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Studies on Plasminogen Activators

IV. An Enzyme Complex with Esterase Activity in Streptokinase-activated Human Plasma

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In previous work it was demonstrated that streptokinase (SK) interacts with normal as well as fibrinogen-free human plasma proteins causing the formation of an enzymatically active complex, which could be separated by gel filtration.[1,2] The molecular size corresponded approximately to the 19 S proteins as shown by sucrose density gradient ultracentrifugation. Recent work [3] demonstrates that the enzyme complex displays esterase activity on α-N-acetyl-L-lysine methyl ester (ALMe), while having no proteolytic effect on fibrin. This indicated that the complex formation may involve a protein binding of a similar type as recently demonstrated with trypsin.[4,5] Mehl *et al.*[5] suggested that the human α_2-macroglobulin may function as the binding protein, reporting a sedimentation constant of 19.8 S. In earlier studies Haverback *et al.*[4] showed that 15–20 S protein in human plasma combines with trypsin and chymotrypsin yielding an enzyme with esterase action but no longer inhibited by soybean. Our results are in agreement with these findings, since the protein complex isolated after incubation of human plasma with SK had esterase action on ALMe, however, was not inhibited by soybean, nor by low concentrations of ε-amino-caproic acid (ε-ACA).[3] When the concentration of ε-ACA was increased to 0.3 to 0.6 M inhibition occurred. The esterase activity was between 3.3 to 5.4 μmoles of ALMe hydrolyzed per hour per mg measured with the protein complex isolated after gel filtration.[3]

Lanchantin *et al.*[7] recently presented evidence for the binding of human thrombin to the trypsin-binding macroglobulin, suggesting that this kind of complex formation occurs with the esterase-type of proteolytic enzymes. As pointed out by Wilding *et al.*[8] α_2-macroglobulin has an established position as a carrier in the transport of several substances in serum. While the reactions leading to the SK-induced fibrinolytic activation in human plasma are yet not known in detail, it seems evident that once activated, plasmin should form a similar complex. Recent work by Ganrot [9] reports that plasmin binds to α_2-macroglobulin, blocking about half of its trypsin binding capacity, which suggests a higher affinity of this protein for trypsin. Recent results revealed that the SK-esterase complex [3] may be dissociated in two protein peaks with esterase activity by ultracentrifugation at pH 7.8 against a sucrose gradient. Compared with standard albumin the component with the slower sedimentation rate behaved as a 7 S protein with an approximate molecular weight comparable to plasmin. However, a proteolytic response could not be shown with the corresponding protein fractions on heated fibrin plates. A proteolytic effect was observed, however, by the decrease in the total protein content during prolonged dialysis of the SK-esterase complex at pH 7.8 and +5°C. This effect was time dependent and could be avoided by lowering the pH to 6.[3] Similarly no dissociation occurred when ultracentrifugation of the enzyme complex was performed at pH 5.3. Previous experiments had shown that the esterase complex was filtered through the Sephadex G-200 gel as a homogeneous peak over the pH range from 8 to 5.5, as concerns the plasminogen activating effect on bovine fibrin plates.

In order to avoid the overlapping by 7 S material after gel filtration [1,2] the SK-esterase complex used for the ultracentrifugation experiment shown in Fig. 1 was a recycled preparation corresponding only to half the active peak filtered immediately following V_0. The apparently homogeneous complex obtained by gel filtration[1-3] over the pH range mentioned above, dissociated in two active components at pH 7.8 after

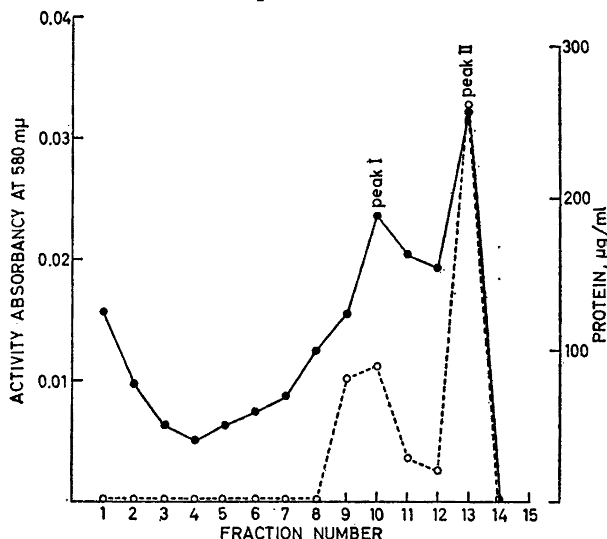

Fig. 1. Dissociation of the SK-esterase complex by ultracentrifugation against a sucrose (5 to 20 %) gradient. Peak I corresponds to 7 S protein, peak II contains the α_2-macroglobulin as ascertained by electrophoretic analyses on agaros gel. Proteins (●) were analyzed by the Folin method and esterase activity (O) α-N-acetyl-L-lysine methyl ester as described in detailed in an earlier publication.[3] The enzyme complex was prepared from normal human plasma by incubation with 1000 units SK per ml and separated by gel filtration. [1,2] Ultracentrifugation (Spinco Model 2 L, SW 25:2) was performed in 0.05 M Tris-HCl buffer pH 7.8 with 1 M NaCl, 90 h.[2] Sucrose was removed by dialysis against 0.15 M NaCl pH 6.0 for 48 h, before the determination of enzyme activity. The material used in this run represents half the active protein peak after recycling on Sephadex G-200, avoiding contamination during gel filtration when collecting the material corresponding to the first plasma sieve fraction.

ultracentrifugation, as demonstrated in Fig. 1 with ALMe. In the higher pH range all SK appeared to remain bound. The amount of SK applied in these studies is furthermore higher than generally applied clinically to achieve thrombolytic activity in blood. The plasma used was of the mixed blood bank type which may be considered as an average material as concerns the immunological response to SK. Dissociation of SK was detected after ultracentrifugation at pH 5.3, and it is assumed that it may have derived from the 7 S protein material.[3] These results suggest that the peak I esterase (Fig. 1) may represent the plasminogen-SK complex also found in the macroglobulin fraction, as shown by the gel filtration behaviour.[1-3] This was sustained by the autolysis found to occur during dialysis of the complex at pH 7.8, indicating an activation to plasmin. A detailed account of this work will be published separately.

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