EXISTENCE OF TWO FORMS OF TISSUE PLASMINOGEN ACTIVATOR

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At least two forms of tissue plasminogen (cytokinase) activator are present in the lysosomal fraction of rabbit kidney. They differ in their degree of specific activity and they are evidently isoenzymes.

Plasminogen is activated in the body by enzymic activators present in the blood and tissues of man and animals. The mechanism of this activation is not yet fully understood, for the nature of the natural activators still remains unknown. The isolation, purification, and some properties of tissue plasminogen activators were described previously.

During chromatographic purification of tissue activator (cytokinase) isolated from the lysosomal fraction of rabbit kidneys two forms of enzyme catalyzing the reaction of plasminogen activation were obtained. Although differing in several features, the two forms of cytokinase processes similar properties. It was important to discover whether these two forms of cytokinase are different enzymes or different forms of the same enzyme.

The object of the present investigation was to study the enzymic nature of tissue plasminogen activators.

There is no information in the literature on the existence of several forms or types of tissue plasminogen activator. Some investigators [3, 4] obtained several active zones of cytokinase, which may be taken as different forms of the enzyme, by chromatographic purification of a preparation of the activator. However, on treatment of the original material with concentrated solutions of urea the whole of the cytokinase activity was concentrated in one peak. The workers cited explained this fact by the existence of hydrogen bonds in the activator molecule, linking it firmly with the other proteins. In this case the urea liberated the activator molecules from the ballast proteins and led to its elution in one zone. In the present investigation to determine whether several forms of tissue activator exist, the cytokinase was therefore purified with the aid of urea.

EXPERIMENTAL METHOD

The proteins were fractionated by ion-exchange chromatography on columns with DEAE-cellulose in a phosphate buffer gradient with the addition of NaCl or urea. The products obtained were identified by gel-filtration through Sephadex G-100. The specificity of cytokinase activity was judged from the activity of the plasmin formed [1]. The specific cytokinase activity was expressed in casein units per milligram activator protein (c.u./mg). The products were analyzed by electrophoresis in polyacrylamide gel by the disk method [2].

EXPERIMENTAL RESULTS

The experiments of series I were carried out by the following scheme (scheme 1). The lysosomal fraction of rabbit kidney, suspended in 0.25 M sucrose solution (with 0.1% sodium deoxycholate as detergent),

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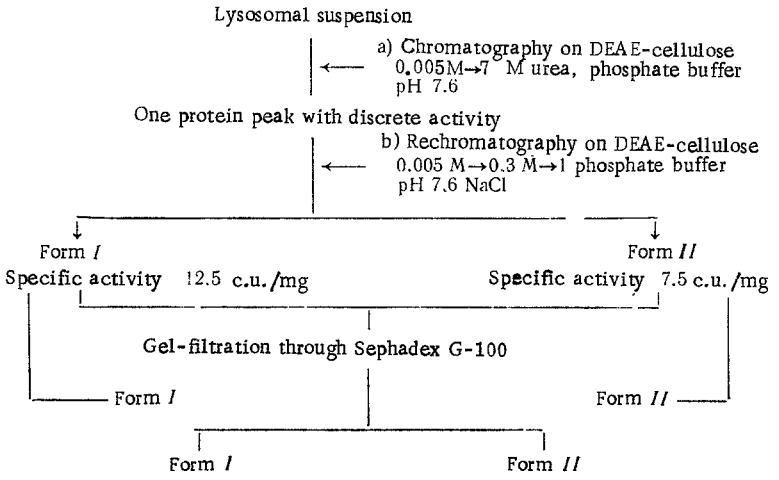
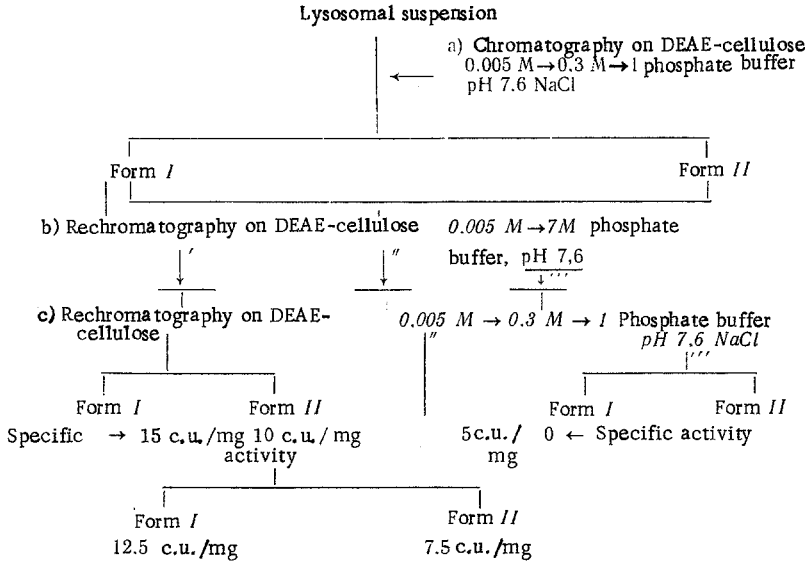
Scheme 1



Scheme 2



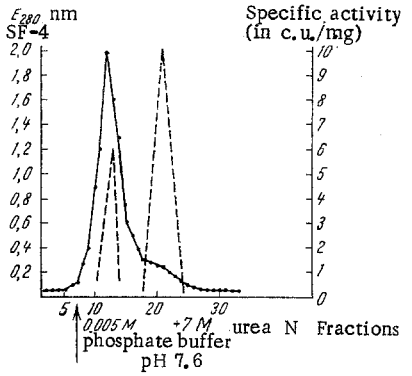


Fig. 1. Chromatography of lysosomal suspension on DEAE-cellulose. Continuous line) protein; broken line) specific cytokinase activity.

was fractionated chromatographically on columns with DEAE-cellulose in a convex gradient of 0.005 M phosphate buffer solution, pH 7.6, the ionic strength of which was gradually increased by the addition of 7 M urea solution. The whole of the protein adsorbed on the ion-exchange resin was eluted as a single peak (Fig. 1). The protein of the fractions corresponding to the peak as it began to decline possessed an activity of 6 c.u./mg. The protein corresponding to the descending slope of the peak was inactive, but cytokinase activity of 10 c.u./mg reappeared in the zone of the tail fractions of the protein peak. The mixture of active zones was then rechromatographed in a phosphate buffer gradient the ionic strength of which was increased by the addition of a 0.3 M solution of the same buffer, followed by a 1 M NaCl solution. As a result the mixture was separated into two protein components: one with specific cytokinase activity of 12.5 c.u./mg emerged when the concentration of the phosphate buffer was about 0.1 M; the other, with specific activity of 7.5 c.u./mg, emerged with NaCl in a concentration of about 1 M. These components corresponded to forms

I and II of cytokinase, as the results of electrophoretic analysis and gel-filtration showed. On filtration of the specimens of forms I and II on columns with Sephadex G-100 in 0.1 M NaCl solution, one protein component was obtained from each. On filtration of a mixture of the two forms of activator under the same conditions two protein components corresponding to forms I and II of cytokinase were eluted from the column.

In the experiments of series II urea was used in the second stage of purification (scheme 2). The lysosomal suspension was fractionated by chromatography on columns with ion-exchange resin under the same conditions as rechromatography of the active zones in the experiments of series I. Two forms of cytokinase were obtained.

The preparations of forms I and II of cytokinase and a mixture of the two were then rechromatographed (rechromatography I) in a phosphate buffer gradient with gradual addition of concentrated urea solution, as in the first stage of purification in the experiments of series I (scheme 2, b', b", b"'). Protein was not eluted in any of the three cases, i.e., the whole of the adsorbed protein remained firmly bound to the ion-exchange resin on the columns. To remove the remains of the urea, the columns with the adsorbed protein were carefully washed with 0.005 M phosphate buffer solution, pH 7.6. To elute the protein, the material adsorbed on the columns was rechromatographed, this time without urea.

Rechromatography II was carried out with phosphate buffer the ionic strength of which was gradually increased by the addition of 1M NaCl solution just as in the experiments of series I (scheme 2, c). As the result, the original protein (of form I or II of cytokinase or their mixture) was separated into two components: one, the more active, was eluted with phosphate buffer in a concentration of about 0.1 M; the other, less active, was eluted with NaCl solution in a concentration of about 1 M. The zones of elution of these proteins corresponded to those of forms I and II of the activator (scheme 2).

On treatment of the cytokinase preparations with concentrated urea solutions forms of the enzyme differing in their degree of specific activity toward plasminogen were therefore obtained. This is possible if the forms of cytokinase obtained are derivative of different degrees of polymerization of identical subunits. In that case, treatment of the cytokinase preparations with urea, followed by dialysis, may lead to the appearance of other forms of the enzyme as a result of random recombination of the polypeptide chains and conformational changes in the molecules.

The results of these experiments suggest that the cytokinase molecule has a quaternary structure, i.e., that it consists of identical subunits each of which has its own active center. Forms I and II of cytokinase are probably derivatives of the same enzyme, and they can be regarded as isoenzymes; form I of cytokinase consists of a larger number of subunits and has a higher molecular weight and higher specific activity than form II.

LITERATURE CITED

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