HUMAN PLASMA KALLIDINS: ISOLATION AND CHEMICAL STUDIES

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The action of the kallikreins on plasma to form a hypotensive and smooth muscle stimulating peptide, substance DK, was first reported by Werle and co-workers (Werle, Götze and Keppler, 1937; Werle and Grunz, 1939). Subsequent studies (Werle and Hambuechen, 1943; Werle and Berek, 1950; Werle, 1955) showed that the kallikreins act enzymatically on a plasma α_2-globulin, called kallidinogen, to release the peptide, renamed kallidin (Werle and Berek, 1948).

Simultaneously with these investigations, Rocha e Silva, Beraldo and Rosenfeld (1949) reported that trypsin and some snake venoms are capable of releasing from plasma a hypotensive and smooth muscle stimulating peptide which they called bradykinin. Bradykinin formed by the action of trypsin on a bovine plasma fraction (Elliott, Horton and Lewis, 1961) has been shown to be the nonapeptide H.Arg.Pro.Pro.Gly.Phe.Ser.Pro.Phe.Arg.OH by degradation (Elliott, Lewis and Horton, 1960) and by synthesis (Boissonnas, Guttmann and Jaquenoud, 1960).

Crude preparations of bradykinin and kallidin were found to be chemically and pharmacologically indistinguishable by several investigators (Werle, Kehl and Koebke, 1950; Holdstock, Mathias and Schachter, 1957; Mathias and Schachter, 1958; Gaddum and Horton, 1959; Schachter, 1960). Similar results were obtained by Van Arman and Miller (1961), with the notable exception that two bradykinins and probably two kallidins could be detected.

The present report describes the isolation of two kallidins from the incubation of human urinary kallikrein with acid-treated human plasma.

Kallidin I could not be distinguished from bradykinin, whereas kallidin II differed from bradykinin by several physical and chemical criteria.

Outdated ACD human plasma was incubated at 37° for 10 minutes at pH 3.0 (compare Gaddum and Horton, 1959). The pH was adjusted to 7.4 and 75 Frey units of human urinary kallikrein (6 Frey units/mg: see Webster and Pierce, 1960, 1961) were added per liter of plasma. After incubation at 37° for 2 hours, the jelly-like reaction mixture was diluted with one to four volumes of water and stirred vigorously for 2 hours at pH 4 to 5 (20°) with 2.5 g of Amberlite XE-64(H^+) per liter of starting plasma. The resin, recovered by filtration, was washed thoroughly with water and eluted batchwise at 25°, initially with 0.1 M $HCOONH_4$ (20 ml/g of resin) and aqueous NH_3 to pH 6.5, then with 0.2 M $HCOONH_4$ and NH_3 to pH 8.2. The second eluate, containing the biological activity, was adjusted to pH 4 with HCOOH, freeze-dried, and an aqueous extract of the dried product was chromatographed twice on Sephadex G-25 (Porath and Flodin, 1959). The first column was developed with 0.002 M HCl (Fig. 1A) and the active fractions (37 mg) were chromatographed on a second column (1.25 x 94 cm), developed with 0.005 M $HCOONH_4$ at pH 8.5. The dried product, dissolved in 0.01 M $HCOONH_4$, pH 5.0, was chromatographed on CM (carboxymethyl)-cellulose (0.6 meq/g) by minor modifications of the method of Elliott et al. (1961). Two peaks of activity were obtained (Fig. 1B). These were designated kallidins I and II. The fractionation of 20 liters of plasma is summarized in Table I.

Kallidin II moved more slowly on paper chromatograms developed with 63:10:27 n-butanol-acetic acid water, more rapidly in paper electrophoresis with 2M acetic acid, and was more strongly adsorbed to CM-cellulose than kallidin I and bradykinin, which could not be distinguished from each other in any of these systems. In its ability to contract smooth muscle, kallidin I was about as potent as pure synthetic bradykinin, while kallidin II was about one-half as potent. Both kallidin preparations were found by paper chromatography and electrophoresis to contain inactive, ninhydrin-positive substances. High-voltage paper electrophoresis and two-dimensional paper chromatography of acid hydrolysates of the kallidins and natural and synthetic

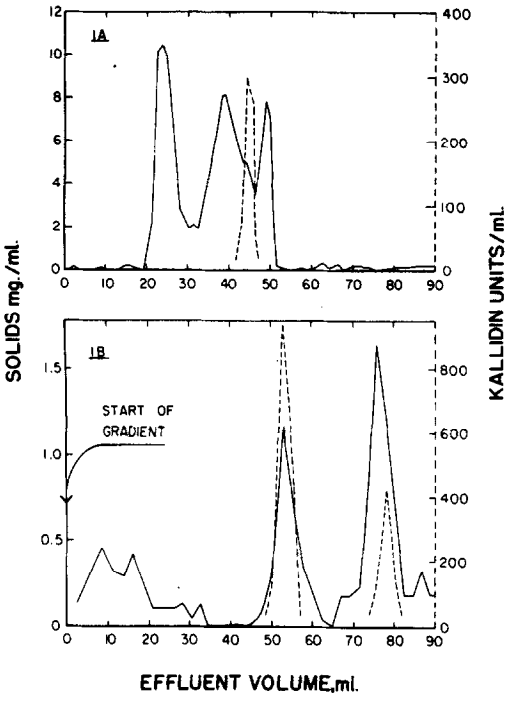

Figure 1. A. Sephadex G-25 chromatography at 4° of the pH 8.2 eluate from XE-64. Sample: 200 mg, from 4 liters of plasma, in 2 ml water. Column: 1.7 x 72 cm. Eluent: 0.002 M HCl. Fraction volume: 1 ml.

B. CM-cellulose chromatography at 4° of product from second Sephadex chromatogram. Sample: 26 mg, from 20 liters of plasma, in 0.5 ml 0.01 M $HCOONH_4$, pH 5.0. Column: 0.67 x 23 cm. Eluents: 80 ml 0.01 M $HCOONH_4$-0.001 M EDTA, pH 5.0; 50 ml 0.01 M $HCOONH_4$, pH 5.0; gradient from 0.01 M to 0.5 M $HCOONH_4$ and from pH 5.0 to 7.5. Volume of mixing chamber: 125 ml. Fraction volume: 2 ml.

bradykinins gave very similar results, except that lysine was found in the kallidin II hydrolysate and small amounts of from one to three amino acids other than the expected ones were found in the hydrolysates of the kallidins and of natural bradykinin.

The N-terminal amino acid residue of kallidin I was identified as arginine by the fluorodinitrobenzene method. Lysine was identified as the N-terminal residue of kallidin II by applying the Edman method as modified by Sjöquist (1959) and comparing the solvent-extractable derivative with authentic N^{ϵ}-phenylthiocarbamyl-lysine-phenylthiohydantoin in three paper chromatographic systems. Paper chromatography of the remaining peptide gave a single active, Sakaguchi-positive spot with the same R_f as bradykinin (Fig. 2).

TABLE I

PURIFICATION OF KALLIDINS FROM 20 LITERS OF HUMAN PLASMA

Description of Fraction	Purity Index, Units/mg*	Purification Factor	Recovery of Activity, %
Acid-treated Plasma + Human Urinary Kallikrein	0.012	1	(100)
XE-64 Adsorption			
Filtrate			10
pH 6.5 Eluate			5
pH 8.2 Eluate	6	430	35
Sephadex G-25 Chromatography			
First	60	4,300	20
Second	180	12,900	20
CM-Cellulose Chromatography			
Kallidin I	500	36,000	15
Kallidin II	225	16,000	5

* One unit of kallidin is defined as that amount of activity found in 1 ml of kallikrein-treated normal human plasma. Activity was determined by ability to contract the large intestine of the guinea pig.

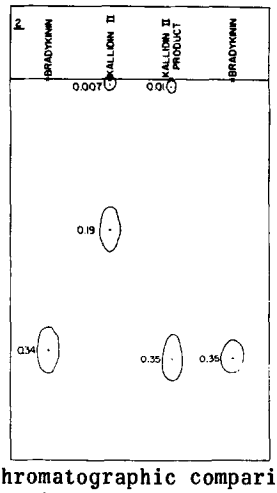

Figure 2. Paper chromatographic comparison of the first stage Edman degradation product from kallidin II with synthetic bradykinin and untreated kallidin II. Spots were detected by Sakaguchi reagent and duplicate, adjacent spots (not shown) were eluted for determination of activity.

These data indicate that kallidin I is identical with bradykinin and that kallidin II is a decapeptide with the sequence H.Lys.Arg.Pro.Pro.Gly.Phe.Ser.Pro.Phe.Arg.OH.

Whether human urinary kallikrein produces two peptides is open to question. Preliminary results suggest that incubation of the pH 3.0-treated outdated human plasma at pH 7.4, without added kallikrein, produces active peptides, presumably by the activation of plasma enzyme(s). Therefore, the kallidins we have isolated probably arose from the combined action of these enzyme(s) and human urinary kallikrein. Isolation of the kallidins by the present procedure from 5 liters of fresh ACD human plasma gave equal yields (15%) of kallidins I and II. Since human urinary kallikrein can hydrolyze synthetic arginine esters but not lysine esters (Webster and Pierce, 1961), this enzyme may produce only kallidin II from kallidinogen.

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