CHROMOGENIC PEPTIDE SUBSTRATES FOR KALLIKREINS AND UROKINASE. G. Claeson, S. Gustavsson, L. Aurell, G. Karlsson, and P. Friberger. AB KABI, Peptide Research, Mölndal, Sweden.

A chromogenic substrate for plasma kallikrein has been modelled after the amino acid sequence preceding one of the scissile bonds of kininogen, that is, the C-terminal part of bradykinin. Similar attempts to use the natural substrates as models for designing synthetic substrates for glandular kallikrein and urokinase have not been successful. However, by means of trial and error (variations of the amino acids in positions P₁, P₂ and P₃) we have obtained correlations between substrate structure and activity. These correlations were then used to construct chromogenic peptide substrates for glandular kallikrein and urokinase.

The new substrates have been utilized in methods for the determination of prekallikrein in plasma, kallikrein in urine and urokinase in urine respectively.

METHODS FOR THE DETERMINATION OF PLASMIN, ANTIPLASMIN AND PLASMINOGEN BY MEANS OF THE SUBSTRATE S-2251. P. Friberger, M. Knös, S. Gustavsson, L. Aurell and G. Claeson. AB KABI, Peptide Research, Mölndal, Sweden.

The chromogenic peptide substrate H-D-Val-L-Leu-L-Lys-pNA (S-2251) has been chosen from a series of potent plasmin substrates. It is a highly specific and sensitive substrate towards plasmin (K_m $2.5 \cdot 10^{-4}$ mol/l, V $0.5 \cdot 10^{-6}$ mol/min CTA-U) and its solubility in tris buffer ($1 \cdot 10^{-2}$ mol/l) is sufficient to obtain substrate saturation. Tris buffer pH 7.4 and I 0.15 serves as an optimal medium for the determination of plasmin. In this buffer, antiplasmins in plasma have been determined by using 0.05 CU of plasmin and 20 ul of plasma. At least two inhibitors have been found. One fast (<15 sec. incubation time) and the other(s) slow (5 min. incubation time).

The substrate has also been used for plasminogen determinations after activation of 10 ul of plasma with UK (500 Ploug-U) or an excess (40 times) of SK. It was found that neither plasminogen free plasma (100% antiplasmin activity) nor an excess (>1000 times) of SBTI inhibited the substrate activity of the SK activated plasminogen. Optimal conditions for the SK-activated plasminogen was tris buffer pH 7.4, I 0.05. $K_m = 2.4 \cdot 10^{-4}$ mol/l for both purified plasminogen and plasma and $V = 1 \cdot 10^{-4}$ mol/min·CU or $3 \cdot 10^{-6}$ mol/min·ml plasma.