Plasminogen and Plasmin as Key-Substances within the Fibrinolytic System *

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Abstract

Plasminogen and plasmin are important proteins of the fibrinolytic system since they constitute a proteolytic mechanism which is responsible for the digestion of fibrin. The central event is the activation of plasminogen to the active protease plasmin by the action of activators which are widely distributed in the organism. Fibrinolysis is regulated by inhibitors influencing either the activation step or the generated plasmin activity.

Plasminogen is isolated by affinity chromatography using as the functional group of the adsorbent 6-aminohexanoic acid which is known to form complexes with plasminogen and plasmin. Plasminogen (mol. weight ~92000) consists of a single polypeptide chain with NH_2-terminal Glu and COOH-terminal Asn. During the two-step activation, peptide material is liberated from the NH_2-terminal end of the original polypeptide chain and the cleavage of an Arg-Val bond in its COOH-terminal half produces the active, two-chain plasmin. The heavy chain (mol. weight ~59000) has NH_2-terminal Lys and COOH-terminal Arg and the light chain (mol. weight ~25000) which is the carrier of the active center, has NH_2-terminal Val and COOH-terminal Asn. The two chains are covalently linked apparently by two disulfide bridges. Two mechanisms of activation are discussed which differ in the nature of an intermediate product and in the sequence of events, but finally lead to the same end product. The primary structure of plasminogen and plasmin is known to a large extent. The catalytically functional part, the light chain, shows similarities to trypsin and other pancreatic proteases which are particularly evident from sequence homology in the vicinity of the essential Ser and His residues of the active center. The heavy chain contains up to five regions with an internal sequence homology which are in addition homologous to two regions in the "pro-part" of prothrombin.

Introduction

The plasma proteins plaminogen and plasmin—a proenzyme/enzyme pair—are essential constituents of a proteolytic mechanism which is known as the fibrinolytic system. This system plays a role antagonistic to that of the blood clotting mechanism. During the latter process the soluble clotting protein fibrinogen polymerizes to a network of insoluble fibrin which contributes to the mechanical strength of the haemostatic plug. The fibrin deposition which normally develops as a natural counter-measure against lesions leads, as a pathological manifestation, also to the formation of intravascular thrombi which cause an impairment of the blood circulation and may be the origin of additional hazardous events (embolism, cardiac infarction, etc.). The fibrinolytic system is responsible for maintaining the functioning and patency of the vascular system and also for the regulation of tissue repair by eliminating intra- and extravascular fibrin deposits and obstructions.

* Lecture given in German at the Fall-Meeting of the Swiss Chemists Association, November 5th, 1977, in Berne

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The Fibrinolytic System

The elimination of fibrin occurs via proteolytic degradation into smaller soluble fragments by the action of plasmin. The fibrinolytic system is complicated by the fact that the substrate specificity of plasmin is relatively broad and that its proteolytic action is not restricted to the digestion of fibrin alone. To avoid random proteolysis of other plasma proteins, plasmin normally does not exist in the blood in its enzymatically active state, but occurs in the inactive proenzyme form (plasminogen). This precautionary measure, however, implies that the organism must have substances available, so-called activators, capable of converting plasminogen to plasmin and which can be released whenever plasminogen activation is required.

Fig. 1 shows a schematic representation of the fibrinolytic system. In the organism plasminogen activation can be produced by a variety of activators of which only the most important ones are listed.

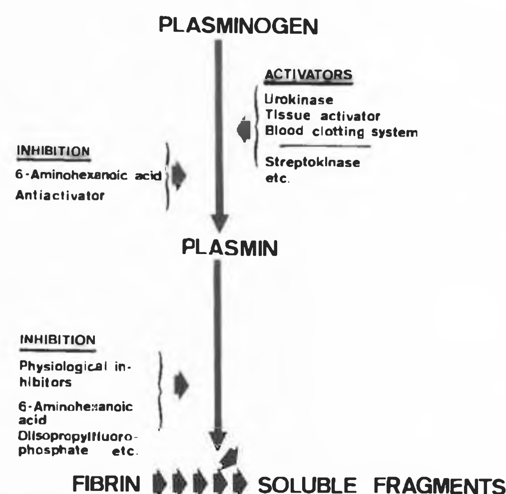

Fig. 1: Schematic representation of the fibrinolytic system.

Tissue activator is found in the tissue of most organs in various amounts [1–3]. In general, there exists a correlation between the activator content and the degree of vascularization of the organ tissue. This type of activator is tissue-bound and requires special techniques for its solubilization.
Urokinase is a plasminogen activating substance found in urine [4]. Its identity with activator isolated from human kidney tissue cultures strongly suggests that urokinase is synthesized in the kidneys [5–8]. This seems to disprove the hypothesis which assumed urokinase to be the excretory form of blood activator.
The possibility also exists of activating the fibrinolytic mechanism by the activated blood clotting system. This mechanism depends on activated Hageman factor (factor XII), prekallikrein and other factors and documents the close relation between blood coagulation, the kinin system and fibrinolysis [9–12].
These endogenous activators behave as very specific proteases with plasminogen and activate it according to the general pattern of a limited proteolysis.
It is interesting to note that streptokinase, an exogenous substance isolated from cultures of Streptococcus haemolyticus also has powerful plasminogen activating properties [13, 14]. Streptokinase differs from the other activators, such as urokinase, in being devoid of protease and esterase activity. However, streptokinase is capable of forming a stoichiometric 1 : 1 complex with plasminogen [15–17], and the conformational changes resulting from this interaction are apparently responsible for the induction of the active site in plasminogen. The end product of this activation, plasmin, is apparently the same as that produced by plasminogen activation with e.g. urokinase [18, 19].
The activation reaction of plasminogen and the enzymatic activity and action of the plasmin generated are regulated by a number of inhibitors.
In several reports physiological inhibitors of a protein nature are described which are supposed to act mainly or even exclusively on the activation mechanism [20, 21, 22]. The plasminogen activation is also inhibited by 6-aminohexanoic acid and similar compounds of comparable structure [23]. The manifestation of inhibitor activity by these substances depends on the presence of free terminal NH_2- and COOH-groups in the same molecule and in addition these functional groups must be separated from each other by a certain distance as in 6-aminohexanoic acid. These requirements are not only fulfilled by the straight-chain molecule of 6-aminohexanoic acid but also by certain cyclic compounds, such as p-aminomethyl benzoic acid [24] and 4-aminomethyl cyclohexane carboxylic acid [25]. The antifibrinolytic drug 6-aminohexanoic acid is also used in the isolation of plasminogen by affinity chromatography where it serves as the prosthetic group of the affinity adsorbent.
The spectrum of plasmin inhibitors is rather wide. It comprises physiological as well as unphysiological inhibitors on a polypeptide and protein basis [26–29]. In addition, 6-aminohexanoic acid and analogous compounds have also an inhibitory effect against plasmin activity although higher concentrations of inhibitor are required compared to the inhibition of the plasminogen activation. Plasmin is also inhibited by diisopropyl fluorophosphate indicating a close relation between this enzyme and trypsin or trypsin-like enzymes [30]. The active center serine residue which is characteristic for this group of enzymes was directly identified by *Summaria* et al. [31].

Isolation of Plasminogen

Plasminogen is isolated by affinity chromatography. This technique has displaced the hitherto usual, mostly complicated methods of preparation which employed steps such as extractions and precipitations under a variety of conditions, ion exchange chromatography and gel filtration. The technique of affinity chromatography used here is based on the complex formation between plasminogen and 6-aminohexanoic acid (and similar aminocarboxylic acids). The affinity adsorbent is obtained by coupling lysine covalently via its α-amino group to a suitable carrier, such as Sepharose [32] or polyacrylamide (Biogel P) [33]. Consequently the α-carboxyl and ε-amino groups of the coupled lysine remain free and thus represent the functional groups of the complex forming 6-aminohexanoic acid. The lysine-substituted adsorbents allow the specific isolation of plasminogen directly from plasma or plasminogen containing plasma fractions in a fast and simple way under mild conditions. The isolation procedure consists of an adsorption step, followed by washing off the unadsorbed plasma protein. The adsorbed plasminogen is eluted by displacement with phosphate buffer containing e.g. 6-aminohexanoic acid or by acidic buffer alone. The affinity chromatographic method yields intact, native plasminogen which is characterized by NH_2-terminal glutamic acid [33], whereas the other methods—if no special precautions are taken—easily lead to the formation of proteolytically altered plasminogen with lysine in the NH_2-terminal position.

Molecular parameters of human plasminogen and plasmin

In Table 1 some of the most important molecular parameters of human plasminogen and plasmin are summarized. According to end group analyses plasminogen consists of a single polypeptide chain with approx. 820 amino acid residues.
Plasmin, however, comprises two polypeptide chains of different size (see fig. 2). The heavy chain (mol. wt. ~59000) has NH_2-terminal Lys and COOH-terminal Arg and the light chain (mol. wt. ~25000) is characterized by NH_2-terminal Val and COOH-terminal Asn.

Table 1: Molecular parameters of human plasminogen and plasmin (for refs. see e.g. [18, 33–38])

Plasminogen	
Molecular weight	~92 000
NH_2-Terminus	Glu
COOH-Terminus	Asn
Isoelectric point	6.0—6.6 (with multiple molecular forms)
Plasmin	
Molecular weight	~84 000
Molecular weight of heavy chain	~59 000
Molecular weight of light chain	~25 000
NH_2-Termini	Lys, Val
COOH-Termini	Arg, Asn

PLASMINOGEN
Molecular weight ~ 92000

PLASMIN
Molecular weight ~ 82000

Heavy chain
Molecular weight ~ 59000

Light chain
Molecular weight ~ 25000

Fig. 2: Polypeptide chain models of plasminogen (top) and of plasmin (bottom).

According to recent results [39] the two chains are covalently linked by two interchain disulfide bridges. In addition, each chain contains a number of stabilizing intrachain disulfide bridges [18] which, for reasons of clarity, are not shown in the drawing. The active site of plasmin with a Ser and His residue was shown to be located in the light chain [31, 40].

From the changes in the spectrum of terminal amino acid residues it becomes evident that certain peptide bonds are cleaved during the activation process. Moreover, a comparison of the molecular weight values between proenzyme and enzyme indicates the release of a peptide fragment during this process.

Activation Mechanism of Human Plasminogen

The conversion of plasminogen to plasmin is characterized by at least two important events. They comprise the cleavage of an Arg-Val bond in the COOH-terminal half and the cleavage of at least one additional bond in the NH_2-terminal region of plasminogen, accompanied by the release of a NH_2-terminal peptide fragment. The enzymatic hydrolysis of the Arg-Val bond is of great importance since the generation of plasmin activity is directly related to the scission of this peptide bond and the concomitant formation of the two-chain plasmin molecule.

These facts are well established and generally accepted. However, there still exists some controversy about details of the activation mechanism, notably about the sequence of peptide bond cleavage during this process. In fig. 3 two mechanisms are schematically shown which are open to discussion. In both instances the starting material is the same, native plasminogen with NH_2-terminal glutamic acid (Glu-plasminogen). Also the end product of the activation, plasmin, is identical in both cases, but it is reached via two different pathways.

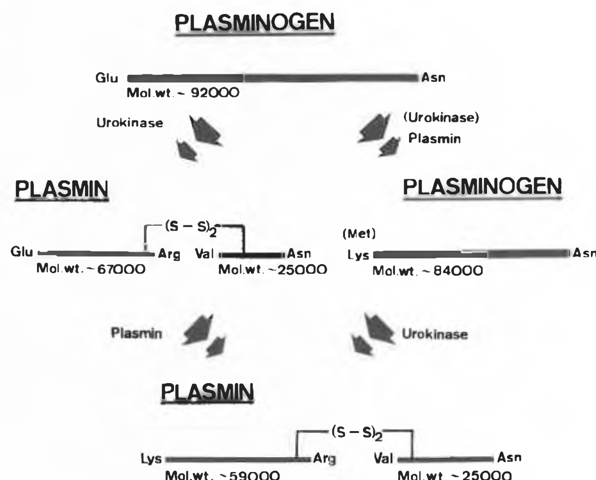

Fig. 3: Two possible pathways of the activation of human plasminogen to plasmin.

When activating plasminogen with urokinase (pathway to the left) in the presence of pancreatic trypsin inhibitor (to inhibit plasmin activity generated) *Summaria* et al. [41] and also Violand and Castellino [38] observed only the cleavage of the Arg-Val bond thus yielding a two-chain plasmin molecule with an intact heavy chain containing the entire NH_2-terminal portion of the original Glu-plasminogen. The activation at high levels of urokinase, but in the absence of inhibitor, produced at least initially the same type of plasmin molecule. From these observations it follows that plasmin with an intact heavy chain is unstable and can only be preserved as long as the plasmin activity remains efficiently inhibited. The final stable plasmin with a shortened heavy chain bearing NH_2-terminal Lys is obtained by plasminolysis, in an autolytic process, accompanied by the liberation of peptide material from the NH_2-terminal end of the heavy chain.

Wiman and *Wallén* [42] and also *Rickli* and *Otavsky* [43, 44] (pathway to the right) observed during the plasminogen activation with urokinase (in the absence of inhibitor) the liberation of peptide material from the NH_2-terminal portion of Glu-plasminogen prior to the urokinase catalyzed cleavage of the Arg-Val

bond in the second step. The liberation of the peptide moiety in the first step (later confirmed by *Walther* et al. [45, 46]) leads to an enzymatically inactive, modified one-chain plasminogen (molecular weight ~84000). The original NH_2-terminal amino acid residue of this urokinase induced intermediate is according to *Wiman* and *Wallén* [42] Met, whereas other NH_2-terminal residues observed must be ascribed to secondary proteolysis under the influence of traces of already generated plasmin. Once plasmin is formed it will apparently compete with the action of urokinase in the first step and give rise to the formation of an intermediate, one-chain plasminogen with NH_2-terminal Lys (Lys-plasminogen). It represents a well known proteolytically altered species of plasminogen which, as *Claeys* et al. [47] demonstrated, is rapidly generated in solutions of Glu-plasminogen under the action of traces of active plasmin with the concomitant liberation of peptide material originating from the NH_2-terminal region of the native proenzyme.

The questions which arise concerning the two mechanisms are whether the cleavage of the NH_2-terminal peptide material is a prerequisite for the subsequent cleavage of the Arg-Val bond and whether urokinase is actually capable of hydrolyzing the necessary peptide bond(s) in the NH_2-terminal region of Glu-plasminogen. The results of *Summaria* et al. [41] and of *Violand* and *Castellino* [38] obtained in their system obviously negate these questions. On the other hand *Wiman* and *Wallén* [48] showed that in Glu-plasminogen, in which the ε-NH_2 groups of the Lys residues were blocked with maleic anhydride, urokinase indeed cleaved an Arg-Met bond (positions 68 and 69) which is apparently identical to the bond cleaved in the first, urokinase catalyzed step of the activation which produced the intermediate product with NH_2-terminal Met. *Sjöholm* et al. [49] then demonstrated that the liberation of the NH_2-terminal peptide moiety induces conformational changes which may be of importance in the resulting intermediate in view of the subsequent Arg-Val bond cleavage. It has been shown by several authors that the conversion of the native Glu-plasminogen to the degraded form (Met- or Lys-plasminogen), either by plasmin or activator, causes an increase in susceptibility of the proenzyme molecule to activators and thus an enhancement of the activation rate.

The controversy over the points mentioned is not yet settled and can probably only be solved if these questions are treated within the entire context of the fibrinolytic system, notably taking into consideration the presence in vivo of fibrin. At this point it must be born in mind that the reported reactions and sequences of events—each one for itself seemingly correct when considered as a separate entity—were determined with the individual purified proteins in vitro which does not necessarily mean identity with the in vivo process.

Primary Structure of Human Plasminogen and Plasmin

The major features of the primary structure of human plasminogen and plasmin have been elucidated through the contributions of serveral research groups [see e.g. refs. 39, 45, 50–58]. From the diversity of this information only a few charachteristic aspects will be discussed.

The light chain as the carrier of the catalytic properties of plasmin resembles trypsin and related proteases, not only in the Ser and His residues commonly present in the active center. but also in terms of amino acid sequence. Of particular interest is the sequence constellation in the immediate vicinity of the active center Ser residue, where homology and over a short distance even identity with the corresponding sequence of some pancreatic proteases [59] and also of thrombin [60] are found (see table 2). A similar constellation also exists in the vicinity of the active His residue. Besides these two important regions, additional segments in the enzymes mentioned can be detected which show sequence homology. It was therefore assumed that these enzymes apparently evolved from a common ancestor molecule in which certain important regions such as the catalytic center and its vicinity behaved very conservatively during the evolutionary process and were thus preserved.

The heavy chain of human plasmin contains at least three and probably five regions which show extensive sequence homologies and which in addition are homologous with two regions (residues 138–147 and 243–252) in the so-called "pro-part" of prothrombin [50]. These two segments form the COOH-terminal part of two internally homologous kringle regions [60], pretzel-shaped structures in which the loops of the polypeptide chain are stabilized by three disulfide bridges. According to the sequence homology with the "pro-part" of prothrombin, *Claeys* et al. [50] postulated that the heavy chain of human plasmin must be arranged in possibly as many as five discrete kringle regions, indicating that a partial gene "quintiplication" has occurred during the evolution of plasminogen. This involved the same basic structure as found in duplicated form in pro-

Table 2: Amino acid sequence in the vicinity of the active center Ser residue (*) of some pancreatic proteases [59] and of human plasmin [39, 40]

Porcine elastase:	– Gly – Cys – Gln – Gly – Asp – Ser * – Gly – Gly – Pro – Leu – His – Cys –
Bovine trypsin:	– Ser – Cys – Gln – Gly – Asp – Ser * – Gly – Gly – Pro – Val – Val – Cys –
Bovine chymotrypsin A:	– Ser – Cys – Met – Gly – Asp – Ser * – Gly – Gly – Pro – Leu – Val – Cys –
Bovine chymotrypsin B:	– Ser – Cys – Met – Gly – Asp – Ser * – Gly – Gly – Pro – Leu – Val – Cys –
Human plasmin:	– Ser – Cys – Gln – Gly – Asp – Ser * – Gly – Gly – Pro – Leu – Val – Cys –

thrombin, documenting the close relationship between the two proteins which may be attributed to a common ancestry.

Concluding Remarks

The data available so far allow a limited insight into the central process of a complex system which, as most biochemical mechanisms, is characterized by the subtle interaction of different factors governing the nature and sequence of individual events. Of particular importance is the knowledge of the amino acid sequence of plasminogen and plasmin which for instance now enables the precise location of the peptide bonds hydrolyzed during the activation and which also provides the basis for relating on a molecular basis biological functions to structural aspects of the two proteins.

Acknowledgments

The author is indebted to Drs. *R. Strässle* and *R. Studer*, Diagnostic Research Department, F. Hoffmann-La Roche, Basel, for the generous gift of human urokinase, and to Dr. *D. Gillessen*, Mr. *W. Lergier* and Mr. *M. Manneberg* of the same department, F. Hoffmann-La Roche, for amino acid analyses and sequence determinations.
The skilful technical assistance of Mrs. *Wendy E. Otavsky*, Mrs. *Edith Moser* and Miss *Beatrice Meier* is gratefully acknowledged. I wish to thank the Central Laboratory, Blood Transfusion Service of the Swiss Red Cross for the supply of human plasma.
Contributions of the author cited in this article were supported by grants 3.525.71 and 3.273.74 of the Swiss National Science Foundation.

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