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Human Renal Kallikrein: Purification and Some Properties[1]

Human renal kallikrein (HRK, E.C. 3.4.21.8) was purified about 540-fold by using chromatographic techniques with an overall yield of 4.6% from crude preparation of HRK being prepared with acetone activation of human kidney cortex extract. The activities of this kallikrein were 3.2 KU/A_{280} of vasodilator and 0.27 μmol/min/A_{280} of TAME esterolytic activities. This purified HRK was observed to be nearly single band with faint subband on disc gel electrophoresis. The approximate molecular weight of HRK was estimated to be 4.7 to 4.9×10^4 by gel filtration on Sephadex G-100 and G-150 columns.

Keywords——purification of kallikrein; human kidney; vasodilator activity; esterolytic activity; acetone activation

The functional relationship between the renal and urinary kallikreins (E.C. 3.4.21.8) has been studied by a few investigators.[2] Recently, the renal kallikreins were purified with rat and dog, and their biochemical and physiological properties have been getting revealed.[3] On the other hand, the study on the human renal kallikrein (HRK) was somewhat difficult becaues of its low content in the human gland. In order to get a clue to elucidate the renal and urinary kallikrein system in human, the purification of HRK is at first important.

The present paper deals with the purification and some properties of HRK.

Materials and Methods

Materials——The human kidney, starting material in this study, was washed and perfused with saline, and stored at −20°C.

DEAE-cellulose was obtained from Green Cross Drug Mfg. Co., Osaka, Japan. Sephadex G-100 and G-150 were supplied from Pharmacia AB, Sweden. N-α-Tosyl-L-arginine methyl ester (TAME) and synthetic bradykinin from Protein Research Foundation, Osaka, Japan, were used.

Assay of Kallikrein Activities——Vasodilator activity was assayed by measuring the increase in femoral arterial blood flow following the injection of samples in the dog,[4] and was expressed as kallikrein unit(KU). Esterolytic activity was measured by the fluorometric method[5] using TAME as substrate and was expressed in terms of μmol TAME hydrolyzed/min at pH 8.0, 30°C.

Protein Determination——The absorbancy at 280 nm in a 1 cm width cuvette was routinely measured to estimate protein concentrations of various solutions.

Disc Electrophoresis——This was performed to the modified method of Davis,[6] using 7% (w/v) polyacrylamide gel columns and 0.04 M Tris-glycinate buffer at pH 8.6.

Results and Discussion

The activation of HRK by acetone was investigated under the same method described in the previous paper with dog renal kallikrein,[3b] using acetone concentration of 0 to 50% (v/v). The maximum relative activity, measured by vasodilator assay, was found at 30% (v/v) as approximately 1.4 times more than that of the water extract without activation treatment. Therefore, the acetone concentration for activation was fixed at 30% (v/v)

through the experiment. However, the ratio of the vasodilator activity of HRK with activation treatment to without treatment was slightly low in comparison with dog renal kallikrein.[3b] This difference could be ascribed to species differences.

The purification procedures of HRK were correspondingly applied to the methods described with dog renal kallikrein,[3b] and the elution buffer of 0.05 M Tris-HCl (pH 7.5) was used at all chromatography steps. Three hundred fortytwo g of kidney cortex was obtained and used, and the results of purification procedures were summarized in Table I. Most of non kallikrein arginine esterase was removed by DEAE-cellulose adsorption and elution step using above mentioned buffer containing 0.05 M NaCl for elute solution. This enzyme had not vasodilator activity and thus non kallikrein arginine esterase had not been contained in the human urine[7] but in the dog kidney[3b] and urine.[8] The specific activities of finally purified preparation of HRK were 3.2 KU/A_{280} of vasodilator and 0.27 μmol/min/A_{280} of TAME esterolytic activities and the ratio of vasodilator to esterolytic activities was 11.9 KU per μmol TAME/min. This value differed from those observed with human urinary and dog renal kallikreins as calculated to be 23.5[7] and 58,[3b] respectively. The final preparation of HRK was purified 540-times more than that of first step of acetone activation. This preparation was observed to be nearly single band with faint subband on disc gel electrophoresis.

Table I. Summary of Purification of HRK

Step	Procedure	Protein Recovery (%)	Vasodilator activity			Esterolytic activity		
			Recovery (%)	KU/A_{280}	P.F.[a]	Recovery (%)	μmol TAME /min/A_{280}	P.F.[a]
1	Activation of acetone	100	100	0.0059	1	100	0.0029	1
2	DEAE-cellulose adsorption							
	0.05 M NaCl elution	0.94	N.D.[b]	N.D.[b]	N.D.[b]	9.7	0.030	10.3
	0.5 M NaCl elution	16.2	28.5	0.011	1.8	11.2	0.0020	0.69
3	Acetone fractionation (50—80%)	3.7	24.2	0.038	6.5	4.7	0.0027	0.93
4	DEAE-Sephadex A-50 chromatography	0.28	21.4	0.46	78	2.7	0.028	9.7
5	Sephadex G-100 gel filtration	0.034	14.9	2.6	440	2.5	0.21	72
6	Sephadex G-150 gel filtration	0.0085	4.6	3.2	540	0.80	0.27	93

a) P.F.: Purification factor.
b) N.D.: Not detected.

Approximate molecular weight of purified HRK was estimated by the method of Andrews[9] using Sephadex G-100 and G-150 columns, being estimated to be 4.7 to 4.9×10^4. This values was somewhat larger than that of human urinary kallikrein (2.7 to 2.9×10^4).[7] This observation with the molecular weights of renal and urinary kallikreins was quite noticeable, showing the matter in which the molecular weight of renal kallikrein had been usually a little larger in compared with that of urinary kallikrein within the same species.[3,7,10] This matter might be suggested that the kallikrein molecule would be transformed getting small during the excretion into the urine.

For the study of HRK, now we noted a few results, such as purification and estimation of molecular weight in this investigation. In spite of some difficulty of collecting human kidney, the authors would like to make study of further properties of HRK in order to elucidate the renal and urinary kallikrein system in human.

References and Notes

1) Abbreviations: HRK, human renal kallikrein; KU, kallikrein unit; TAME, N-α-tosyl-L-arginine methyl ester.
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