Evidence of the Different Nature of Human Plasminogen and Proactivator

MOGENS LASSEN

From the Biological Institute of the Carlsberg Foundation, and the University Institute of Physical Chemistry, Copenhagen, Denmark

Human plasminogen and proactivator were found to have different thermal stabilities at 50° and pH 10.6. This observation supports the assumption that these components are chemically different.

The fibrinolytic activity induced in human blood by streptokinase, an extra-cellular streptococcal product, is due the conversion of plasminogen into the proteolytic enzyme, plasmin. Studies by Christensen ¹ and Christensen and MacLeod ² indicated a direct catalytic effect of streptokinase on plasminogen. Müllertz and Lassen ³ suggested that streptokinase does not react directly with human plasminogen, but that an activator of plasminogen is formed through a reaction between streptokinase and a substance, termed proactivator, which is present in large amounts in human plasma. Troll and Sherry ⁴ and Müllertz ⁵ independently suggested the reaction between streptokinase and proactivator to be a stoichiometric and reversible reaction, while the effect of the activator on plasminogen was catalytic:

This concept was rejected again by Sherry. He now assumes identity between plasminogen and proactivator and supposes the activator to be a complex of streptokinase with plasminogen or plasmin (Sherry and Alkjaersig ^{6,7}), and his concept has been accepted by several investigators. The present work gives evidence for the original suggestion that proactivator and plasminogen are two different factors.

Fig. 1. Inactivation of proactivator at 50° and pH 10.6. Abscissa: heating time. Ordinate: residual concentration of proactivator in per cent of the concentration in the unheated solution.

Fig. 2. Inactivation of plasminogen at 50° and pH 10.6. Abscissa: heating time. Ordinate: residual concentration of spontaneously occurring plasmin (curve 1), of plasminogen + plasmin (curve 2), and of plasminogen (curve 3). The last curve was obtained by subtracting curve 1 from curve 2. The activation of plasminogen with urine activator is described in the text. The estimations were performed by the manometric method using 0.25 M NaHCO₃ in the liquid phase and 50 % carbon dioxide in the gas phase, giving the pH 7.34. In the side arms: 25 mg BAEe. In the main compartment of flasks: Curve 1; 40 μl of the heated solution + 1 960 μl 0.25 M NaHCO₃. Curve 2; 40 μl of the heated solution + 20 μl of the urine activator solution + 1 940 μl 0.25 M NaHCO₃.

MATERIALS AND METHODS

Streptokinase. "Varidase" was kindly supplied by the Lederle Laboratories Division, American Cyanamid Company. The units are those stated on the packages as determined according to Christensen⁸.

Urine activator. A preparation of human urokinase (Ploug and Kjeldgaard⁹) was kindly supplied by Løvens kemiske Fabrik, Copenhagen. By means of the lysis time method (Lassen¹⁰) the activating power of this preparation was compared with that of streptokinase activated human plasma. 1 mg urine activator preparation was found to

Fig. 3. Denaturation of plasminogen (O) and plasmin (●) at 50° and pH 10.6. First order rate constants: plasminogen, 0.0198 min^{-1} ; plasmin 0.059 min^{-1} . $t_{1/2}$: plasminogen, 36 min; plasmin 12.5 min.

correspond in activity to approximately 1 500 units of streptokinase mixed with 1 ml of human plasma (excess).

Plasminogen and proactivator. 100 ml freshly prepared human serum was diluted with 1 900 ml icecold distilled water and adjusted to pH 5.5 with acetic acid. After standing for 30 min at 0° the precipitate was collected by centrifuging and dissolved in 0.9 % NaCl to a final volume of 16 ml. This solution was adjusted to pH 2.0 with 1 M HCl and heated at 70° for 30 min. The heated solution was then mixed with 4 ml veronal-gelatin buffer (Lassen ¹⁰), adjusted to pH 7.7, and clarified by centrifuging. The procedure caused some loss in plasminogen and proactivator, but it removed the bulk of inhibitory substances.

Manometric determination of plasmin. Plasmin was measured by the method previously reported (Lassen ¹¹), using benzoyl-L-arginine ethylester (BAEe) as substrate.

Determination of proactivator. Proactivator was measured by the lysis time method as described by Lassen ¹⁰.

RESULTS

Advantage was taken of the difference between the stabilities of the components at alkaline reaction. The heat inactivation was performed as follows:

10 ml of the plasminogen and proactivator solution was mixed with 0.5 ml 0.1 M NaOH. The resulting pH was 10.6. 1 ml aliquots were then heated at 50° for different periods of time, whereafter they were cooled, neutralized by adding 50 μl 0.1 M HCl, and cleared by centrifuging. The final determinations of proactivator and plasminogen were performed as follows:

Proactivator was estimated as described using the unheated solution for the standard curve (Fig. 1). Plasminogen was converted into plasmin (see below) and estimated by the manometric method. Because of the low content of proactivator in the heated solutions, streptokinase was considered unsuitable for the activation of plasminogen. Instead 200 μl of the samples

were activated for 30 min at room temperature with 100 μ l of a solution containing 4 mg urine activator per ml. The unheated solutions of proactivator and plasminogen contained small amounts of plasmin. The activity of this spontaneously occurring plasmin was determined separately, and subtracted from the activities obtained after activation with urine activator (Fig. 2).

The figures show that by heating at pH 10.6 proactivator disappears more rapidly than plasminogen, indicating that we are concerned with two different compounds. The denaturations of plasminogen and of the spontaneously occurring plasmin are first order reactions (Fig. 3), while the denaturation of proactivator seems to be more complicated. The relative decreases of plasmin and proactivator during heating for 30 min are in accordance with the results obtained by Müllertz^{5,12}.

DISCUSSION

The experimental results indicate the presence of at least two fibrinolytic precursors in human plasma. One of these is plasminogen. About the other can only be said that it is essential for the formation of the activator by streptokinase. On the basis of our present knowledge it seems most natural to identify this factor with the proactivator presented in the introductory scheme.

Experimental evidence of different heat stabilities of human plasminogen and proactivator has been presented by Troll, Sherry and Wachman¹³ and by Troll and Sherry⁴. The first authors found proactivator to be labile in plasma at pH 7.0 and a temperature of 50°, while plasminogen was found to be stable. The present author has tried to reproduce this experiment, but found both precursors to be stable at the above conditions. A less marked, although obviously still significant difference between the heat stabilities of the two precursors were found at 100° and pH 2 and at 50° and pH 9 by Troll and Sherry⁴. The results of the last experiment resemble the results presented in this paper (50° and pH 10.6). However, Sherry later abandoned the two component-theory, which means that he questions the results of these experiments (Sherry and Alkjaersig^{6,7}). Ablondi and Hagan¹⁴ who used a more purified preparation of human plasminogen found identical heat stabilities of plasminogen and proactivator at 50° and pH 6.4. However, they filtered the heated aliquots before the assays. In a similar experiment at 50° and pH 7.0 where filtration was omitted, a greater stability of plasminogen than of proactivator was apparent from their results. The authors ascribe this deviation to a different solubility of the heated proteins in different assay methods, and suggest that this phenomenon might be interfering in similar experiments made by other investigators. In the present investigation the neutralized aliquots were cleared by centrifuging, and no precipitation occurred during the assays.

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