Cell. Mol. Life Sci. 66 (2009) 1782 – 1783
1420-682X/09/101782-2
DOI 10.1007/s00018-009-9039-0
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Cellular and Molecular Life Sciences

# Letters and Comments

# Conformational rearrangements of plasminogen activator inhibitor type 2

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Received 15 January 2009; received after revision 07 March 2009; accepted 30 March 2009
Online First 29 April 2009

This comment letter refers to the review article "Recent progress in understanding the diversity of the human ov-serpin/clade B serpin family" by K. Izuhara, S. Ohta, S. Kanaji, H. Shiraishi and K. Arima in Cell. Mol. Life Sci. 65, 2541 – 2553 (2008).

Dear Editor,

The review "Recent progress in understanding the diversity of the human ov-serpin/clade B serpin family" by K. Izuhara et al. is an interesting and timely analysis of structure-function relations within this protein family. However, in their discussion of the conformational changes and polymerization of plasminogen activator inhibitor type 2 (PAI-2) with potential outcomes on the biological functions of the serpin, these authors drew some erroneous conclusions from data in papers published by our group. We therefore would like to use this opportunity to correct any potential misunderstandings drawn from our published results.

We have shown that native PAI-2, in contrast to other serpins, can exist in two different forms, both of which are monomeric and inhibitory active: the stable monomeric and the polymerogenic form [1, ref. no. 67 in Izuhara et al.]. The stable monomeric form of PAI-2 remains as a monomer under physiological conditions, unlike the polymerogenic form, which is stabilized by a disulfide bond and prone to polymerization [1 and 2, ref. no. 66 and 67 in Izuhara et al.]. Therefore, the last sentence of the last paragraph in the section "PAI-2 (SerpinB2)", page 2547, stating "…turned out that monomeric and polymerogenic forms are interconvertible…" is not strictly speaking correct, as the polymerogenic form is also monomeric. That is, the stable monomeric (non-polymerizing) and polymerogenic forms are interconvertible, depending on redox potential of the environment [3, ref. no. 68 in Izuhara et al.]. This mechanism is further clarified below. Furthermore, the authors' statement in the second to last sentence of the paragraph "…once PAI-2 is secreted from the cells into the extracellular milieu, it is converted from an active monomeric form to an inactive polymerogenic form [67]" is incorrect since the polymerogenic form of PAI-2 is also active [1, ref. no. 67 in Izuhara et al.]. In addition, PAI-2 that is processed via the ER secretory pathway is converted into the polymerogenic form within the ER (i.e., intracellularly), where it partially polymerizes, and not "…once PAI-2 is secreted…" (i.e., outside the cell) [1, ref. no. 67 in Izuhara et al.]. Moreover, we did not publish any data on secreted PAI-2.

To date, PAI-2 remains the most enigmatic serpin and its physiological role(s) is not well understood [4]. The

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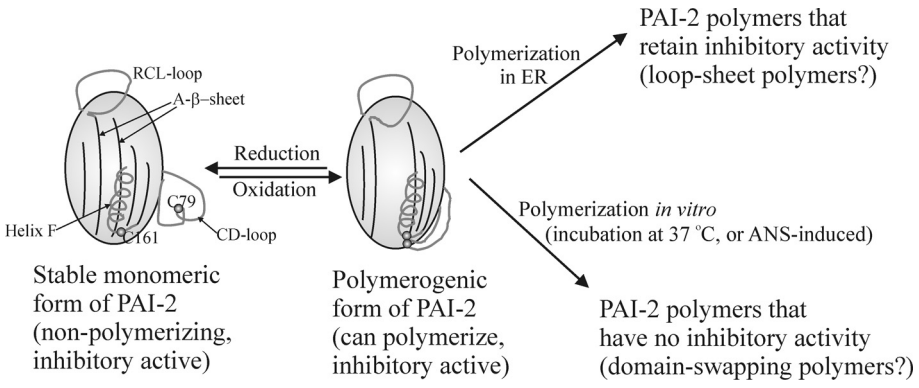


**Figure 1.** Schematic representation of PAI-2 molecular rearrangements.

fact that PAI-2 is localized in both the nucleus and cytoplasm, and can also be secreted, suggests that PAI-2 may have several different functions. In short, nuclear PAI-2 has been shown to protect the retinoblastoma protein from proteolysis [reviewed by Izuhara et al. and 5], the role of cytoplasmic PAI-2 is unknown [discussed in 5], and the extracellular PAI-2 has been proposed to regulate cell migration/invasion [5]. We have shown that PAI-2 directed to the secretory pathway forms polymers [1 and 2, ref. no. 66 and 67 in Izuhara et al.]. However, in contrast to other serpins, PAI-2 polymerization has not been linked with any pathological outcomes, and the function of PAI-2 polymers is not known.

While little is known about the role(s) of PAI-2 conformational forms, the phenomenon of interconversion between them is well understood [3, ref. no. 68 in Izuhara et al.] (Fig. 1). Among serpins, PAI-2 has the longest loop connecting helices C and D (the CD-loop). This is not required for the inhibitory function of PAI-2 but is responsible for PAI-2 interaction with other proteins [4, 5 and 3, ref. no. 68 in Izuhara et al.]. The CD-loop of PAI-2 also functions as a redox-sensitive molecular switch that regulates the conversion between the stable monomeric and polymerogenic forms of the serpin. In the stable monomeric form, the CD-loop folds on the side of the molecule [3, ref. no. 68 in Izuhara et al.] (Fig.1). However, due to inherent mobility, the loop can translocate to the bottom of PAI-2 where it can be stabilized by a disulfide bond (C79-C161), thereby converting PAI-2 to the polymerogenic form [3, ref. no. 68 in Izuhara et al.] (Fig.1). Thus, in contrast to other human serpins, wild-type PAI-2 can pre-exist in a ready-to-polymerize conformation [6].

Surprisingly, polymers of PAI-2 formed in the cell secretory pathway retain their inhibitory activity (our unpublished data), whereas polymers of recombinant PAI-2 obtained upon incubation at 37 °C or induced by bis-ANS have no inhibitory activity [1 and 6, ref. no. 67 in Izuhara et al., and unpublished results] (Fig.1). This discrepancy might be due to different types of polymers formed by PAI-2 under various conditions. Different mechanisms for serpin polymers have been proposed already, including the so called "loop-sheet" and "domain-swapping" polymers [7]. However, the mechanisms of PAI-2 polymerization and the activity of its different polymer types require further investigation.

We trust that clarification of these points regarding the current understanding of the conformational rearrangements of PAI-2 will eliminate further misunderstandings related to the different conformational forms of PAI-2 and their inhibitory activities.

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