ISOLATION OF PORCINE URINARY KALLIKREIN

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Kallikrein from porcine urine was isolated by aceton precipitation, Sephadex G-75 gel filtration and affinity chromatography on a column of bovine trypsin-kallikrein inhibitor bound to BrCN-activated Sepharose 4 B. The preparation was further purified by Sephadex G-50 gel filtration and characterized by ion exchange chromatography, gel electrophoresis, molecular weight determination (gel filtration method), amino acid and carbohydrate composition and biological activity in the ileum test.

A SENSITIVE KININ LIBERATING ASSAY FOR KININOGENASE IN RAT URINE, ISOLATED GLOMERULI AND TUBULI OF RAT KIDNEY

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There is no Kinin liberating assay for rat urine kininogenase available, which is sensitive enough to measure the enzyme activity in isolated structures of the kidney.
First of all we developped a well reproduceable rat uterus test, which allows to measure Bradykinin equivalents from 0,4 ng to 4 ng and doses differences of 0,13 ng. The variation coefficient of the method is about 5%.
Substrate was prepared in a modification of the method, suggested by Habal, Movat (Biochem. Pharmacol. 23, 2291, 1974) and Spragg, Austen (Biochem. Pharmacol. 23,721, 1974). A fairly simple one-step procedure yields a human HMW-Kininogen, sufficient purified for a sensitive assay. "Spontaneous Kinin forming activity" is about 1% of total Kinin liberating activity (150 ng/mg protein). α_2-macroglobulin Cl inactivator and α_1-Antitrypsin could not be detected in Ouchterlony-double diffusion test. Kininases were completely inhibited by Na_2 EDTA ($4x10^{/3}$ M) and 8-Hydroxychinolin ($1 \times 10^{/2}$ M).
The kininogenase activity of rat urine was determined by incubating 0,1 – 1,2 μl with 600 ng substrate in a total volume of 250 μl for 4 hours. The Kinin formation was directly proportional to substrate – enzyme concentration and time. Optimal conditions: pH 8,6; ion strength 0,15 M, temperature 37°C.