# INTERACTION OF STREPTOKINASE AND PLASMINOGEN

## Demonstration of a Reaction Product by Ultracentrifugation and Starch-Gel Electrophoresis

E. C. De Renzo, W. Barg, Jr., E. Boggiano, M. E. Englert and M. C. Davies

Biochemistry Research Department, Biochemical Research Section,
Lederle Laboratories, American Cyanamid Company,
Pearl River, New York

Received May 29, 1963

Activation of human plasminogen by streptokinase has been extensively studied (Alkjaersig et al., 1958; Kline and Fishman, 1961; Marcus and Ambrus, 1960; Mullertz, 1955), but the chemical sequence of events which occurs in the interaction of these proteins and the physical and chemical properties of the reaction products have not been elucidated. At least two enzymatic activities are generated when streptokinase and human plasminogen react. One is an activator of a species of plasminogen, such as bovine plasminogen, which is refractory to activation by streptokinase alone (Ablondi and Hagan, 1956; Mullertz, 1955). The other is plasmin, a proteolytic enzyme which hydrolyzes fibrin and casein. Both enzymes hydrolyze basic amino acid esters (Ablondi and Hagan, 1956; Alkjaersig et al., 1958; Kline and Fishman, 1961; Marcus and Ambrus, 1960).

We have been studying the interaction of streptokinase and human plasminogen at definite molar ratios using highly purified preparations of both proteins in systems containing inhibitors of plasminogen activation and of plasmin. This communication describes the results of ultracentrifuge and starch-gel electrophoresis experiments which demonstrate the formation of a reaction product which appears to be an equimolar complex of streptokinase and plasminogen.

For ultracentrifuge experiments plasminogen (Kline and Fishman, 1961) and streptokinase (unpublished) were dissolved in 0.1 M phosphate buffer, pH 7.5,

containing 0.01 M di-isopropylphosphofluoridate (DFP) and/or 0.1 M 6-amino-caproic acid (6-ACA). Centrifugation was carried out in double sector cells so that the sample under study and the buffer baseline were simultaneously recorded. The sedimentation pattern of each protein was determined individually and protein concentrations were calculated from the areas under the curves. Schlieren patterns showed a high degree of homogeneity for both proteins. Under the conditions of these experiments S* values for streptokinase and plasminogen were approximately 3.0 and 4.3 respectively. Mixtures comprising definite molar ratios were then analyzed. Molecular weight determinations by sedimentation and diffusion were found to be 84,000 for plasminogen (Davies and Englert, 1960) and 47,000 for streptokinase (unpublished). When equimolar mixtures were examined a new product with an S of about 5.2, which accounted for the total protein present, was observed in buffers containing 6-ACA alone or 6-ACA and DFP. Figure 1 shows the schlieren patterns for plasminogen, streptokinase and an equimolar mixture in 0.1 M phosphate buffer, containing 0.1 M 6-ACA. The molecular weight of the product was found to be about 130,000 by the Yphantis equilibrium sedimentation method (Yphantis, 1960). In either system when the streptokinase-plasminogen molar ratio was 2:1 the schlieren pattern revealed two peaks which were resolved into a 3 S component and a 5.2 S component. The area of the smaller component completely accounted for the molar excess of streptokinase. When the streptokinase-plasminogen ratio was 1:2 the pattern observed in buffer containing 6-ACA alone was resolved into a 4 S component which accounted for the molar excess of plasminogen and a 5.3 S component corresponding to the reaction product. For unknown reasons, with both DFP and 6-ACA present the pattern could not be readily resolved when plasminogen was in excess.

*S values reported are at 20°C in the buffer systems described and have not been extrapolated to zero concentration.

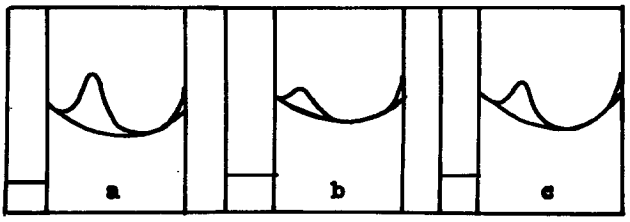


Figure 1. Schlieren patterns of streptokinase, plasminogen and a mixture of streptokinase and plasminogen at a 1:1 molar ratio.

Conditions: Photographs taken after 72 minutes at 52,640 rpm. Buffer - 0.1 M phosphate, pH 7.5, 0.1 M 6-ACA.

(a) Plasminogen, 0.41%, 20°C $S_{20} = 4.1$

(b) Streptokinase, 0.23%, 20°C $S_{20} = 2.9$

(c) Streptokinase and plasminogen at a 1:1 molar ratio, 0.33%, 8.5°C $S_{20} = 5.2$

---

For starch-gel electrophoresis experiments streptokinase and plasminogen were dissolved in tris buffers containing varying concentrations of 6-ACA over a wide pH range. The buffer composition of the gel was similar to the reaction mixture and electrophoresis was carried out at several pHs. Streptokinase and plasminogen were examined individually and in mixtures of varying molar ratios. Figure 2 shows a schematic representation of results at pH 8.4 in 0.4 M 6-ACA. Plasminogen appeared heterogeneous consisting of several components in about equal concentration whereas streptokinase was essentially homogeneous. The pattern observed with plasminogen alone was dependent upon the pH of electrophoresis and to a lesser extent on the 6-ACA concentration of the gel. Between pH 4.5 and 6.0 plasminogen migrated essentially as one component but at neutral or slightly alkaline pH several distinct bands were observed in a diffuse background. At pH 2.3, 6-ACA concentration 0.04 M, plasminogen migrated as a single major component with several trace components in evidence. A satisfactory explanation for these findings is lacking but it is a matter of interest that the plasminogen preparations appeared homogeneous in the ultracentrifuge at pH 3.0 in 0.1N NaCl and at pH 7.5 in 0.1M phosphate buffer containing 6-ACA.

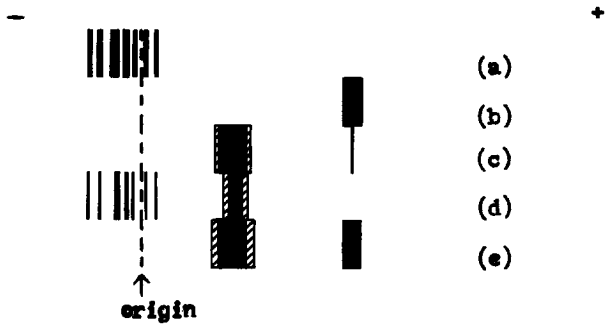


Figure 2. Starch-gel Electrophoretic Patterns of Streptokinase, Plasminogen and Reaction Mixtures of Streptokinase and Plasminogen.

Conditions: Gel and protein samples in 0.4 M 6-ACA, 0.015 M tris, pH 8.4; Electrode buffer - 0.05 M tris, 0.07 M boric acid, pH 8.2; Potential - 17 volts per cm.; Duration - 4 hours; (a) plasminogen (b) streptokinase (c) streptokinase-plasminogen mixture at a 1:1 molar ratio. (d) streptokinase-plasminogen mixture at a 1:2 molar ratio (e) streptokinase-plasminogen mixture at a 2:1 molar ratio. Plasminogen concentration was kept constant and the streptokinase concentration was varied to produce the desired molar ratio. For purposes of clarity, very faint protein zones present in the streptokinase preparation have not been shown.

---

In spite of the anomalous results described for plasminogen, Figure 2 shows that an equimolar mixture of streptokinase and plasminogen, subjected to electrophoresis, revealed a new zone with a mobility intermediate to both proteins. Furthermore, essentially all of the stainable protein in the plasminogen preparation reacted with streptokinase. When either component was in excess of a 1:1 molar ratio, an excess was observed with its expected mobility.

When ovalbumin was added to a reaction mixture it migrated with its normal mobility and did not influence the mobility of the streptokinase-plasminogen reaction product. Significantly, when 6-ACA was absent from the gel buffer the reaction product zone was not seen nor was intact plasminogen or streptokinase in evidence. In the absence of 6-ACA little of the stainable protein present in the reaction mixture was accounted for indicating that extensive breakdown occurred.

The reaction product had a diffuse appearance when electrophoresis was carried out at neutral or slightly alkaline pH and in some experiments discrete zones could be seen within the diffuse area. When electrophoresis was carried out at pH 5.0-6.0 the reaction product zone appeared to be homogeneous, paralleling the apparent increase in homogeneity of the plasminogen observed under weakly acidic conditions.

In the experiment shown in Figure 2 an unstained slice of gel was cut transversely in 2 mm. segments and enzymatic activities were determined in eluates from each section. Agar films containing casein or purified bovine fibrinogen (Bergström and Wallen, 1961) and fibrin films made from such fibrinogen served as substrates either alone or in the presence of bovine plasminogen. For a semi-quantitative comparison dilutions of each eluate in 0.1 M phosphate buffer, pH 7.5, were added to the surface of the substrates. After a period of incubation the diameter of a clear zone of lysis gave a measure of enzymatic activity. Eluates from the reaction product zone displayed activator activity (activity with substrates containing bovine plasminogen) at dilutions at which proteolytic activity (activity in the absence of bovine plasminogen) was not detected.

The data presented above demonstrate that streptokinase and plasminogen react at a 1:1 molar ratio in the presence of 6-ACA to form a product which has a molecular weight approximately equal to the sum of the molecular weights of the individual proteins and an electrophoretic mobility intermediate to that of the two proteins. 6-ACA is apparently essential for demonstration of the product at pH 8.5 since in its absence the product was not observed in starch-gel electrophoresis. Eluates of the reaction product zone observed in starch-gel electrophoresis displayed activator activity at concentrations at which proteolytic activity was not detected. It would be premature to conclude that the complex is in fact the activator enzyme. It seems reasonable however, to suggest, as a working hypothesis, that the observed reaction-product represents an intermediate molecular species in the formation of the

activator enzyme. The fact that highly purified plasminogen appeared multicomponent at slightly alkaline pH but nonetheless quantitatively reacted with streptokinase on an equimolar basis further suggests the existence of several molecular forms of plasminogen. A similar conclusion was reached by other investigators (Rybák and Petáková, 1963) on the basis of immunolectrophetic data.

Details and further implications of these studies will be published separately.

## References

Ablondi, F. B. and Hagan, J. J., Proc. Soc. Exper. Biol. and Med. 93, 414 (1956).
Alkjaersig, N., Fletcher, A. P. and Sherry, S., J. Biol. Chem. 233, 86 (1958).
Bergström, K. and Wallen, P., Arkiv für Kemi 17, 503 (1961).
Davies, M. C. and Englert, M. E., J. Biol. Chem. 235, 1011 (1960).
Kline, D. L. and Fishman, J. B., ibid. 236, 2807 (1961).
Kline, D. L. and Fishman, J. B., ibid. 236, 3232 (1961).
Marcus, G. and Ambrus, C. M., ibid. 235, 1673 (1960).
Müllertz, S., Biochem. J. 61, 424 (1955).
Rybák, M. and Petáková, M., Clin. Chim. Acta 8, 133 (1963).
Yphantis, D. A., Annals N.Y. Acad. of Sci. 88, 586 (1960).