PLASMINOGEN ACTIVATOR INHIBITOR TYPE-2

A Spontaneously Polymerizing Serpin that Exists in Two Topological Forms

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1. INTRODUCTION

Plasminogen activator inhibitor type-2 (PAI-2) is a quite unusual Serpin. Unlike most other Serpins, which are secreted proteins with well characterized target proteinases and function, PAI-2 exists in both cytosolic and secreted forms; its physiological role is not well understood.

PAI-2 belongs to a subgroup of Serpins denoted the ovalbumin related Serpin (OV-Serpin) family, which reveal a particularly high degree of amino-acid sequence homology and seem to have a similar gene organization. OV-Serpins lack cleavable NH₂-terminal signal sequences and some of them utilize unconventional internal non-cleavable secretion signals for their translocation to the extracellular compartment. For several members of the OV-Serpin family, a functional role remains to be established.

The internal non-cleaved translocation signal of PAI-2 seems to be inefficient by design thereby allowing the synthesis of both intracellular and extracellular forms of the protein. In the extracellular environment, PAI-2 is thought to play an important role in the control of pericellular proteolysis by inhibiting urokinase-type plasminogen activator (uPA) and the two-chain type of tissue-type PA (two-chain tPA).

The functional role of intracellular PAI-2 is a subject for speculation as no intracellular target protein has been identified. However, recent studies suggest that PAI-2 may play a role in protecting cells against programmed cell death. In this review we will summarize recent findings on the properties of PAI-2 and discuss functional and biochemical aspects of PAI-2, especially relating to its secretion pattern and its ability to spontaneously

* This work was supported by the Swedish Natural Science Foundation Research Grants NFR BU 8473-308.
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Chemistry and Biology of Serpins, edited by Church et al.
form polymers. In addition, extensive reviews covering biological and clinical aspects of PAI-2, as well as its regulation, have also been published recently (Dear & Medcalf, 1995; Kruithof et al., 1995).

2. BIOCHEMISTRY AND STRUCTURE OF PAI-2

The presence of uPA-inhibitor activity in partially purified extracts from placenta was first reported by Kawano and coworkers (Kawano et al., 1968; Kawano et al., 1970). This activity was initially named placental-type plasminogen activator inhibitor after the source of its discovery but was later renamed PAI-2.

PAI-2 exists in two distinct molecular mass forms (47 kDa and 60 kDa) that differ in their topology and in their degree of glycosylation (Genton et al., 1987; Wohlwend et al., 1987).

In most cellular sources used for purification the majority of PAI-2 remains intracellular and appears to be unglycosylated. This low molecular mass form of PAI-2, which has an isoelectric point of 5.0, (Kruithof et al., 1986) has been purified and characterized from human placenta (Åstedt et al., 1985; Wun & Reich, 1987), phorbol ester stimulated human U937 cells (Kruithof et al., 1986), and from recombinant bacterial (Mikus et al., 1993) and yeast expression systems (Steven et al., 1991).

For the production and purification of the secreted high molecular mass form of PAI-2, an artificial signal sequence was in-frame fused to the coding sequence of PAI-2 in order to direct a larger portion of PAI-2 protein to the secretory pathway (Mikus et al., 1993). By using this expression system the secreted glycosylated form of PAI-2 could be produced and biochemically characterized. Apart from differences in molecular weight, isoelectric point and glycosylation, both forms of PAI-2 appear to have similar characteristics (Kruithof et al., 1986; Åstedt et al., 1985; Mikus et al., 1993; Thorsen et al., 1988; Wun & Reich, 1987; Steven et al., 1991). Both secreted and intracellular forms of PAI-2 inhibit PAs by forming SDS-resistant complexes. They are efficient inhibitors of uPA (second order rate constants of $10^6$ M$^{-1}$ s$^{-1}$), and two-chain tPA (second order rate constants of $2 \times 10^5$ M$^{-1}$ s$^{-1}$) but react very poorly with single-chain tPA (second order rate constants of $10^4$ M$^{-1}$ s$^{-1}$). As a comparison, PAI-1 inhibits both tPA and uPA much faster than PAI-2 (second order rate constants of $10^7$ M$^{-1}$ s$^{-1}$). The difference in rate is especially large with single-chain tPA, which makes it unlikely that PAI-2 contributes to the inhibition of this activator in vivo.

PAI-2 forms 1:1 SDS-resistant complexes with target proteases and treatment with nucleophilic agents yields reactive center cleaved PAI-2 (Kruithof et al., 1986 and Kiso et al., 1988). Although the structure of the native inhibitory complex has not been studied in detail, the P1-P1' peptide bond of the reactive center of PAI-2 is most likely cleaved, leaving the inhibitor in an acyl-complex with cognate protease (Wilczynska et al., 1995; Lawrence et al., 1995). In accordance with that proposed for PAI-1 and other Serpins, the mechanism of PAI-2 action therefore most likely involves complex formation and reactive center cleavage. The subsequent rapid insertion of the reactive center loop then induces the conformational changes required to lock the PAI-2--protease complex (Lawrence et al., 1990; Fa et al., 1995; Wilczynska et al., 1995; Lawrence et al., 1995).

Fibrin bound tPA appears to be protected from inhibition by PAI-2 (Leung et al., 1987). It is therefore unlikely that PAI-2 plays any major role in tPA-mediated vascular fibrinolysis. Although it is generally accepted that PAI-2 functions primarily as a uPA inhibitor and plays only a minor role in controlling tPA mediated proteolysis, the finding