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Abstracts

The Mechanism of Action of Streptokinase

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Streptokinase (SK) is one of the catabolic byproducts of β-haemolytic streptococci; it is a protein comprised exclusively of amino acids which are arranged in a long peptide-chain with a molecular weight of 47 000 and an electrophoretic mobility corresponding to the serum α_2-globulins.

In contrast to the plasminogen activators of mammalian origin, the enzymatic nature of SK has still to be proven. However, there are also important pointers to a non-enzymatic character, eg, SK does not activate plasminogen directly and furthermore this effect is only seen in a few mammalian species, particularly man; bovine plasminogen, for example, is not activated by SK. During the interaction of human plasminogen with SK an activator is generated first of all and then plasmin, ie, human plasminogen has the ability to react both with SK, activator, and plasmin (Fig. 1). With respect to the twofold function of the molecule the term proactivator-plasminogen (PP) is used in order to differentiate it from animal plasminogen.

The proportions in which activator and plasmin are generated from a fixed amount of PP has been shown to be a function of SK: maximal plasmin activity is formed by PP and SK at a molar ratio of

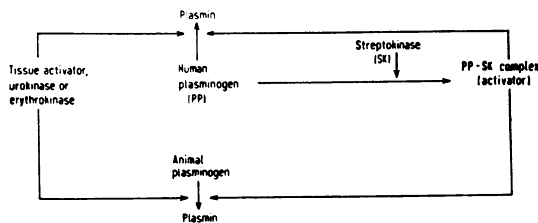

Fig. 1 *Activation of animal and human plasminogen with endogenous activators and with streptokinase*

10:1, while maximal activator efficiency is obtained at an equimolar ratio, eg higher concentrations of SK inhibit the formation of plasmin. This can be demonstrated by various electrophoretic and immunological methods and quantitated by enzymatic assays using casein and bovine fibrin as substrate.

The SK-induced activator is characterized as a molar complex of PP and SK. On electrophoresis it migrates homogeneously in polyacrylamide-gel, however, faster than plasmin and intermediate to PP and SK.

The activator formed by the interaction of SK and PP is characterized thus. In contrast to SK its specificity is the same as that of naturally occurring activators, eg, it catalyzes the conversion of human

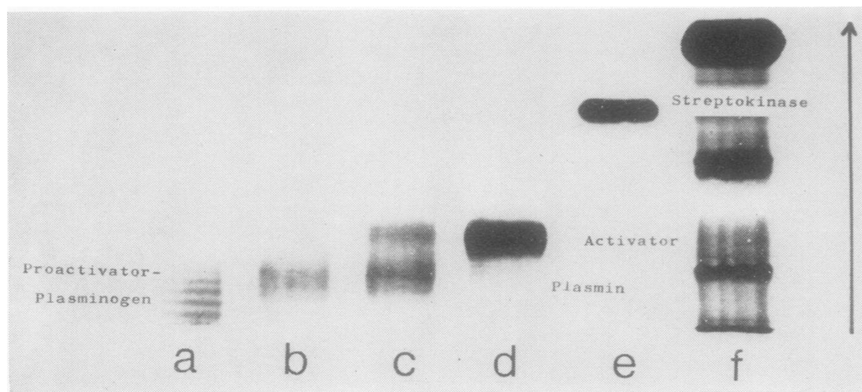

Fig. 2 *Electrophoresis in polyacrylamide gel. Proactivator plasminogen (PP) 1·3% in each sample. All mixtures were prepared in 0·1 M phosphate buffer,* pH *7·0, and incubated at 37°C for 10 min prior to insertion and electrophoresis at* pH *8·6* (a) *PP;* (b) *PP activated by 2 500 CTA-units urokinase per ml;* (c) *PP-SK mixture, molar ratio 10:2;* (d) *equimolar mixture of PP and SK;* (e) *SK 0·68%;* (f) *human serum.*

and animal plasminogen into plasmin (Fig. 1) as well as the hydrolysis of lysine and arginine esters; the esterolytic activity of the activator, in contrast to plasmin, is not inhibited by soybean trypsin-inhibitor. Its active site is not accessible to bigger molecules, as evidenced by a strong reduction of its proteolytic activity compared with plasmin. Fibrin, for example, is cleaved not at all by the SK-induced activator.

Plasmin also combines with SK to form an activator complex. It is suggested that the activator has an active site in common with plasmin, with the difference that it is partially sterically blocked by SK. This would explain that the esterolytic activity of the SK-induced activator equals the activity of plasmin, but that the esterolytic activity of the activator is not abolished by soybean trypsin-inhibitor and that large protein molecules are not cleaved in contrast to plasmin.

It remains still to be explained how the active site is generated from PP by SK. An autocatalytic process triggered by SK may be assumed.

Pharmacology of Thrombolysis: Urokinase

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Urokinase of human origin, the naturally occurring plasminogen activator found in human urine, possesses many of the biochemical properties required of an ideal thrombolytic agent.

First prepared on a pilot planned scale by Ploug and Kjeldegaard some 14 years ago, serious study of this agent in man only became possible in 1961 when two pharmaceutical companies successfully prepared material conforming to the following specifications: (1) The material was of high-specific activity, 35 000 CTA urokinase units/mg protein (crystalline urokinase has a specific activity approximately three-fold greater than the clinical grade material). (2) The preparation was essentially non-toxic in the experimental animal. (3) The preparation had been heat-treated at 56°C for 10 hours for the purpose of virus inactivation, and, (4) it passed stringent specifications for absence of thromboplastic components (components which contaminate the urine, from which it was prepared).

Studies in man have shown: (1) the material to produce highly-controllable and predictable states of enhanced plasma thrombolytic activity when administered on a dose/body weight basis; (2) have shown the material to be essentially non-toxic in man when administered in therapeutic doses; (3) human urokinase is non-antigenic to man; (4) human urokinase has an extremely high therapeutic ratio; that is, it produces a maximal increase in plasma thrombolytic activity with only a mild induced blood coagulation defect; and, (5) as a consequence of (4), plasma-plasminogen levels are well maintained (approximately 30% of normal), even in states of greatly enhanced plasma thrombolytic activity, (6) Finally, urokinase possesses a most valuable property not found in any other thrombolytic agent; that of being a most flexible drug capable of inducing either a mild prolonged thrombolytic state with minimal disturbance to the blood coagulation system or a state of intense plasma thrombolytic activity for shorter periods of time.

Altogether, clinical experience with urokinase has shown this drug to approximate to that of the ideal thrombolytic agent. Unfortunately, urokinase is a most expensive drug, and, consequently, financial and administrative difficulties have greatly impeded developmental work at the clinical level. It should, however, be emphasized that the slow pace of development has been entirely due to administrative and financial difficulties and not due to any defect in the drug or its preparations.

A substantial possibility exists that urokinase may ultimately be produced on a commercial scale by tissue culture methods with a substantial reduction in cost.

Basic Biochemical and Pharmacological Properties of Brinase

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Brinase is a fibrinolytic and thrombolytic enzyme from the mould *Aspergillus oryzae*. Like plasmin it is not specific for fibrin only, but attacks proteinaceous substrates like casein, gelatin, and denatured haemoglobulin. Brinase degrades fibrinogen roughly six times faster than it lyses fibrin, which compares favourably with trypsin, chymotrypsin, and the other proteolytic enzymes from *Aspergillus oryzae*, all having significantly higher fibrinogenolytic activity.

On fibrin plates brinase produces more marked lysis zones on heated than on unheated plates, suggesting that the enzyme is devoid of activator activity, which is verified using purified plasminogen. This implies that brinase does not consume the endogenous plasminogen, when used for thrombolysis.

The proteolytic enzyme inhibitors soybean trypsin inhibitor, ϵ aminocaproic acid, and Trasylol do not affect the enzymatic activity of brinase. The only efficient antidote at present known is plasma.

Electrophoresis shows that inhibitors towards brinase are localized in the α_1 and α_2 globulin

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