STARCH GEL AND MOVING BOUNDARY ELECTROPHORESIS OF HIGHLY PURIFIED PROFIBRINOLYSIN[1,2]

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Profibrinolysin (plasminogen) has been prepared by the method of Sgouris, Inman and McCall (1959) from Fr. III separated from human plasma. This preparation has a specific activity (92 caseinolytic units/mg nitrogen) equal to any reported to date. It has been studied by moving boundary electrophoresis in a pH 2.1, $\Gamma/2$ 0.1 glycine hydrochloride buffer. The results compare closely with those of Shulman (1958). Two components were identified with mobilities of 7.0 and 5.2 x 10^{-5} cm^2 per volt per second. The major, faster component constituted 75-80 per cent of the total and is thought to include or represent the profibrinolysin boundary.

This preparation was analysed by starch gel electrophoresis by a modification of the method of Smithies (1955) using commercially available hydrolyzed starch (Connaught Research Laboratories, Toronto). The modification was dictated by the relative insolubility and instability of profibrinolysin in an alkaline buffer. In place of the borate buffer we used pH 2.1, $\Gamma/2$ 0.1 glycine hydrochloride buffer in which profibrinolysin is stable and soluble. No difficulty was encountered in forming a gel with this acid buffer. Electrophoresis was carried out for 18 hours at a current density of 4.8 ma/cm^2, in a 0° to 2°C room.

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The starch gel was divided into two comparable strips by splitting the gel horizontally with a fine wire. One strip was frozen to impart a sponge-like structure to the gel. The second strip was stained with amido black 10B in 50 per cent methanol as described by Smithies (1955). Five components were detected in the stained strips. Three bands were slow moving, trace components. The two faster components formed a pattern similar to that observed in moving boundary electrophoresis. Quantitation of the major components was achieved after rendering the gel transparent by placing it in glycerol at 70°C and then wrapping it in Saran wrap. A densitometer tracing showed 80 per cent of the area lying under the larger and faster of the two components.

With the stained strip as a guide it was possible to cut out the two major areas on the frozen strip. These sections were then individually extracted with pH 7.4, 0.1 M sodium phosphate, 0.15 M sodium chloride buffer by centrifuging them in conical tubes. The eluates were assayed by a modification of the caseinolytic assay of Remmert and Cohen (1949). All of the caseinolytic activity was detected in the larger, faster component.

Fibrinolytic activity was also determined by a modification of Lassen's (1952) heated fibrin plate method. The plate was prepared as follows: Agar (1.2 per cent) was prepared in pH 7.4, 0.1 M phosphate saline buffer, cooled to 40-45°C and added to an equal volume of 0.4 per cent human fibrinogen (previously dialysed against pH 7.4, 0.1 M phosphate saline buffer). With each 10 ml of mixture was mixed 0.1 ml (10 units) of Parke-Davis Thrombin. This was then poured immediately into the Petri dish and allowed to clot. The plates were heated for 15 minutes at 80°C, then cooled. Single drops (0.08 ml each) of the eluates, with and without streptokinase, were placed on the plates and incubated at 32°C for 18 hours. The reaction was stopped by flooding the plates with 10 per cent trichloroacetic acid. This resulted in excellent contrast between the lysed and unlysed areas. Streptokinase or the eluates alone did not show any lytic activity; however, streptokinase with the eluate of the fastest component showed fibrinolytic activity.

In summary, it has been shown that the streptokinase induced caseinolytic and fibrinolytic activities of purified profibrinolysin definitely are associated with the major, faster component in starch gel electrophoresis at pH 2.1. The similarity of moving boundary (Tiselius) and starch gel electrophoretic patterns suggests that profibrinolysin also is associated with the faster component of the former method.

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