

## Protein C inhibitor (PCI)

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### Abstract

PCI is a non-specific serpin that inhibits several proteases of the coagulation and fibrinolytic systems as well as plasma- and tissue kallikreins and the sperm protease acrosin. The precise physiological role of PCI has not been defined yet. Heparin stimulates most PCI/protease reactions, but interferes with the tissue kallikrein/PCI-interaction. Thereby heparin not only regulates PCI-activity but also its specificity in systems containing two or more of its target proteases. This effect is not restricted to heparin, but is also seen with other glycosaminoglycans (GAGs) and large, negatively charged molecules. PCI also binds to GAGs present on the surface of epithelial kidney cells, and GAGs isolated from these cells have a similar effect on PCI activity as heparin. Studies analyzing the role of PCI as an acrosin inhibitor revealed that endogenous PCI is immunocytochemically localized to disrupted acrosomal membranes of morphologically abnormal sperms, while intact sperms are negative for PCI-antigen. In a mouse *in vitro* fertilization model human PCI inhibited sperm/egg binding and decreased the fertilization rate. Northern blotting of human and mouse mRNA using human and mouse PCI-cDNA probes revealed that in the mouse PCI is exclusively synthesized in the genital tract (testis, seminal vesicle, ovary), while in humans PCI is additionally synthesized in many other organs (e.g., liver, pancreas, heart). Therefore PCI might regulate enzymes involved in fertilization (e.g. acrosin) in both species. Other proteases (e.g., tissue kallikrein) are possibly regulated in a species specific manner by different inhibitors.

*Keywords:* Protein C inhibitor; Tissue kallikrein; Serine protease; Serpin

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### 1. Introduction

Protein C inhibitor (PCI) is a member of the serpin (*serine protease inhibitor*) superfamily (Suzuki et al., 1987) and has an  $M_r$  of 57 000 and  $pI$  values of between 4.3 and 4.5. PCI has been originally described as an inhibitor of the anticoagulant serine protease activated protein C (aPC) in plasma (Marlar and Griffin, 1980). Since then it has been shown that PCI additionally inhibits a variety of

other serine proteases involved in blood coagulation and fibrinolysis (e.g., thrombin, factor Xa, factor XIa, plasma kallikrein, and urokinase; for review see Geiger, 1988, and Suzuki et al., 1989). Complexes of PCI with aPC have been shown in plasma samples from patients with disseminated intravascular coagulation (DIC) and deep venous thrombosis (España et al., 1990); complexes between PCI and urokinase are present in plasma samples from patient receiving systemic urokinase therapy for thrombolysis after myocardial infarction (Geiger et al., 1989). These findings suggest that PCI could play a role in the

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regulation of hemostasis. However, most coagulation and fibrinolytic enzymes are inactivated much more efficiently by other serpins, and the precise physiological role of PCI has still not been defined. Therefore, in order to analyze the *in vivo* function of this inhibitor we studied the tissue distribution of PCI and investigated the interaction of PCI with possible new target enzymes. Here we present recent data that indicate that the main function of PCI might not be – as initially assumed – the regulation of hemostasis. Furthermore, they suggest that the physiological function of this serpin might be different in different species.

## 2. Interaction of PCI with target proteases outside the hemostatic system

### 2.1. PCI in the reproductive system

PCI is synthesized throughout the male reproductive tract, and high concentrations (3–4  $\mu\text{M}$ ) are present in seminal plasma (España et al., 1991; Laurell et al., 1992). Seminal plasma PCI is partially cleaved and/or complexed to serine proteases (España et al., 1991), suggesting that PCI plays a role as a protease inhibitor in the male reproductive tract. Christensson and Lilja (1994) and España et al. (1991) have shown that PCI in seminal plasma forms complexes with prostate specific antigen, a tissue kallikrein like serine protease. However, PCI does not seem to have a regulatory function for this protease, since the concentration of prostate specific antigen in seminal plasma (15–60  $\mu\text{M}$ ) exceeds the concentration of PCI by far.

We (Zheng et al., 1994) and others (Hermans et al., 1994) have shown that PCI is also a very efficient inhibitor of the sperm protease acrosin. Acrosin inhibition by PCI might be important in the male reproductive tract, since prematurely released and activated acrosin could cause proteolytic damage of other spermatozoa, of surrounding tissues and of seminal plasma proteins. On the other hand, inhibition of acrosin (and other sperm proteases) might also occur in the female reproductive tract, where acrosin is physiologically activated and released in the immediate vicinity of the ovum. Since acrosin is thought to be involved in the fertilization process by

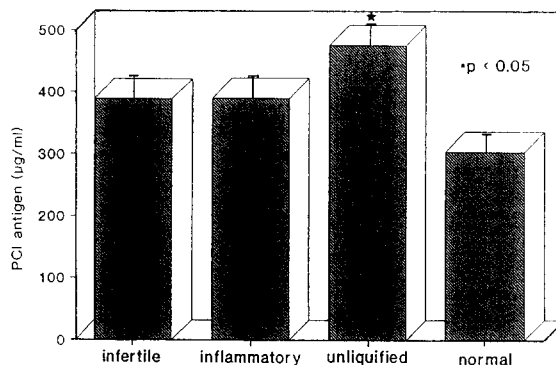


Fig. 1.

digesting a pathway for the sperm through the zona pellucida of the ovum, inhibition of acrosin could have an influence on the fertilization process. As shown elsewhere (Zheng et al., 1996) PCI inhibits in fact sperm-egg binding and decreases the rate of fertilization in a mouse *in vitro* fertilization model. These findings are in agreement with data published by Moore et al. (1993) who found that human sperm-egg binding is inhibited by peptides corresponding to the core region of an acrosomal serine protease inhibitor identified as PCI. PCI is not only synthesized in the male reproductive tract but also in the ovary (Zechmeister-Machhart et al., 1996; Zheng et al., 1996) and should therefore be present in the surroundings of the ovum.

In order to further analyze the role of PCI in the reproductive system we measured PCI-antigen in seminal plasma samples from healthy donors and from selected patients. Preliminary data revealed that one group of infertile patients with non- or incompletely liquifying semen exhibited significantly higher PCI levels as compared to controls (Fig. 1), suggesting that PCI might regulate enzymes involved in semen liquification.

### 2.2. PCI and the tissue kallikrein-kinin system

We have shown that PCI inhibits tissue kallikrein with a  $k_{app}$  of  $2.3 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$  by forming stable 1:1 complexes (Ecke et al., 1992a). Inhibition and complex formation is inhibited by heparin and other glycosaminoglycans, and high concentrations completely block the tissue kallikrein-PCI interaction. This reaction pattern with tissue kallikrein as well as

several other properties of PCI ( $M_r = 57\,000$ , pI: 4–5, plasma concentration) are very similar to those described by the group of Chao (1986) for the kallikrein binding protein (kallistatin). Therefore, and since plasma immunodepleted of PCI no longer formed complexes with  $^{125}\text{I}$ -tissue kallikrein, we speculated that PCI and kallikrein binding protein (kallistatin) might be identical. However, in the meantime the protein kallistatin (kallikrein binding protein) has been purified (Zhou et al., 1992) and a kallistatin cDNA has been cloned and sequenced (Chai et al., 1993). Results obtained in these studies clearly revealed that PCI and kallistatin are different entities.

So far, little is known about the physiological relevance of tissue kallikrein inhibition by PCI, although tissue kallikrein-PCI complexes can be measured in some urine- and plasma samples (Ecke et al., 1992b). Studying PCI in plasma samples from patients with orthostatic hypotension we found that some of these patients exhibited low plasma PCI activity as determined using an ELISA measuring complex formation between plasma PCI and exogenously added urokinase (Fig. 2). Whether PCI in these patients is intrinsically low or just decreased due to PCI consumption (e.g., by plasma serine proteases) remains to be determined.

### 3. Regulation of PCI activity and target enzyme specificity by glycosaminoglycans (GAGs)

PCI – like antithrombin III and heparin cofactor II – belongs to the subgroup of heparin binding serpins, and heparin stimulates many PCI-protease interactions (Geiger, 1988). On the other hand heparin interferes with the inhibition of tissue kallikrein by PCI (Ecke et al., 1992a). This inhibitory effect does not seem to be GAG-specific, but is also seen with other negatively charged high- $M_r$  compounds (not shown). It is most likely caused by steric hindrance. PCI-binding GAGs are present on epithelial kidney cells (Priglinger et al., 1994) and have a similar effect on PCI activity as heparin (Geiger et al., 1991), indicating that such cellular GAGs might regulate PCI-activity and target enzyme specificity *in vivo*.

### 4. Tissue-specific gene expression of human and mouse PCI

Transgenic mouse models (e.g., knock-out mice) are widely used for studying the biological roles of proteins, generally assuming that the protein studied fulfils the same or similar functions in mice and in humans. We therefore isolated and analyzed the gene encoding for mouse PCI and studied expression of PCI using human and mouse tissues (Zechmeister-Machhart et al., 1996). As the human counterpart, the mouse PCI-gene is composed of five exons and four introns. It encodes for a polypeptide of 405 amino acids. On the protein level mouse PCI is ~63% homologous to human PCI. Tissue specific expression of murine and human PCI was studied by Northern blotting using mouse and human cDNA probes. PCI mRNA was detected in many human organs including liver, heart, pancreas, ovary and testis, with the highest concentrations in pancreas. In the mouse, however, PCI expression was exclusively detected in the reproductive system (testis, seminal vesicle, ovary). No PCI was found in mouse liver, suggesting that in the mouse PCI is not a plasma protein. Therefore PCI seems to fulfil different biological functions in mice and in humans, although the proteins are highly homologous.

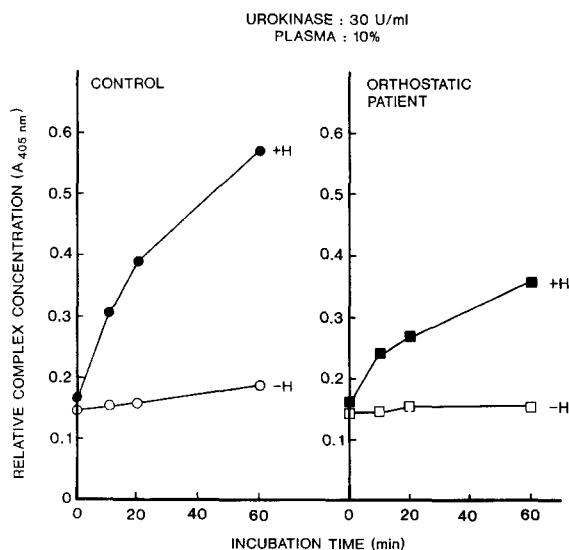


Fig. 2.

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