvantages of a two point assay.

The radioimmunoassay for human urinary kallikrein was also applied to clarify, whether endogenous glandular kallikrein is present in the blood. We found glandular kallikrein in human serum in concentrations of 10-15 ng/ml. In order to ascertain that the radioimmunoassayable substance was not a low molecular degradation product, serum samples were subjected to gel filtration and the fractions tested by radioimmunoassay (Fig. 4). After gel filtration one peak was detected in the position of a molecular weight of about 80,000 whereas our kallikrein preparation was eluted in the position of about 50,000. The exact origin of the glandular kallikrein in blood is unknown, a discrimination by radioimmunoassay is impossible because of the immunological cross-

Table 6.

Assay of human urinary kallikrein using the substrate D-Val-Leu-ArgNHNp

0.4 ml	0.4 M TRIS/HCl, pH 8.2
x ml	urine
(0.5-x) ml	water
	5 min preincubation at 37°C
0.1 ml	0.001 M D-Val-Leu-ArgNHNp
	Incubation at 37°C

The absorbance increase (405 nm) after 30 min. is read.

reactivity of the various tissue kallikreins. The presence of glandular kallikrein in blood leads to the assumption that renal filtration might contribute to some extent to the amount of kallikrein found in urine.

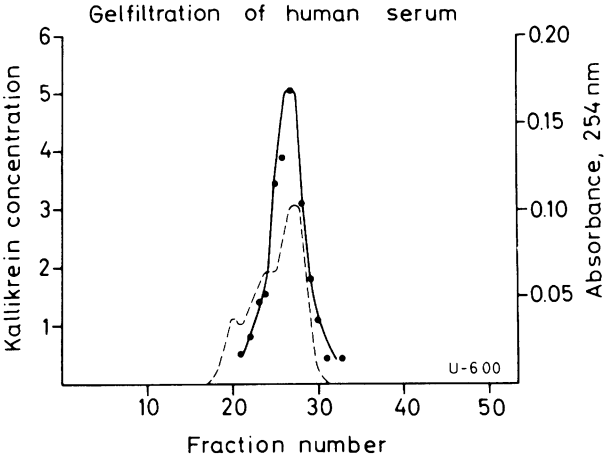

Fig. 4. Gel filtration of human serum through Sephacryl S-200.

The fractions were assayed by a radioimmunoassay for human urinary kallikrein.

ABBREVIATIONS

Bz-ArgOEt: N^{α}-Benzoyl-arginine ethyl ester, Z-TyrONp: Carbobenzoxytyrosine nitrophenyl ester, Ac-Phe-ArgOMe: Acetyl-phenylalanyl-arginine methyl ester, Ac-Phe-ArgOEt: Acetyl-phenylalanyl-arginine ethyl ester, Z-Ser-Pro-Phe-Arg-MNA: Carbobenzoxy-seryl-prolyl-phenylalanyl-arginine-p-nitroanilide, D-Val-Leu-ArgOEt: D-valyl-leucyl-arginine ethyl ester.

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