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Current Opinion in Structural Biology

New insights into the structure and function of the plasminogen/plasmin system

Ruby HP Law, Diana Abu-Ssaydeh and James C Whisstock

Plasminogen is the zymogen form of plasmin, an enzyme that plays a fundamental role in the dissolution of fibrin clots, the extracellular matrix and other key proteins involved in immunity and tissue repair. Comprising seven distinct domains (an N-terminal Pan-apple domain (PAp), 5 kringle domains (KR) and the serine protease domain (SP)), plasminogen undergoes a complex, incompletely understood conformational change that is key to its activation. Here, we review our current understanding of the structural basis for plasminogen activation with regard to new insights derived from crystallographic and biochemical studies.

Addresses
Department of Biochemistry and Molecular Biology, Monash University, Clayton, Melbourne, VIC 3800, Australia

Corresponding authors: Whisstock, James C (James.Whisstock@monash.edu)

Current Opinion in Structural Biology 2013, **23**:836–841

This review comes from a themed issue on **Catalysis and regulation**

Edited by **Ben M Dunn** and **Alexander Wlodawer**

For a complete overview see the Issue and the Editorial

Available online 16th November 2013

0959-440X/$ – see front matter,

http://dx.doi.org/10.1016/j.sbi.2013.10.006

Plasminogen is the inactive zymogen form of plasmin. It is synthesized primarily in the liver [1], as well as in all major organs and tissues [2] and is found in significant quantities in extravascular fluids. Under physiological conditions, plasminogen is converted to plasmin through cleavage in the activation loop (between Arg_{561} and Val_{562}) by tissue plasminogen activator (tPA) or urokinase plasminogen activator (uPA). Other proteases, including kallikrein, factors XIa and XIIa are also reported as being capable of functioning as plasminogen activators [3,4].

Biological activity, deficiency and disease

Active plasmin possesses exceptionally broad specificity for target substrates [5]. Accordingly, plasmin targets include fibrin, fibrinogen, complement component 3 (C3), complement component 5 (C5) [6], vitronectin [7], osteocalcin [8], factors V, VIII [9] and X [10], protease-activated receptor 1 [11], injury-induced aggregated proteins [12••] and some collagenases [13,14] (Figure 1). Plasmin can also target the key plasminogen activators tPA and uPA [15] to create a positive feedback loop. Plasminogen and its active form plasmin thus play important physiological and pathological roles in fibrinolysis and haemostasis, degradation of extracellular matrix, cell migration, tissue remodeling, wound healing, angiogenesis, inflammation and tumor cell migration [16].

Additional insight into the plasminogen/plasmin system comes from the study of deficiency of this key protein. Murine knockout studies reveal that plasminogen-deficient mice develop spontaneous fibrin deposition and severe thrombosis [17]. Conversely, humans with low levels of circulating plasminogen can be asymptomatic [18•]. However, a severe reduction in plasminogen levels (type I deficiency or hypoplasminogenemia) is associated with difficult to treat inflammatory conditions such as ligneous conjunctivitis and ligneous gingivitis [18•,19]. Interestingly, true (Type I) plasminogen deficiency is not associated with pathological thrombus formation [20,21•]. Accordingly, it is suggested that the minimal level of plasminogen required to sustain essential intravascular thrombolysis is very low. Furthermore, it has been postulated that the reduction in the activity of the plasminogen/plasmin system is counterbalanced by the upregulation of other proteases such as cathepsins and elastase [22,23].

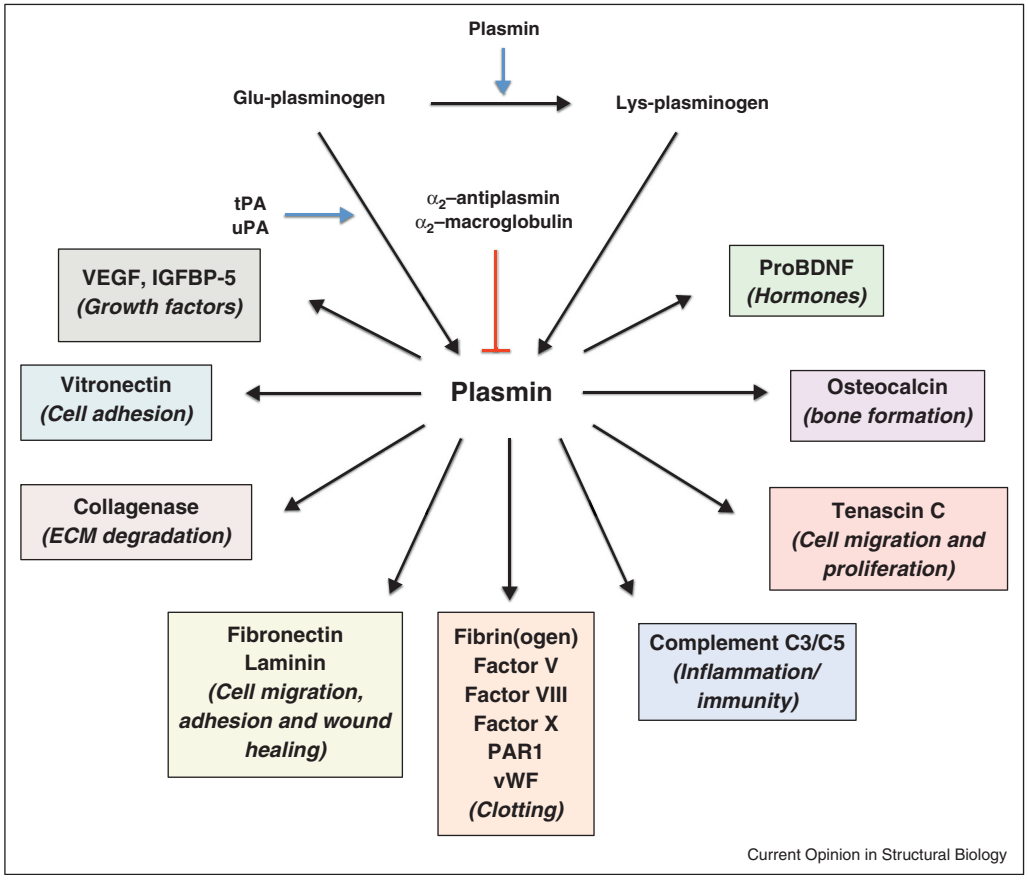
Inhibition of the plasmin system

Plasmin is a highly efficient enzyme, such that unchecked plasmin can rapidly exhaust the entire reserve of circulating fibrinogen, resulting in a generalized hemorrhagic state within minutes [24]. As such, under normal physiological conditions, active plasmin is only present on the surface of target sites such as fibrin clots or cell surfaces, creating a burst of localized plasmin activity that is resistant to inhibition. Free plasmin released from target surfaces is almost instantaneously neutralized by protease inhibitors via the formation of irreversible complexes. Indeed, in these regards, the interaction of plasmin with its primary inhibitor, α_2-antiplasmin [25•], is one of the fastest known protein–protein interactions with a K_a of $2 \times 10^7\ mol^{-1}\ s^{-1}$ [26]. If α_2-antiplasmin is exhausted from the system, the non-specific protease inhibitor, α_2-macroglobulin, can inhibit plasmin to a lesser extent. Mutations in α_2-antiplasmin, such as $V_{385}M$ and *Enschede* variant (where an alanine insertion occurs at position 366), thus lead to trauma induced hemorrhagic complications and serious bleeding, respectively [26].

Structural biology of the plasminogen system — maintaining the closed form

Full-length plasminogen (Glu-plasminogen) comprises 791 residues and seven domains — an N-terminal

Figure 1



Schematic illustration of plasminogen activation and functional roles. Plasminogen is the inactive precursor form of plasmin found in circulation. Full-length Glu-plasminogen can be converted by plasmin to Lys-plasminogen during preactivation. Tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA) convert both Glu-plasminogen and Lys-plasminogen to plasmin on target sites. Upon degradation of the targets, active plasmin is inhibited by α_2-antiplasmin and α_2-macroglobulin. Plasmin possesses broad substrate specificity. Examples of substrates that plasmin targets and their physiological functions are shown. VEGF, vascular endothelial growth factor; IGFBP-5, insulin-like growth factor-binding protein 5; PAR1, protease-activated receptor 1; vWF, von Willebrand factor; ECM, extracellular matrix; proBDNF, pro-brain-derived neurotrophic factor.

Pan-apple domain (PAp), 5 kringle domains (KR1-5) and the serine protease domain (SP) (Figure 2).

Plasminogen can adopt two distinct conformations, termed closed and open. Glu-plasminogen circulates in a closed conformation that cannot be readily activated by tPA or uPA. Glu-plasminogen can adopt an open conformation when bound to fibrin or the cell surface. Removal of the PAp domain by plasmin during pre-activation produces an alternative zymogen form called Lys-plasminogen, which also adopts an open conformation [4]. Glu or Lys-plasminogen in the open conformation assumes a flexible 'beads on a string' formation where the activation loop is exposed for cleavage by tPA or uPA.

Recent crystal structures [27,28••], together with a wealth of previous biochemical insights, have permitted a detailed understanding of how plasminogen is maintained in the closed conformation. Of key importance are the five tandem kringle domains; four of which are capable of binding to lysine residues, a characteristic of this domain family. In closed plasminogen, lysine and arginine residues (in particular Lys_{50}, Arg_{68} and Arg_{70}) present on the N-terminal PAp domain bind to the ligand-binding sites of KR-4 and KR-5 (Figure 2). Thus the conformation of closed plasminogen is partially mediated by inter-domain interactions via lysine-binding sites [29••]. These structural data also explain how the removal of the PAp domain (in Lys-plasminogen) results in conformational change to an open, extended state.

Additional major inter-domain interactions in the closed form include an interface between KR-2 and Lys_{708} from the SP domain. Both the PAp, KR-4/KR-5 and KR-2/SP interfaces feature additional interactions mediated by chloride ions (Figure 2), which have been shown to be important to maintain the stability of closed plasminogen [29••].