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Review

Structural insights into the multiple functions of protein C inhibitor

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Abstract. Protein C inhibitor (PCI) is a widely distributed, multifunctional member of the serpin family of protease inhibitors, and has been implicated in several physiological processes and disease states. Its inhibitory activity and specificity are regulated by binding to cofactors such as heparin, thrombomodulin and phospholipids, and it also appears to have non-inhibitory functions related to hormone and lipid binding. Just how the highly conserved serpin architecture can support the multiple diverse functions of PCI is a riddle best addressed by protein crystallography. Over the last few years we have solved the structure of PCI in its native, cleaved and protein-complexed states. They reveal a conserved serpin fold and general mechanism of protease inhibition, but with some unique features relating to inhibitory specificity/promiscuity, cofactor binding and hydrophobic ligand transport.

Keywords. Cofactor, haemostasis, heparin, phospholipid, serpin, structure, thrombin.

Biological functions of protein C inhibitor

The actual biological function of protein C inhibitor (PCI) in human health and disease has been hard to pinpoint, and it is even unclear whether the main role of PCI has even been discovered. This is due in large part to the wide tissue distribution of PCI, its ability to inhibit many different serine proteases, the effect of cofactors on localisation and specificity, the ability to bind to and transport hydrophobic compounds, and the fact that no appropriate animal model exists. Even so, many published reports show a correlation between PCI levels and disease, and the mouse knockout firmly links PCI with male fertility (for a recent review see [1]). Figure 1 illustrates several of the putative functions of PCI in human biology. Of particular relevance for this review is the role of PCI in blood coagulation (haemostasis).

PCI was discovered as the principal plasma protein inhibitor of activated protein C (APC), and it is from this activity that its name is derived [2, 3]. APC plays an important role in limiting blood coagulation by cleaving factors (f) Va and VIIIa, and thus down-regulating thrombin formation. The importance of the protein C anticoagulant pathway is evident from the thrombotic tendency of carriers of the Leiden mutation of fVa, where cleavage by APC is affected (for recent reviews see [4, 5]). In addition to inhibiting APC itself, PCI regulates the formation of APC by inhibiting the thrombin-thrombomodulin (TM) complex, responsible for activating protein C [6]. Thus, PCI appears to play a haemostatic role in the blood by inhibiting the protein C anticoagulant pathway. This

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function is further supported by the fact that PCI is expressed in megakaryocytes and platelets, where it is stored in α-granules [7, 8]. Approximately one third of this PCI is released upon platelet activation, and the secreted PCI rapidly associates to the platelet surface, where it keeps the Xase (fVIIIa/fIXa) and prothrombinase (fVa/fXa) complexes intact and functional. In addition to the potential pro-haemostatic role of PCI, it may also play an anticoagulant role by directly inhibiting thrombin, fXa and fXIa [9]. This may be counterintuitive, but there are other examples of coagulation factors playing dual roles in coagulation, with function depending on cofactor binding and localisation (*e.g.* thrombin [10]).

In this review we discuss the subset of PCI functions related to protease recognition and inhibition, cofactor binding and their effect on inhibitory specificity, and the unusual ability of PCI to bind to and transport hydrophobic compounds. These functions exploit three unique features of PCI: a long and flexible reactive centre loop (RCL); a large basic patch adjacent to the RCL; and a deep bifurcated hydrophobic channel next to the basic patch.

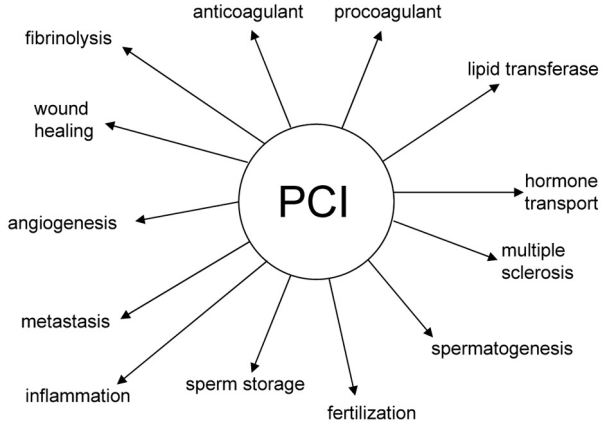

Figure 1. Multiple putative functions of protein C inhibitor (PCI). Although the precise roles of PCI in human health and disease are unclear, the inhibitory and non-inhibitory functions of PCI have been implicated in a diverse range of biological processes and disease states.

PCI is a serpin

The inhibitory promiscuity of PCI is less surprising when understood in the context of the conserved serpin mechanism of protease inhibition (Fig. 2) (for good reviews see [11, 12]). Serpins utilise a metaphorical mousetrap mechanism (known as a suicide-substrate mechanism), where an energetically strained trap (serpin) attracts the mouse (protease) with an appealing morsel of cheese (the RCL). Once the bait has been nibbled, the trap is sprung and the mouse is crushed (described in detail in the legend of Fig. 2) [13]. The metastable native state is composed of three β-sheets and nine α-helices, and the main features relevant to protease inhibition are the 20-residue RCL and the five-stranded β-sheet A (Fig. 2, left panel). The rapid incorporation of the RCL into β-sheet A is the conformational event that leads to the more stable state (more than doubling the melting temperature), and is responsible for crushing the protease. The final complex is a kinetically trapped acyl enzyme intermediate, with an ester bond between the catalytic Ser of the protease and the P1 residue of the serpin.

As shown in Figure 2, the RCL is exposed as a substrate loop and is the principal recognition site for target proteases. The most important residue in determining protease specificity is the P1 residue (after the nomenclature of Schechter and Berger [14], where the scissile bond is denoted P1-P1′, and residues to the N- and C-terminal sides are numbered sequentially), which fits neatly into the active site of the protease to align the scissile bond for cleavage. The importance of the P1 residue is illustrated by the Pittsburgh variant of α_1-antitrypsin (α_1AT), where the change of a Met for an Arg switches the specificity from chymotrypsin-like proteases such as elastase, to trypsin-like proteases such as thrombin. The result of the heterozygous lesion was death through massive haemorrhage at a very young age [15]. PCI is highly unusual in possessing two P1 residues, Arg354 and Phe353, so that it is able to recognise and inhibit both classes of serine proteases [16]. However, crystal structures of serpins with their physiological targets have revealed a dependence on exosites (binding regions outside the RCL and the active site) in the formation of recognition (Michaelis) complexes [17–19].

Structures of native and RCL-cleaved PCI

The structures of native [20] and RCL-cleaved [21] PCI are shown in Figure 3. A quick look at the two ribbon diagrams (Fig. 3A and B) confirms the conserved serpin architecture and suggests a normal inhibitory mechanism involving full RCL incorporation into β-sheet A. Closer examination, however, shows some rather interesting differences of functional relevance: the RCL is exceptionally long and flexible, with four extra residues C-terminal to the scissile bond; helix A is shorter by two turns (7 residues) at the N terminus; and helices D and H are rotated relative to α_1AT (Fig. 3C). In total, five different forms of native PCI were observed in our crystal structures, each with a different position of the RCL and all but one with unresolved RCL residues

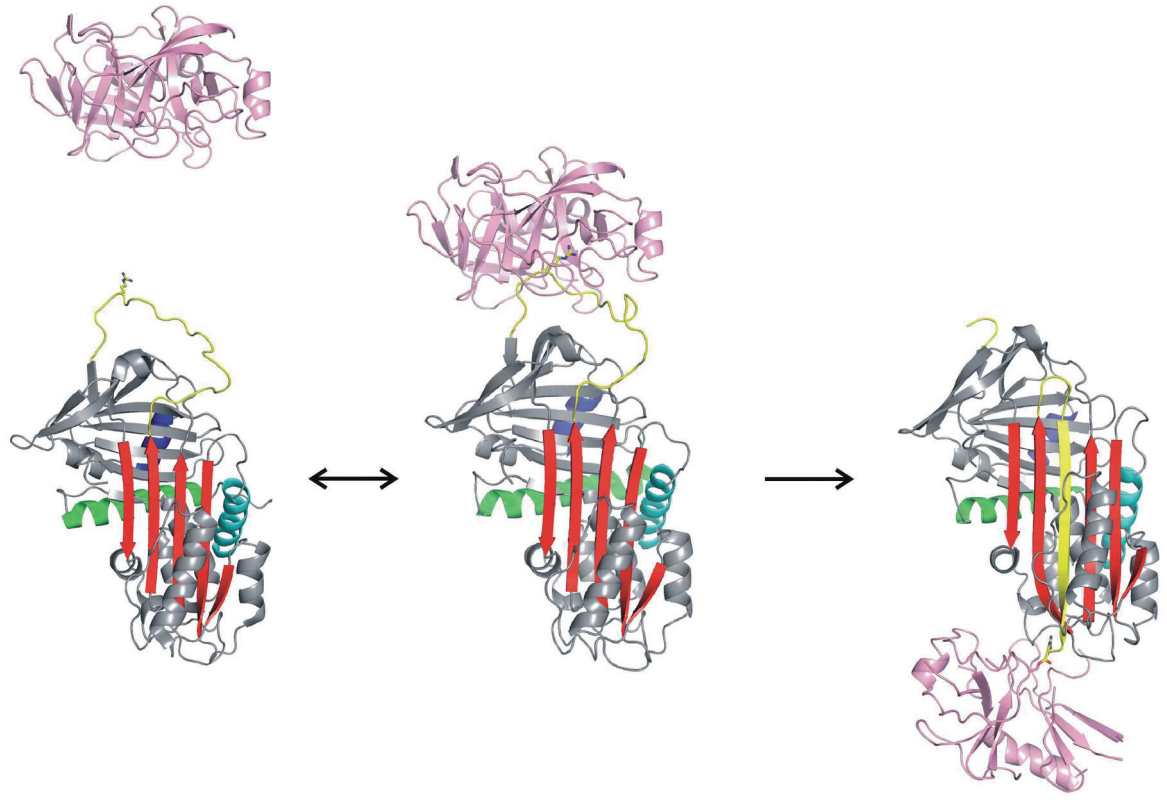

Figure 2. The serpin mechanism of protease inhibition. Protease inhibition by serpins proceeds through the formation of an initial recognition complex (Michaelis complex, centre) followed by an irreversible conformational change step leading to the final covalent complex (right). The serpin is shown in the classic orientation with β-sheet A facing (red), the reactive centre loop (RCL; yellow) on top, helix A in the back (green), helix D on the side (cyan) and helix H (blue) at the top in the back. The protease (pink) recognizes the sequence in the RCL (P1 residue is of principal importance and the side chain is shown as sticks), and then begins to cut the peptide bond between the P1 and P1′ residues. At the acyl-enzyme intermediate step, where an ester bond exists between the protease and the serpin (shown as sticks), the protease is flung to the opposite pole of the serpin through rapid incorporation of the RCL as strand 4 of β-sheet A. Deacylation is prevented by disruption of the structure of the protease (40 % of the protease structure is missing).

due to conformational flexibility. Although there is some flexibility inherent in the RCLs of most serpins, the degree seen for native PCI was unusually high due to a paucity of interactions in the hinge region (the N-terminal portion) and the additional four residues at the C terminus of the RCL. These features effectively lift the P1 residue away from the surface of PCI and confer upon it a greater degree of conformational freedom. This length and flexibility allows the RCL to interact with proteases as a relatively isolated peptide loop, which would predictably reduce the need for surface charge complementarity between the body of PCI and a protease. It will also allow the protease to rotate or twist while still engaging the RCL within its active site, to permit exosite contacts that would not otherwise be accessible. There is precedence for this in the structures of the Michaelis complexes of antithrombin (AT) with targets thrombin and fXa, where the conformational freedom of the RCL directly impacts which exosites on the protease and serpin are engaged [22].

The other unique features of native PCI are on the back of the molecule (oriented as in Fig. 3C), and are best illustrated by surface representations in stereo, coloured according to electrostatic and hydrophobic properties (Fig. 4). There is a large basic patch near helix H, and this region has been implicated in protease recognition and cofactor binding (discussed in detail in the following section). Just below the basic patch is a large hydrophobic pocket (Fig. 4B), formed by the shortening of helix A and the rotation of the D and H helices relative to other serpins (see Fig. 3C). The shape of the pocket is rather unusual, with a large hole at the bottom (helix A gap) and two linear channels radiating from the hole along helix D and helix H (the D′ and H′ channels). These channels are separated by the loop between strands 4 and 5 of β-sheet B, which is shorter in PCI by three residues relative to α_1AT. Mutagenesis and molecular modelling studies predict that this is the site of binding for retinoic acid and phospholipids (discussed in detail in later sections).

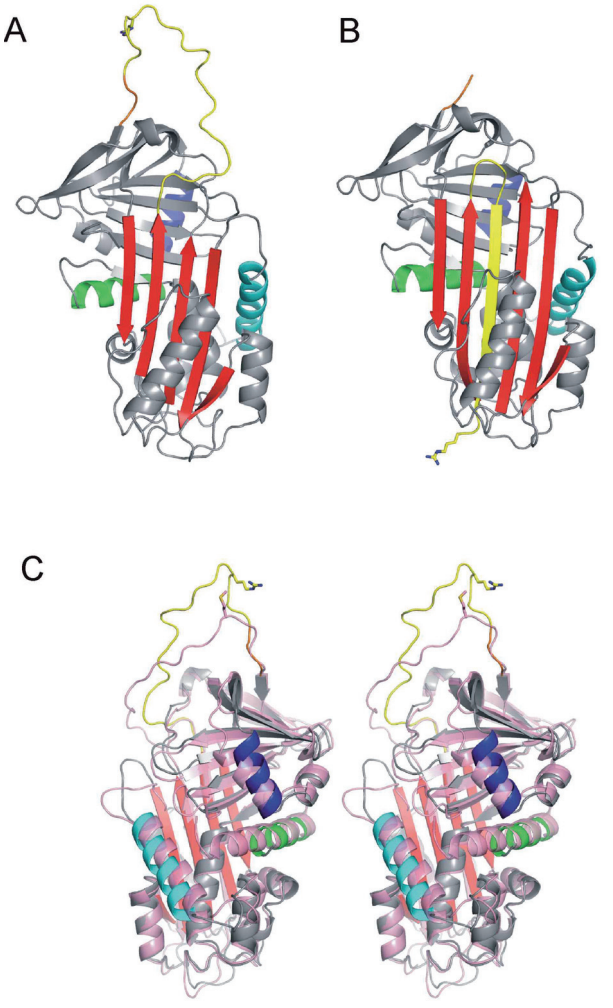

Figure 3. Structural features of PCI. (*A*) Ribbon diagram of native PCI shown in the classic orientation (coloured as previously) reveals the normal serpin fold, but with an extra-long RCL due to the insertion of four residues on the P′ side (orange). (*B*) The structure of RCL-cleaved PCI has its RCL incorporated into β-sheet A, as do all inhibitory serpins. (*C*) A stereo representation of native PCI rotated to illustrate the main structural differences with the prototypical serpin α_1-antitrypsin (α_1AT; semitransparent pink with the P1 Met in sticks). The main differences are the length of the RCL, the shortening of helix A, and the rotation of both the D and H helices (coloured as previously).

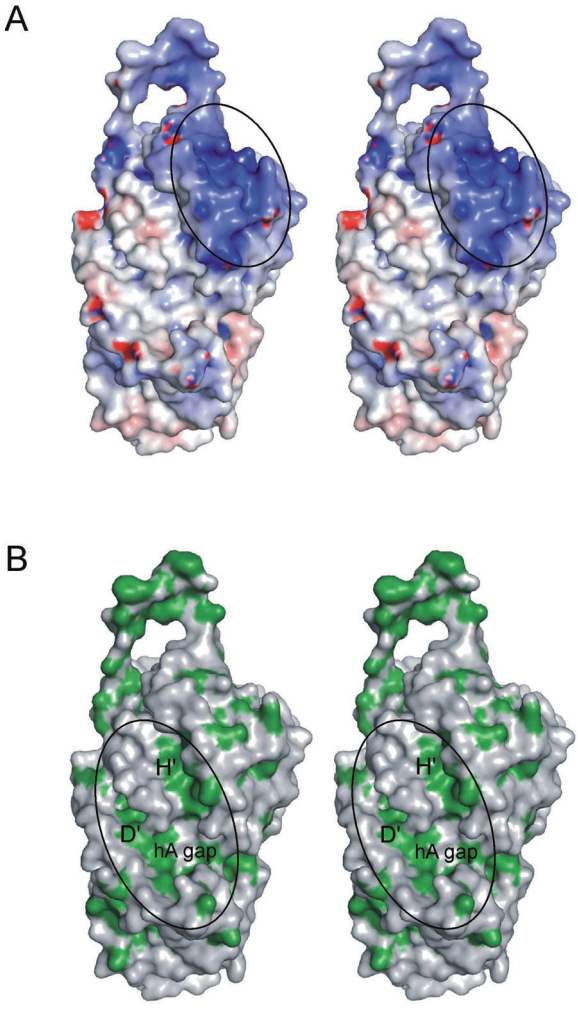

Figure 4. Stereo surface representations of native PCI. (*A*) The electrostatic properties of PCI (oriented as in Fig. 3C, with positive blue and negative red) are important for understanding protease specificity. Just adjacent to the RCL (top) is a large basic patch (indicated by the oval) composed of helix H and other regions. (*B*) The same surface coloured according to hydrophobic properties (green) reveals the hydrophobic helix A gap and the D′ and H′ channels (indicated). This is the putative hydrophobic compound and phospholipid-binding site of PCI.

Heparin-binding site

PCI belongs to a subclass of serpins that bind to heparin and other glycosaminoglycans (GAGs) [23]. These 'heparin-binding serpins' include AT, heparin cofactor II (HCII), protease nexin I and plasminogen activator inhibitor-1, all of which are involved in blood coagulation, are thrombin inhibitors and utilise basic residues along helix D for GAG binding. It is clear from the electrostatic surface representation of PCI in Figure 4A that PCI does not possess a positively charged D helix, and mutagenesis studies confirm that it is not involved in heparin binding [24]. To date, the only crystal structure of a serpin bound to a GAG is that of AT bound to its specific pentasaccharide fragment [25], but it is not clear whether this mode of binding is preserved for other heparin-binding serpins that use helix D. Determining how PCI interacts with heparin is even more difficult due to the overall basic charge of the protein, and the large basic patch contiguous with helix H (indicated by the oval in Fig. 4A). In addition to the basic site on and near helix H, PCI has an N-terminal extension with a signature heparin-binding sequence predicted to form a helix, the so-called A+ helix [26]. Mutagenesis studies by our group and others have shown that neither the A+

helix nor the basic region adjacent to helix H are critical for heparin binding [20, 27]. We also determined the minimum heparin length capable of fully occupying the heparin-binding site of PCI to be an eight monosaccharide chain, which fits nicely along the linear length of helix H [20] (Fig. 5A). While this is likely to be the favoured binding mode for heparin on PCI, PCI may be capable of interacting with heparin in a protease-dependent fashion by using more of its non-linear basic surface.

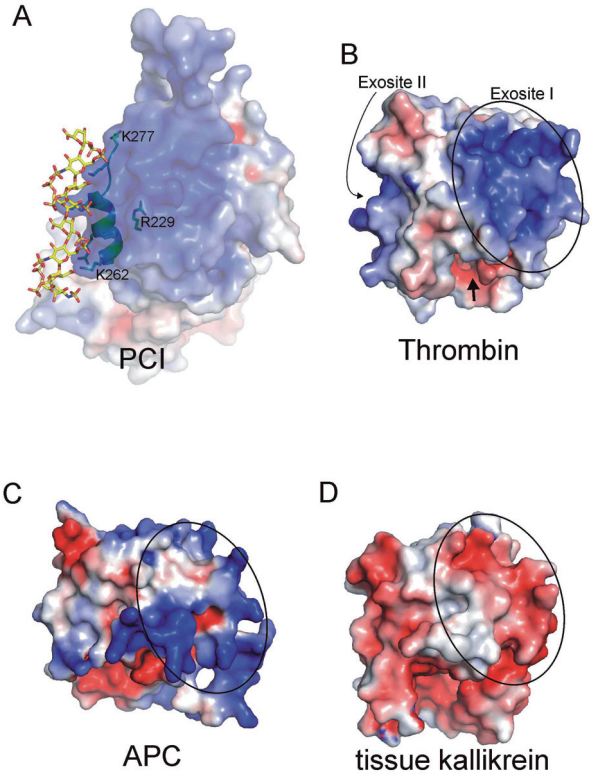

Figure 5. Electrostatic surface representations of the interaction interface between PCI and target proteases. (*A*) The top of PCI is shown as a semitransparent surface coloured according to electrostatic potential (blue positive and red negative). The minimal heparin binding length of eight monosaccharide units is shown (yellow rods) on the putative heparin-binding site along helix H (shown as ribbon, with basic side chains as sticks). Arg 229 (indicated) is nearby, but does not appreciably affect heparin binding. The basic region adjacent to helix H does, however, exert influence on the region of attacking protease indicated by the oval in B, C and D. (*B*) Thrombin surface with the active site indicated by the arrow, and exosites I and II indicated. (*C*) APC surface oriented as thrombin, with its heparin-binding region indicated. (*D*) In contrast to thrombin and APC, tissue kallikrein would present a favourable negatively charged surface toward the top of PCI, and heparin binding by PCI would predictably repulse the protease.

Heparin activation

Because the native structure of the RCL of PCI is not conformationally constrained by hinge-region insertion (as seen for AT and HCII), it is safe to conclude that allostery plays no role in the heparin activation of PCI. Rather, a simple template mechanism, whereby PCI and protease bind to the same heparin molecule, is the mechanism by which protease inhibition is accelerated by heparin. The effect is improved rates of diffusion and encounter, and also stabilisation of the Michaelis complex to ensure that every encounter will proceed to the acyl-enzyme intermediate before dissociation can occur. A bell-shaped dependence of rate of inhibition on heparin concentration has been shown for PCI with thrombin, APC and other proteases [28, 29], and this is the hallmark of the template mechanism. However, as mentioned above, the heparin-binding site of PCI is unusually close to the RCL, and therefore very near the docked protease. Thus, the positively charged region that includes the heparin-binding site exerts an influence on the protease in the absence of heparin, either repulsive or attractive (see Fig. 5), and an opposite influence when heparin is bound. Initial modelling of the PCI-protease Michaelis complexes based on the HCII-thrombin complex showed how thrombin and APC would present a repulsive basic face towards PCI, and we hypothesised that heparin binding to both the protease and the serpin would relieve the repulsion by interposing between the two molecules [21]. This hypothesis is supported by improved rates of inhibition for variants of APC with charge neutralizing or reversing mutations at the heparin-binding site [30, 31]. This model also explains why tissue kallikrein inhibition is abrogated by the binding of heparin to PCI [32], as tissue kallikrein presents a highly negatively charged face to the top of PCI (Fig. 5D).

The structure of the heparin-bridged PCI-thrombin complex

To determine how heparin acts as a cofactor for thrombin inhibition by PCI, we recently solved a crystal structure of the complex between PCI, a catalytically inert form of thrombin (S195A), and a 14mer heparin fragment [33]. The structure provided a satisfying explanation for the extra-long RCL, with the P′ side extended to allow thrombin to rotate towards the back of PCI to align the heparin-binding sites (Fig. 6A and B). We also observed some surprising exosite contacts between the body of PCI and the 60-loop of thrombin, which seem to have been formed

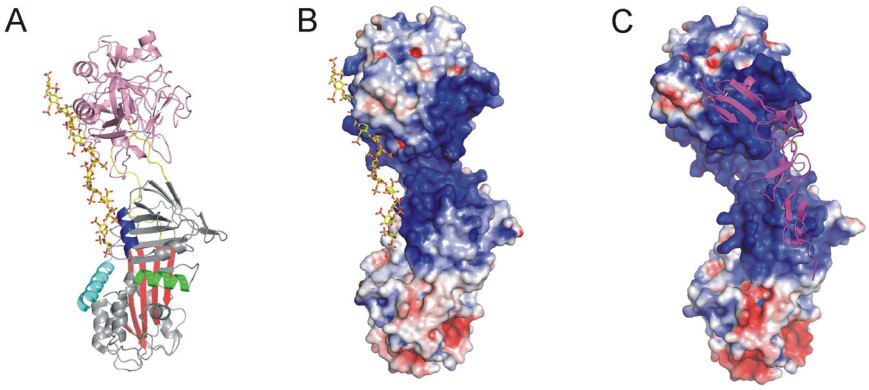

Figure 6. The structure of the PCI-thrombin complex. (*A*) A ribbon depiction of the crystal structure of the Michaelis complex between thrombin (pink) and PCI (coloured as before) reveals how the heparin (yellow) binding sites are aligned. (*B*) A surface representation of the same complex coloured according to electrostatics shows how a linear heparin chain of 14 monosaccharide units can bridge the two proteins (oriented as in A). (*C*) Modelling the PCI-thrombin-thrombomodulin (TM) complex required a small rotation of thrombin from the position seen in the structure (*A*) to place TM (magenta) in contact with residues known to mediate the interaction.

through the influence of the unusual size of the P2 Phe residue of PCI on the conformation of the 60-loop of thrombin. These contacts help orient thrombin on PCI and maintain the proper alignment of the heparin-binding sites of the two proteins (exosite II of thrombin and helix H of PCI).

In spite of the high resolution of the structure and the fact that heparin was clearly present in the crystal, no density could be observed for heparin bound to PCI. This was due to the packing of basic regions around the heparin molecule in the crystal lattice, so that heparin was sampling multiple conformations within the crystal. However, we were able to build a disaccharide of heparin into exosite II of thrombin that is in full agreement with the binding mode observed in the crystal structure of the thrombin-heparin complex [34]. The extension of this heparin chain towards PCI allows for facile interaction with residues along helix H that are known to be involved in heparin binding. This result supports our earlier prediction of a linear heparin-binding mode along helix H (Fig. 5A), and nicely illustrates how the special features of the RCL are exploited to allow the docking of two molecules predicted to be repulsive by electrostatics alone.

Model of the PCI-thrombin-TM complex

One of the interesting features of the PCI-thrombin structure is the alignment of exosite I of thrombin with the basic patch adjacent to helix H (see Fig. 6B). Superposition of the thrombin-TM complex on thrombin in complex with PCI provides a good starting point for understanding how TM accelerates thrombin inhibition by PCI. TM is a transmembrane protein with six epidermal growth factor (EGF)-like domains, three of which, EGF4–6, are sufficient to serve as a cofactor for inhibition of thrombin by PCI [6]. The structure of thrombin bound to EGF4–6 revealed that domains 5 and 6 bind directly to thrombin and are held rigidly in place, while domain 4 has no contact with thrombin and is connected to domain 5 by a flexible linker [35]. We showed that PCI interacts directly with TM4–6 by demonstrating a bell-shaped dependence of inhibition rate on EGF4–6 concentration, and the K_d for PCI was estimated to be ~24 μM [33]. Our initial model predicted contacts between domains 4 and 5 with specific residues on PCI, but mutagenesis forced us to rule them out [33]. A refined model that satisfied the mutagenesis data was created by simply rotating the thrombin-TM complex by 25° (Fig. 6C).

PCI binding to retinoic acid

The serpin fold has evolved to carry out multiple functions, some of which are non-inhibitory. PCI is unique among the inhibitory serpins in its ability to bind to hydrophobic hormones/growth factors such as estradiol, testosterone and retinoic acid, although the biological relevance of this activity is unclear [36]. Corticosteroid-binding globulin (CBG) and thyroxine-binding globulin (TBG) are non-inhibitory serpins that bind to and transport hydrophobic hormones, and seem to utilise the serpin β-sheet expansion mechanism to effect delivery at specific sites [37]. When we solved the structure of cleaved PCI and noticed the large hydrophobic pocket created by the shortening of helix A and the rotation of helices D and H, we hypothesised that the same site would be

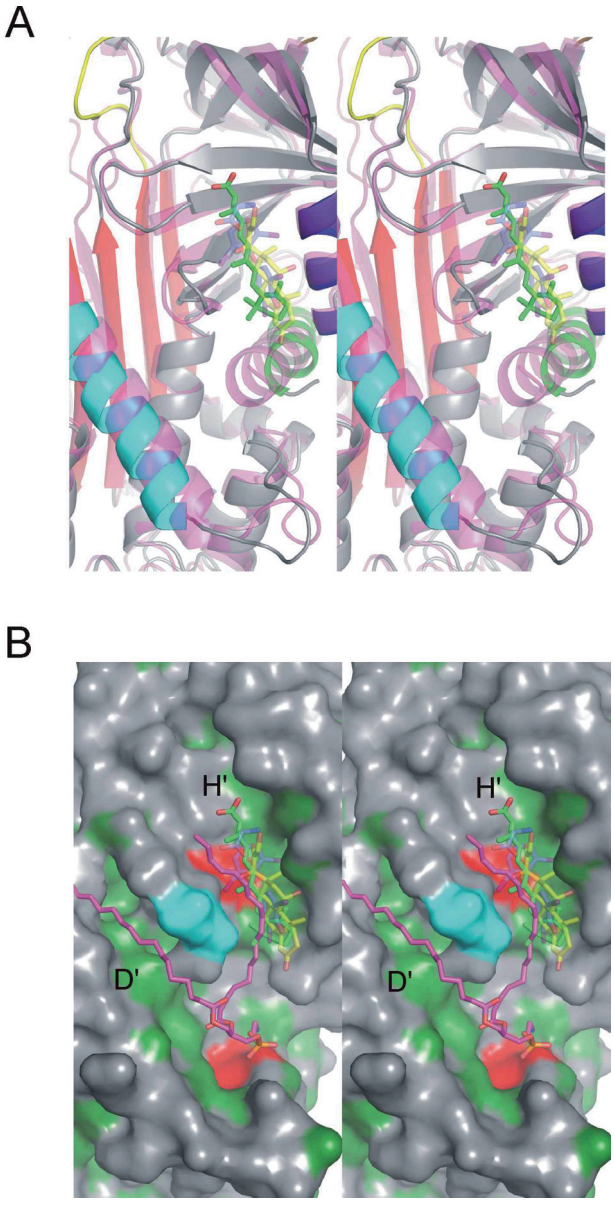

Figure 7. Stereo views of the putative hormone and phospholipid-binding sites of PCI. (*A*) Native PCI (coloured as before) superimposed on thyroxine-binding globulin (TBG; semitransparent magenta) reveals a good fit with respect to helices D and H. Thyroxine bound to native TBG is shown as blue rods, and corresponds well to the position of cortisol in corticosteroid-binding globulin (CBG; yellow sticks) and the proposed binding site of retinoic acid (green sticks). (*B*) The same orientation of PCI with surface coloured according to hydrophobic properties illustrates the depth of the helix A gap and the D′ and H′ channels. The modelled position of phosphatidylethanolamine (PE) (magenta) utilises all of these features, with one of the acyl chains binding in the same region as the hydrophobic ligands (coloured as in A). Mutations that inhibit binding to PE are shown in red, and the loop that separates the channel is cyan.

utilised by CBG and TBG for hormone binding [21]. This has been born out by the recent crystal structures of CBG and TBG bound to their respective ligands [38–40], but in both cases, rotation of helices D and H was sufficient to create the hydrophobic binding pocket and no shortening of helix A was observed. Superposition of native PCI onto native TBG or CBG shows a much closer fit than that seen for the prototypical serpin α_1AT (Fig. 7A *versus* Fig. 3C). The position of cortisol and thyroxine is conserved, and corresponds well to the position of retinoic acid predicted by docking studies (Fig. 7A). Further support for a shared hormone-binding site for CBG, TBG and PCI was provided by a crystal contact seen for native PCI, where a hydrophobic stretch of the RCL of one PCI monomer (IFTFR) was found docked in the same pocket of a crystallographically related monomer [20].

PCI binding to phospholipids

PCI has been shown to interact specifically with phosphatidylethanolamine (PE) [7], a lipid normally found on the inner leaf of the cell membrane that is exposed as a marker of apoptosis or platelet activation. This property has intriguing implications for the role of PCI in haemostasis, since PCI is secreted from α-granules in response to platelet activation. The secreted PCI is subsequently found bound to the platelet surface in a PE-dependent manner, where it can efficiently inhibit APC to preserve the Xase and prothrombinase complexes. However, a recent report suggests that the binding is not mediated by PE itself, but by phosphatidylserine (PS) or by PE in its oxidised form [41]. Oxidation of these phospholipids enhances the stimulation of APC inhibition from about 7-fold to over 100-fold, and may have implications at sites of inflammation, such as atherosclerotic plaques. The PCI-phospholipid interaction is evidently highly sensitive to the properties of the head group, with PE and PS binding with K_ds of approximately 3 nM and twice as tightly to the oxidised forms. Acceleration of APC inhibition is dependent on APC binding to the membrane surface by its Gla-domain, and is thus Ca^{2+} sensitive. The PCI-membrane interaction is competed by heparin but not retinoic acid, and requires an intact helix H. It would thus appear that the cofactor activity of phospholipids is entirely mediated by the head groups interacting with the heparin-binding site of PCI to co-localise PCI and APC on the cell surface. However, a recent report suggests two entirely different activities associated with phospholipid binding to PCI that depend on both the type of head group and the presence of a cone-shaped acyl tail.

In 1998, Engelmann and colleagues [42] published the finding that a protein secreted by stimulated platelets was capable of transferring PE from low-density

lipoprotein (LDL), and that the transferred PE supported improved prothrombinase activity. This factor was later identified by the same group as PCI [43]. PCI could transfer PE from LDL and small unilamellar vesicles to platelets and other cells, and specificity for PE was absolute. This activity implies a binding mode that protects the acyl chains of PE from solvent during transfer, and a model was made based on the original structure of cleaved PCI and *in silico* molecular docking (Fig. 7B). The model placed the head group of PE into the helix A gap and the acyl chains in the hydrophobic D′ and H′ channels. This binding site appears to fit the conical shape of PE perfectly, and was verified by mutagenesis [43] (Fig. 7B). It was also observed that PCI was capable of incorporating into vesicles and cells in a PE-dependent manner, independent of the endocytotic machinery. Interestingly, given time the internalised PCI was found in the nucleus of cultured cells and in mouse leukocytes after tail vein injection. Why it is taken up by cells and why it ends up in the nucleus is unclear; however, PCI uptake by macrophages stimulates phagocytosis and therefore may play a role in host defence. The addition of exogenous heparin inhibits the cell entry of PCI, indicating that once again helix H plays a role in the interaction with PE. The modelled position of PE bound to PCI is in contact with helix H, and may partially explain the competitive effect. It was also suggested that cell entry is a two-step process, with GAG binding preceding the interaction with PE.

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1 Geiger, M. (2007) Protein C inhibitor, a serpin with functions in- and outside vascular biology. Thromb. Haemost. 97, 343–347.
2 Marlar, R. A. and Griffin, J. H. (1980) Deficiency of protein C inhibitor in combined factor V/VIII deficiency disease. J. Clin. Invest. 66, 1186–1189.
3 Suzuki, K., Nishioka, J. and Hashimoto, S. (1983) Protein C inhibitor. Purification from human plasma and characterization. J. Biol. Chem. 258, 163–168.
4 Segers, K., Dahlback, B. and Nicolaes, G. A. (2007) Coagulation factor V and thrombophilia: Background and mechanisms. Thromb. Haemost. 98, 530–542.
5 Dahlback, B. (1908) Early days of APC resistance and FV Leiden. Hamostaseologie 28, 103–109.
6 Rezaie, A. R., Cooper, S. T., Church, F. C. and Esmon, C. T. (1995) Protein C inhibitor is a potent inhibitor of the thrombin-thrombomodulin complex. J. Biol. Chem. 270, 25336–25339.
7 Nishioka, J., Ning, M., Hayashi, T. and Suzuki, K. (1998) Protein C inhibitor secreted from activated platelets efficiently inhibits activated protein C on phosphatidylethanolamine of platelet membrane and microvesicles. J. Biol. Chem. 273, 11281–11287.
8 Prendes, M. J., Bielek, E., Zechmeister-Machhart, M., Vanyek-Zavadil, E., Carroll, V. A., Breuss, J., Binder, B. R. and Geiger, M. (1999) Synthesis and ultrastructural localization of protein C inhibitor in human platelets and megakaryocytes. Blood 94, 1300–1312.
9 Rau, J. C., Beaulieu, L. M., Huntington, J. A. and Church, F. C. (2007) Serpins in thrombosis, hemostasis and fibrinolysis. J. Thromb. Haemost. 5 Suppl. 1, 102–115.
10 DiCera, E. (2008) Thrombin. Mol. Aspects Med. 29, 203–254.
11 Gettins, P. G. (2002) Serpin structure, mechanism, and function. Chem. Rev. 102, 4751–4804.
12 Silverman, G. A., Bird, P., I, Carrell, R. W., Church, F. C., Coughlin, P. B., Gettins, P. G. W., Irving, J. A., Lomas, D. A., Luke, C. J., Moyer, R. W., Pemberton, P. A., Remold-O'Donnell, E., Salvesen, G. S., Travis, J. and Whisstock, J. C. (2001) The serpins are an expanding superfamily of structurally similar but functionally diverse proteins. Evolution, mechanism of inhibition, novel functions, and a revised nomenclature. J. Biol. Chem. 276, 33293–33296.
13 Huntington, J. A., Read, R. J. and Carrell, R. W. (2000) Structure of a serpin-protease complex shows inhibition by deformation. Nature 407, 923–926.
14 Schechter, I. and Berger, A. (1967) On the size of the active site in proteases. I. Papain. Biochem. Biophys. Res. Commun. 27, 157–162.
15 Owen, M. C., Brennan, S. O., Lewis, J. H. and Carrell, R. W. (1983) Mutation of antitrypsin to antithrombin. alpha 1-antitrypsin Pittsburgh (358 Met leads to Arg), a fatal bleeding disorder. N. Engl. J. Med. 309, 694–698.
16 Pratt, C. W. and Church, F. C. (1992) Heparin binding to protein C inhibitor. J. Biol. Chem. 267, 8789–8794.
17 Johnson, D. J., Li, W., Adams, T. E. and Huntington, J. A. (2006) Antithrombin-S195A factor Xa-heparin structure reveals the allosteric mechanism of antithrombin activation. EMBO J. 25, 2029–2037.
18 Li, W., Johnson, D. J., Esmon, C. T. and Huntington, J. A. (2004) Structure of the antithrombin-thrombin-heparin ternary complex reveals the antithrombotic mechanism of heparin. Nat. Struct. Mol. Biol. 11, 857–862.
19 Baglin, T. P., Carrell, R. W., Church, F. C., Esmon, C. T. and Huntington, J. A. (2002) Crystal structures of native and thrombin-complexed heparin cofactor II reveal a multistep allosteric mechanism. Proc. Natl. Acad. Sci. USA 99, 11079–11084.
20 Li, W., Adams, T. E., Kjellberg, M., Stenflo, J. and Huntington, J. A. (2007) Structure of native protein C inhibitor provides insight into its multiple functions. J. Biol. Chem. 282, 13759–13768.
21 Huntington, J. A., Kjellberg, M. and Stenflo, J. (2003) Crystal structure of protein C inhibitor provides insights into hormone binding and heparin activation. Structure (Camb) 11, 205–215.
22 Huntington, J. A. (2006) Shape-shifting serpins – Advantages of a mobile mechanism. Trends Biochem. Sci. 31, 427–435.
23 Huntington, J. A. (2003) Mechanisms of glycosaminoglycan activation of the serpins in hemostasis. J. Thromb. Haemost. 1, 1535–1549.
24 Neese, L. L., Wolfe, C. A. and Church, F. C. (1998) Contribution of basic residues of the D and H helices in heparin binding to protein C inhibitor. Arch. Biochem. Biophys. 355, 101–108.
25 Jin, L., Abrahams, J. P., Skinner, R., Petitou, M., Pike, R. N. and Carrell, R. W. (1997) The anticoagulant activation of antithrombin by heparin. Proc. Natl. Acad. Sci. USA 94, 14683–14688.
26 Kuhn, L. A., Griffin, J. H., Fisher, C. L., Greengard, J. S., Bouma, B. N., Espana, F. and Tainer, J. A. (1990) Elucidating the structural chemistry of glycosaminoglycan recognition by protein C inhibitor. Proc. Natl. Acad. Sci. USA 87, 8506–8510.
27 Elisen, M. G., Maseland, M. H., Church, F. C., Bouma, B. N. and Meijers, J. C. (1996) Role of the A+ helix in heparin binding to protein C inhibitor. Thromb. Haemost. 75, 760–766.
28 Espana, F., Berrettini, M. and Griffin, J. H. (1989) Purification and characterization of plasma protein C inhibitor. Thromb. Res. 55, 369–384.
29 Aznar, J., Espana, F., Estelles, A. and Royo, M. (1996) Heparin stimulation of the inhibition of activated protein C and other

enzymes by human protein C inhibitor – Influence of the molecular weight of heparin and ionic strength. Thromb. Haemost. 76, 983–988.
30 Friedrich, U., Blom, A. M., Dahlback, B. and Villoutreix, B. O. (2001) Structural and energetic characteristics of the heparin-binding site in antithrombotic protein C. J. Biol. Chem. 276, 24122–24128.
31 Yang, L., Manithody, C. and Rezaie, A. R. (2002) Contribution of basic residues of the 70-80-loop to heparin binding and anticoagulant function of activated protein C. Biochemistry 41, 6149–6157.
32 Ecke, S., Geiger, M., Resch, I., Jerabek, I., Sting, L., Maier, M. and Binder, B. R. (1992) Inhibition of tissue kallikrein by protein C inhibitor. Evidence for identity of protein C inhibitor with the kallikrein binding protein. J. Biol. Chem. 267, 7048–7052.
33 Li, W., Adams, T. E., Nangalia, J., Esmon, C. T. and Huntington, J. A. (2008) Molecular basis of thrombin recognition by protein C inhibitor revealed by the 1.6-A structure of the heparin-bridged complex. Proc. Natl. Acad. Sci. USA 105, 4661–4666.
34 Carter, W. J., Cama, E. and Huntington, J. A. (2004) Crystal structure of thrombin bound to heparin. J. Biol. Chem. 280, 2745–2749.
35 Fuentes-Prior, P., Iwanaga, Y., Huber, R., Pagila, R., Rumennik, G., Seto, M., Morser, J., Light, D. R. and Bode, W. (2000) Structural basis for the anticoagulant activity of the thrombin-thrombomodulin complex. Nature 404, 518–525.
36 Jerabek, I., Zechmeister-Machhart, M., Binder, B. R. and Geiger, M. (2001) Binding of retinoic acid by the inhibitory serpin protein C inhibitor. Eur. J. Biochem. 268, 5989–5996.
37 Pemberton, P. A., Stein, P. E., Pepys, M. B., Potter, J. M. and Carrell, R. W. (1988) Hormone binding globulins undergo serpin conformational change in inflammation. Nature 336, 257–258.
38 Zhou, A., Wei, Z., Read, R. J. and Carrell, R. W. (2006) Structural mechanism for the carriage and release of thyroxine in the blood. Proc. Natl. Acad. Sci. USA 103, 13321–13326.
39 Klieber, M. A., Underhill, C., Hammond, G. L. and Muller, Y. A. (2007) Corticosteroid-binding globulin, a structural basis for steroid transport and proteinase-triggered release. J. Biol. Chem. 282, 29594–29603.
40 Zhou, A., Wei, Z., Stanley, P. L., Read, R. J., Stein, P. E. and Carrell, R. W. (2008) The S-to-R transition of corticosteroid-binding globulin and the mechanism of hormone release. J. Mol. Biol. 380, 244–251.
41 Malleier, J. M., Oskolkova, O., Bochkov, V., Jerabek, I., Sokolikova, B., Perkmann, T., Breuss, J., Binder, B. R. and Geiger, M. (2007) Regulation of protein C inhibitor (PCI) activity by specific oxidized and negatively charged phospholipids. Blood 109, 4769–4776.
42 Engelmann, B., Schaipp, B., Dobner, P., Stoeckelhuber, M., Kogl, C., Siess, W. and Hermetter, A. (1998) Platelet agonists enhance the import of phosphatidylethanolamine into human platelets. J. Biol. Chem. 273, 27800–27808.
43 Baumgartner, P., Geiger, M., Zieseniss, S., Malleier, J., Huntington, J. A., Hochrainer, K., Bielek, E., Stoeckelhuber, M., Lauber, K., Scherfeld, D., Schwille, P., Waldele, K., Beyer, K. and Engelmann, B. (2007) Phosphatidylethanolamine critically supports internalization of cell-penetrating protein C inhibitor. J. Cell. Biol. 179, 793–804.