

Biochemistry of the plasmin system

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The fibrinolytic enzyme system is designed to release the protease plasmin from its inactive precursor plasminogen and to localise its action to the site of its physiological substrate fibrin. The basic components of the system are, therefore, plasminogen and plasmin; agents, collectively termed plasminogen activators, which convert plasminogen to plasmin; and inhibitors of activators or plasmin which ensure that proteolysis of other susceptible proteins does not take place. This review considers the structure and properties of plasminogen and plasmin and the processes involved in the activation of plasminogen, and outlines their relationship to recent concepts of thrombolysis.

Plasminogen

SYNTHESIS AND METABOLISM

The plasma concentration of plasminogen is around 200 $\mu\text{g}/\text{ml}$.^{1,2} Its half-life in healthy men is between 1.75 and 2.65 days.² A rapid rate of synthesis is inferred from the restoration of normal plasma concentrations within 12-24 hours of depletion during thrombolytic therapy with streptokinase.

The site of synthesis of plasminogen has not been established. Using a fluorescent antibody technique Barnhart and Riddle³ concluded that plasminogen is synthesised in bone marrow eosinophils. All types of human granulocytes have subsequently been found to contain a high concentration of plasminogen⁴ while treatment of leucocytes with cytostatic agents was observed to result in the disappearance of the plasminogen, suggesting that the granulocytes may be a site of synthesis.⁵ The liver, shown to be the site of formation of the α - and β -globulins,⁶ has not been excluded as a source of the β -globulin plasminogen, and the kidney may be a further source.⁷

STRUCTURE AND PROPERTIES

Human plasminogen is a single-chain glycoprotein with 791 amino-acid residues.⁸ It was initially believed to have lysine as the NH_2 -terminal amino-acid,⁹ but it was found subsequently that native plasminogen has glutamic acid rather than lysine in the NH_2 -terminal position.¹⁰ It is now clear that

plasminogen with NH_2 -terminal lysine (lys-plasminogen) results from proteolytic degradation of the native form (glu-plasminogen) with removal of peptide material from this part of the molecule during preparation. The COOH -terminal amino-acid is asparagine.⁹ Based on the amino-acid composition and assuming a carbohydrate content of 1.5% the molecular weight of glu-plasminogen would be about 90 000.¹¹ Through the endeavours of a number of laboratories the primary structure of plasminogen is now known.⁸

A variety of physicochemical findings have shown conformational differences between the glu- and lys-forms of plasminogen. Circular dichroism spectra in the near ultraviolet region differ significantly¹² and sedimentation analysis has also indicated differing conformational states.¹³

Apart from glu- and lys-plasminogen a number of different molecular forms of plasminogen have been demonstrated by electrophoretic techniques.^{10,14} Using affinity chromatography on lysine-Sepharose both glu- and lys-plasminogen have been separated into two forms with differing molecular size and charge.¹⁵ The cause of the microheterogeneity is uncertain; sialic acid content differences may contribute.¹⁶

Lysine-binding sites

Structures in plasminogen which specifically bind omega amino-acids such as lysine and 6-amino-hexanoic acid are termed the lysine-binding sites. Plasminogen forms a 1:1 stoichiometric complex with these amino-acids¹⁷ with the production of striking conformational changes in the plasminogen molecule^{12,18}—changes seen also on the conversion of glu- to lys-plasminogen. There are several lysine-binding sites on plasminogen, one with high affinity and four or five with low affinity for 6-aminohexanoic acid.¹⁹ The site which strongly binds 6-aminohexanoic acid is located in the NH_2 -terminal part of the molecule²⁰ and the conformational change is believed to arise from the dissociation of a non-covalent interaction between a site in the NH_2 -terminal part and a lysine-binding site elsewhere in the molecule.²¹

There has been debate over the years whether

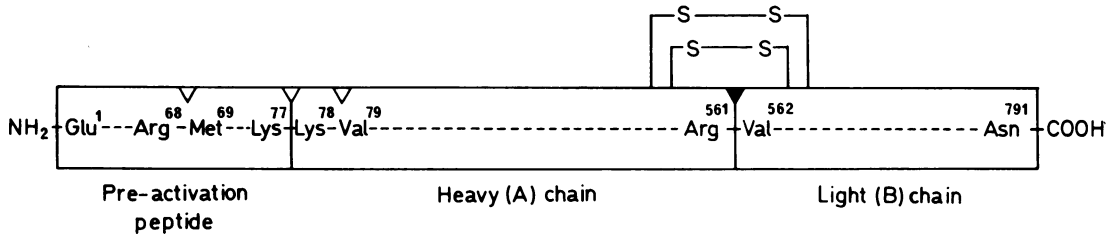


Fig. 1 Gross structural features of human plasminogen to plasmin conversion. ▼ Site cleaved by activator. ▽ Sites cleaved by plasmin.

plasminogen is specifically adsorbed to fibrin. There is now substantial evidence that such adsorption does take place and that the quantities involved, although relatively small, are of functional significance. Lys-plasminogen is adsorbed to fibrin more efficiently than glu-plasminogen.^{22, 23} In recently described experiments some 4% of native plasma plasminogen (glu-plasminogen) binds to fibrin compared with 8% for lys-plasminogen.²³ The only fragments of plasminogen which adsorb to fibrin are those which also adsorb to lysine-Sepharose, indicating a likely identity between lysine-binding sites and the sites responsible for the association of plasminogen with fibrin.²⁰

Fibrinogen contains several sites which can interact with the lysine-binding sites in plasminogen. These are present in purified fragment E and fragment D demonstrating location in both the NH₂-terminal and COOH-terminal regions of fibrinogen.²⁴

Lysine-binding sites on plasminogen are also involved in the interaction of plasmin with the fast-acting α_2 -antiplasmin.²⁴

ACTIVATION

Some features of the human plasminogen to plasmin conversion are represented in Fig. 1. The activation of plasminogen by urokinase requires the cleavage of a specific sensitive arginyl-valyl peptide bond (Arg₅₆₁-Val) in the COOH-terminal portion of the molecule with the production of a two-chain molecule.²⁵ While there is universal agreement on the requirement for hydrolysis of this bond subsequent studies have suggested that the activation of plasminogen is a two-step mechanism. In the first step it was proposed that a peptide bond in the NH₂-terminal part of the glu-plasminogen molecule is cleaved leaving Met₆₉ as the NH₂-terminal amino-acid.²⁶ In the second step cleavage of the arginyl-valyl bond takes place as originally described. Finally, further proteolysis in the NH₂-terminal portion of the Lys₇₇-Lys bond results in the formation of lys-plasmin. More recently it has been shown that the only bond cleaved by urokinase in the presence of the plasma plasmin inhibitors or Trasylol

is Arg₅₆₁-Val.^{27, 28}

In the absence of specific inhibitors a second bond is cleaved, Arg₆₈-Met or Lys₇₇-Lys. It is now accepted that these cleavages in the NH₂-terminal part of the molecule are due to plasmin rather than to activator.⁸ While splitting of the Arg₅₆₁-Val bond in glu-plasminogen alone provides a plasmin molecule with protease activity the activation rate of lys-plasminogen, formed by the cleavage of the plasmin-sensitive bonds in the NH₂-terminal part of the molecule, is many times faster.²⁹

Probably the increased rate of activation of lys-plasminogen is determined by the conformational change which follows the release of peptide material (preactivation peptide) from the NH₂-terminus, a conformational change which is also induced by the addition of omega amino-acids. The conformational change resulting from removal of the preactivation peptide may, by uncovering fibrin-binding sites, be responsible also for the more efficient adsorption of lys-plasminogen to fibrin.

The precise pathway for the activation of plasminogen is not finally established. One possibility which takes account of much of the experimental findings is represented diagrammatically in Fig. 2. The initial event in this scheme is the formation of catalytic quantities of glu-plasmin from glu-plasminogen by the action of activator on the Arg₅₆₁-Val bond. The plasmin formed cleaves the preactivation peptide from the NH₂-terminal portion of the molecule to form lys-plasminogen, and the accompanying conformational change facilitates its further cleavage by activator and the formation of the two-chain lys-plasmin molecule. This is essentially

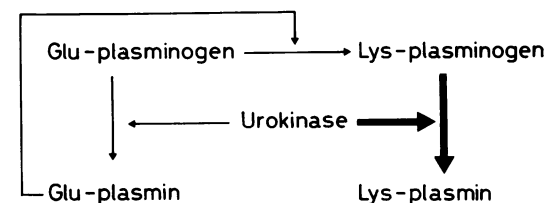


Fig. 2 Scheme for pathway of activation of plasminogen.

the pathway proposed by Violand and Castellino.³⁰ Lys-plasmin is the usual final form in activation systems *in vitro*, but the physiological form of plasmin has not been ascertained.

Plasmin

STRUCTURE AND PROPERTIES

Plasmin is a proteolytic enzyme with trypsin-like specificity which cleaves proteins and peptides at arginyl and lysyl bonds,³¹ basic amino esters,³² and amides.³³ It appears to have a preference for lysyl bonds.³⁴

Plasmin consists of two polypeptide chains, the heavy (A) chain and the light (B) chain,⁹ connected by two interchain disulphide bridges.³⁵ The molecular weight of the heavy chain from the NH₂-terminal part of the plasminogen molecule has been estimated at around 60 000,³⁶ and the light chain from the COOH-terminal end has a molecular weight of about 25 000.³⁶ The primary structure of the heavy chain of human plasmin has been established by Sottrup-Jensen and colleagues,³⁷ while Wiman³⁵ has detailed the primary structure of the light chain.

The active centre of plasmin contains a DFP-sensitive serine residue and a single TLCK-sensitive histidine residue; both are sited on the light chain of the molecule.³⁶

Plasmin is a protease of broad specificity. A number of plasma proteins are susceptible to its proteolytic action including coagulation factors V and VIII;^{38,39} components of the complement system;⁴⁰ the hormones ACTH, glucagon, and somatotrophin;⁴¹ and, in purified systems, fibrinogen is also digested. Its principal physiological substrate is presumed, however, to be fibrin.

Mechanism of thrombolysis

The recent accumulation of knowledge on the biochemistry of plasminogen and its activation together with the concurrent discovery of the fast-acting α_2 -antiplasmin has led to new concepts of the mechanism of thrombolysis.⁴²

The principal physiological activator is probably that derived from the vascular wall. This activator is identical or closely similar to tissue activator,⁴³ which has been shown to be strongly adsorbed to fibrin.²² Through its lysine-binding sites plasminogen is adsorbed to specific sites on fibrinogen and is therefore present on the fibrin formed from it. In addition, plasminogen may have a somewhat higher affinity for fibrin than for fibrinogen. In the presence of fibrin and its associated plasminogen the activating properties of tissue activator are much enhanced,⁴⁴ leading to the rapid localised formation of plasmin

at sites appropriate for the cleavage of fibrin. *In-vitro* studies have shown that the formation of lys-plasminogen with its associated conformational change increases its affinity for fibrin and makes it more readily activated. There is, however, no available evidence for the formation of the lys- form of plasminogen in the circulation. The physiological significance of this form of plasminogen is therefore uncertain.

When associated with fibrin the lysine-binding sites of plasmin are occupied and, as a result, its inactivation by α_2 -antiplasmin is very slow. In contrast, any free plasmin released into the plasma will be rapidly neutralised and the circulating fibrinogen and other susceptible proteins protected from proteolysis.

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