Angiotensin (bovine)	Asp-Arg-Val-Tyr-Val-His-Pro-Phe-His-Leu
Gallus gallus (Angiotensin-F)	Asp-Arg-Val-Tyr-Val-His-Pro-Phe-Ser-Leu
Elaphe climocophora (Angiotensin-S)	X-Asp-Arg-Val-Tyr-Val-His-Pro-Phe-Tyr-Leu

CHROMATOGRAPHIC SYSTEMS FOR DESALTING AND SEPARATING KININS: APPLICATION TO TRYPSIN-TREATED HUMAN PLASMA

M.U. Sampaio, M.L. Reis, E. Fink, A.C.M. Camargo and L.J. Greene

Biology Department, Brookhaven National Laboratory, Upton, New York
and
Department of Pharmacology, Faculty of Medicine of Ribeirão Preto, University of Sao Paulo, 14100 Ribeirao Preto, ESP, Brasil

An analytical method is presented for the separation of Bradykinin (K-9), Lys-Bradykinin (K-10), and Met-Lys-Bradykinin (K-11) by equilibrium chromatography on SP-Sephadex, C-25. The column (0.3 x 50.0 cm) is operated at room temperature under equilibrium conditions with 0.02 M, Tris-HCl buffer, pH 8.10, containing 0.12 M NaCl. The kinins are identified on the basis of elution volume and quantitated by bioassay with the isolated guinea pig ileum.

The system was applied to trypsin-treated human plasma (1). The ethanol soluble fraction was desalted on AG 11-A 8 resin (0.9 x 200 cm) eluted with H_2O and chromatographed on SP-Sephadex at pH 8.1. Bradykinin was the only kinin detected in the effluent. However, the bradykinin was not completely separated from potentiating peptides also released from plasma proteins by trypsin treatment (2). Bradykinin was separated from the mixture of potentiating peptides by chromatography on SP-Sephadex developed with 0.02 M Tris-HCl buffer, pH 7.7, containing 0.08 M NaCl. On this basis, plasma-derived bradykinin potentiating peptides account for 30 to 40 percent of the "apparent" kinin activity when kinin activity is assayed with the isolated guinea pig ileum.

The pH 8.1 system has been used to purify commercially available synthetic K-10 and K-11, which usually contain the lower kinin homologues as well as oxidized forms of the sulfur in K-11. Elution profiles of 5-20 micrograms of synthetic kinins have been obtained by transmission measurements at 206 nm. These systems can be used to measure the kinins released both as intermediates and as limit products from kininogen and from plasma by treatment with proteolytic enzymes.

(This research was supported by the United States Atomic Energy Commission, Fundacão de Amparo à Pesquisa do Estado de São Paulo and Deutche Forschungsgemeinschaft).

---

1. C.R. DINIZ and I.F. CARVALHO, *Ann. N.Y. Acad. Sci. 104* 77 (1963).
2. P.N. AARSEN, *Brit. J. Pharmacol. & Chemotherap. 32* 453 (1968).