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Minireview

Structural aspects of serpin inhibition

Andreas J. Schulze*, Robert Huber, Wolfram Bode, Richard A. Engh

Max-Planck-Institut für Biochemie, Am Klopferspitz 18 A, D-82152 Planegg-Martinsried bei München, Germany

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Abstract

The essential roles of proteins of the serpin family in many physiological processes, along with new discoveries of their unique folding properties, have attracted intense interest in recent years. Many serpins display unusual mobile behavior attributed to rearrangements of α-helical or β-sheet domains, whereby large scale transitions accompany a variety of functions, including inactivation. This unusual behavior was first recognized with the X-ray structure of modified α1-proteinase inhibitor. Subsequent experiments, including new X-ray structures, have revealed a surprising variety of conformations which are functionally important but only partially understood. We review here experimental evidence for conformations relevant to the serpin inhibitory mechanism.

Key words: Serpin; X-ray structure; β-sheet; Canonical conformation; Structural transition

1. Introduction

The search for the spatial structure of a serpin (the acronym denotes one superfamily of SERine Proteinase INhibitors) in an active conformation began with the determination of the X-ray structure of proteolytically modified α1-proteinase inhibitor (α1PI) [1], which revealed the occurrence of a large scale transition after cleavage. A variety of X-ray structures have been determined since, including that of the common deficiency S-variant of α1PI [2], the inhibitor α1-antichymotrypsin (ACHY [3]), equine elastase inhibitor (HLEI [4]), the non-inhibitory serpin ovalbumin (PLAK [5]; OVAL [6]), the latent form of plasminogen activator inhibitor PAI-1 [7], uncleaved antichymotrypsin ACHY [8], and uncleaved antithrombin III (ATIII [9,10]). Of these structures, only the latter three (PAI-1, ACHY, and ATIII) are inhibitory serpins not modified by proteolytic cleavage. The structures distinguished the serpins from other standard mechanism serine proteinase inhibitors (BPTI, among others; see Bode and Huber [11]), which otherwise are typically rigid with a single, well defined 'canonical conformation' at the binding site. None of the serpin structures, however, show a canonical conformation at the binding site. Instead, they show a binding loop which can adopt a variety of conformations. The structure of ATIII [9,10] shows a structure most compatible with binding to its target protease, and demonstrates also a conformation suggested from a variety of experiments [12–17]. In this minireview, we summarize these experiments, together with the serpin crystal structures, while attempting to rationalize aspects of the serpin inhibitory mechanism not yet observed by direct structural determination.

1.1. Serpin discovery

A proteinase inhibitor in human plasma was discovered in 1894 [18], isolated in 1955 [19] and later named α1PI. Hunt and Dayhoff [20] recognized its sequence homology to ovalbumin and ATIII, thus identifying the serpin superfamily. Most serpins have been isolated from plasma, are variously glycosylated and have molecular weights from 45 to 100 kDa. HLEI and PAI-2 have been shown to exist in unglycosylated forms in the cytosolic fraction [21], and still others, such as ATIII, PAI-1 and proteinase nexin I, can be found in the extracellular matrix.

A recently discovered serpin is cowpox virus crmA (cytokine response modifier) [22], which suppresses host response to infection via inhibition of interleukin-1-β-converting enzyme [23]. Its apparent molecular weight is 38 kDa due to several deletions, mostly on the N-terminal side of the active site, among them the deletion of helix D (see Fig. 1). Others include 'Serp1' from myxoma virus and malignant rabbit fibroma pox virus [24].

Although many serpins function as proteinase inhibitors, others are hormone transporters, peptide hormone precursors or have no known function. Clinical studies

*Corresponding author. Fax: (49) (89) 8578 3516.

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have revealed many naturally occurring deficiency mutants of serpins, primarily of α1PI and ATIII, showing the importance of these serpins in lung and blood processes (for details and further references see [25,26]).

1.2. Receptor binding and plasma clearance

A major function of serpins is to provide a tag for the removal of proteolytic enzymes from circulation via cellular receptors, sometimes supposed to occur when complexation with the serpin reveals a receptor recognition site [27]. Three types of receptor have been described: (i) the α2-macroglobulin/low density lipoprotein (α2M/LDL) related protein receptor, (ii) a proposed serpin-enzyme complex (SEC) receptor, and (iii) the urokinase receptor [28]; the latter recognizes and binds urokinase independent of serpin binding (PAI-1).

The α2M/LDL receptor is implicated in hepatic uptake of proteinase–inhibitor complexes, as well as proteinase–inhibitor–receptor complexes, from circulation and extracellular space. Evidence for its involvement in internalization and degradation of complexes comes from experiments that show blocking of PAI-1-enhanced degradation by addition of anti-α2M receptor antibody in monocytes [29].

A serpin–enzyme complex (SEC) receptor has been proposed to recognize a specific pentapeptide sequence highly conserved among serpins, based on studies showing that analogous synthetic peptides block the binding and internalization of serpin–enzyme complexes by HepG2 cells, and by the observation that such peptides bind specifically and saturably to neutrophils, monocytes, and hepatoma derived hepatocytes. The recognition sequence, buried and completely inaccessible in crystal structures of serpins, is supposed to become accessible after a serpin conformational change upon complexation with the protease. This putative change must be related to the transition after cleavage, since cleaved α1PI competes with the α1PI–elastase complex for binding ([30] and refs. therein), which is not easily reconcilable with the known crystal structure of α1PI.

1.3. Serpin interaction with heparin

ATIII, heparin cofactor II (HCII), protease nexin I, protein C inhibitor, and PAI-1 are relatively weak binding inhibitors of thrombin but bind tightly after addition of heparin. The heparin–ATIII complex binds thrombin 1,000-fold more tightly than ATIII alone. A heparin pentasaccharide interacts with serpins via four of its sulfate groups, probably at helix D in ATIII and HCII ([25] and refs. therein), and at helix H in protein C inhibitor ([31a,b]; Fig. 1), as identified by chemical modification and naturally occurring mutant studies. The enhancement of thrombin–serpin binding with heparin involves both induced conformational changes and bridging interactions. The minimal pentasaccharide heparin accounts for nearly all of the binding energy of heparin to

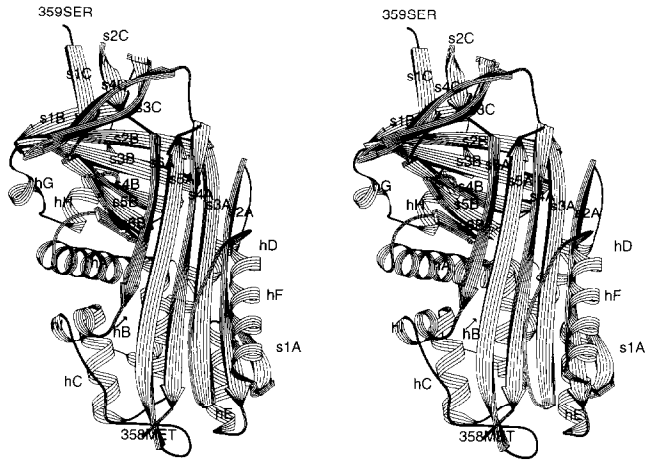

Fig. 1. Ribbon-type stereo representation [56] of α1PI.

ATIII, accelerates thrombin inhibition and promotes interaction with factor Xa, attributed to the induction of a conformational change in ATIII [32]. Heparin binding also depends on the degree of glycosylation of ATIII [33], and involves the N-terminus and other regions of the protein [34]. There is also evidence of heparin dependent domain rearrangement or unfolding [35].

1.4. Serpin interaction with vitronectin

Vitronectin, reviewed in detail by Preissner [36], forms complexes with serpins, heparin, and target proteases. It acts as a non-competitive inhibitor of the heparin-accelerated reaction of ATIII with thrombin and factor Xa. Interactions of vitronectin in ternary complexes of serpin, thrombin and vitronectin presumably involve disulfide bridges with thrombin. Vitronectin binds directly to PAI-1, however, mainly through ionic interactions, which stabilizes PAI-1 in its inhibitory form, although other sites have also been suggested ([36] and refs. therein). Vitronectin itself binds heparin at a site adjacent to its PAI-1 binding site.

2. X-ray structures

The first X-ray structure determination of a serpin was of the reactive site modified M-form of human α1-antitrypsin (α1PI*, * denotes cleavage at the reactive site Met^{358}–Ser^{359} [1,37]). The structure showed that the cleavage is followed by a conformational rearrangement whereby the residues at the cleavage site (P1, P1′) become separated by 70 Å after incorporation into β-sheet structures. The nature of this rearrangement was suggested by Löbermann et al., but was first observed in part in the X-ray structure of proteolytically modified ovalbumin, PLAK [5]. The structure of intact ovalbumin followed [6], showing an α-helical structure for the strand corresponding to the binding site of serpin inhibitors, similar

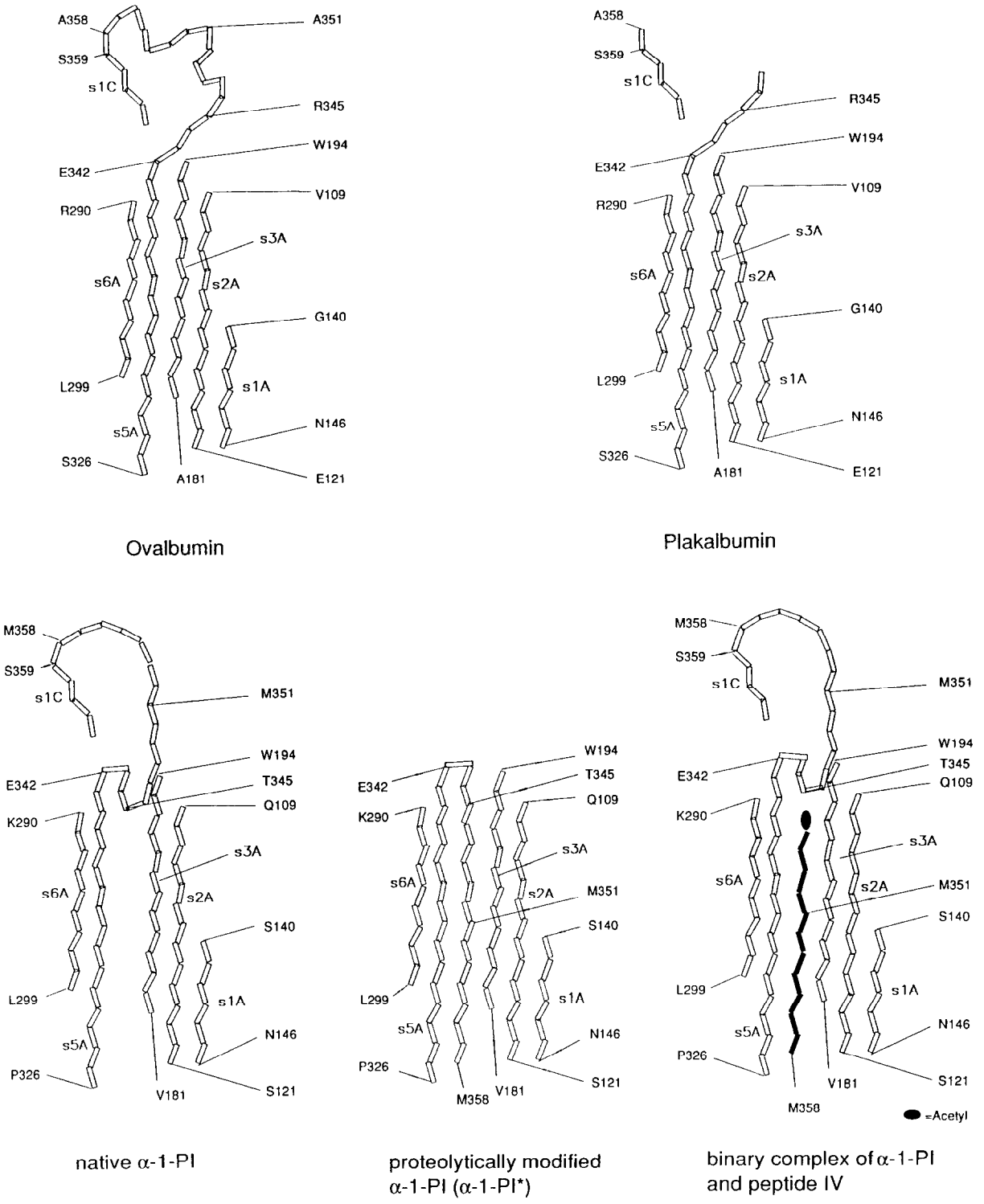

Fig. 2. Schematic diagrams of β-sheet A configurations in different serpins. The binding loop and strand s4A, partially absent in plakalbumin, adopts a helical conformation in ovalbumin but is an integral part of sheet A in cleaved α1PI. Experimental results suggest that a partial insertion of s4A into sheet A is required for inhibitory activity of α1PI.

to the structure of intact ACHY [8]. The structure of the latent form of PAI-1 [7] showed incorporation of the binding loop into a β-sheet without cleavage, demonstrating the structural basis for latency. The recently described structures of intact ATIII [9,10] show partial incorporation, consistent with proposals based

on a variety of experiments [14], although these structures also leave unanswered many questions regarding the serpin inhibitory mechanism. We have recently reviewed the structures of α1PI*, PLAK, OVAL, and latent PAI-1 [26].

2.1. *Cleaved* α1PI*

The cleavage at the binding loop of α1PI by chymotrypsinogen A [37] facilitates crystallization, probably by allowing a normally flexible binding loop to be incorporated into existing secondary structures, a transition often referred to as the S→R (strained→relaxed) transition [38,39]. Cleaved α1PI* is folded into a highly ordered structure, with three β-sheets (A–C), nine α-helices (A–I) and six helical turns (Fig. 1; h stands for helix, s for β-sheet). The six stranded, mostly antiparallel β-sheets A and B are the most extensive structures of the molecule. Three strands of sheet B, along with helix B, provide the hydrophobic core highly conserved among serpins. One of these, strand s4B, is entirely buried and contains the putative recognition site for the proposed SEC receptor [30].

Sheet A is packed over the hydrophobic core, with the strands orientated perpendicular to the β-strands of sheet B (Fig. 1). Sheet A has a large number of conserved hydrophobic interactions and several hydrogen bonds with the mostly hydrophobic core. Several conserved hydrophobic contacts exist between strands 1–3 of sheet A and helix F. Prior to proteolytic modification, however, β-sheet A lacks its central strand (s4A), which only after cleavage can adopt the position observed in the crystal structure.

Sheet C is also implicated in this structural transition, since strand 1 includes the residues immediately C-terminal to the cleavage site. Sheet C is exposed to the solvent on one side, and is packed with hydrophobic contacts on the other to sheet B.

Most of the rest of the molecule is folded into α-helices. Helix F partially covers sheet A, including strand s4A, and so must move during or following the S→R transition. Helix D, absent in the viral serpins, forms the likely heparin binding site. Helices G and H are found under and behind β-sheet B (in the orientation of Fig. 1), covering the putative recognition site for the SEC receptor [30]. Any reorganization of the molecule to expose the recognition site must involve the movement of these helices away from their hydrophobic packing against sheet B.

2.2. *Ovalbumin*

Ovalbumin has no known inhibitory function and does not undergo the S→R transition [38,39] characteristic of the inhibitors. Plakalbumin, a cleaved ovalbumin, was therefore expected to provide a model for the overall structure of intact serpins, particularly for β-sheet A. Indeed, its structure [5] differs from α1PI*, primarily through the absence of β-strand s4A in sheet A [1]. Sheet A was found to have a nearly ideal 5 stranded β-sheet geometry (Fig. 2), with antiparallel interactions between strand pairs s5A–s6A and s2A–s3A, and parallel interactions between s3A–s5A and s1A–s2A. Superposition of the conserved hydrophobic core regions of α1PI and plakalbumin showed that the S→R conformational transition is accompanied by a translation of strands s1A–s3A relative to the rest of the molecule; strands s5A and s6A have nearly identical conformations and positions as in α1PI. Helix F is translated in parallel with strands s1A–s3A. The flatness of sheet A may be a prerequisite for this reformation of sheet A to occur.

In plakalbumin, the residues from the turn between s4A and s5A up to the cleavage site emerge from the sheet and lie along the protein surface. The upper segments of strands 3A and 2A have identical positions as in α1PI, leaving a small opening which in α1PI contains the first residues of s4A, but here is filled with ordered water molecules. Residue 345, at the hinge site, is an arginine in ovalbumin and angiotensinogen, but is otherwise conserved as Thr, Ser, Val, or Ala among all inhibitory serpins. Wright et al. [5] pointed out that a small, preferably hydrophobic residue at this site is a prerequisite for the insertion of strand s4A into sheet A.

The 1.9 Å structure of intact ovalbumin was the first X-ray structure of an intact serpin to be determined [6]. The strand from P9 to P1′ adopted an α-helix structure. This was contrary to expectations based on an assumption that all serpins would have the canonical structure of small serine proteinase inhibitors at the binding site, but was consistent with a strongly predicted α-helix propensity for the loop in serpins, including the inhibitor α1PI [40]. Since cleavage is thought to require a canonical conformation, this helix presumably must partially unfold prior to or during protease binding and cleavage.

The similarity of the structure of the ovalbumin reactive loop and that of an intact inhibitory serpin structure is uncertain, since ovalbumin is not an inhibitor and does not undergo the post-cleavage structural rearrangement. All known structures of 'standard mechanism' serine proteinase inhibitors adopt stable canonical conformations [11] at the binding loop. In contrast, inhibitory serpin structures have been determined where the binding loop conformations may have been artificially stabilized, and a helical conformation may be influenced by crystallization conditions [41,42]. A structure of intact antichymotrypsin was recently reported [8] with strand s4A in a helical conformation, so at least a propensity for this conformation [1] may well be characteristic of the serpin family.

2.3. *Latent PAI-1*

The mobility of sheet A, strand s4A, and sheet C was demonstrated anew with the structure of latent human PAI-1 [7]. The crystal structure showed that strand s4A

is integrated into sheet A from the 'hinge' at P15 to residue Val^{355} (P4) in the latent form. The residues C-terminal to the cleavage site, comprising strand s1C in α1PI*, are not part of sheet C and instead approach strand s5B from below the β-ribbon of sheet C, rather than from above as in the α1PI* structure. If the conformation of proteinase bound PAI-1 is to resemble that suggested by the plakalbumin and ovalbumin structures, strands s3C and s4C of the β-ribbon must move to accommodate the transition to the latent form. These were found to be disordered in latent PAI-1. As with ovalbumin, the information from this structure relative to the serpin inhibitory mechanism is unclear, since the spontaneous transition to a 'latent', non-inhibitory state is not a general property of serpins [43], although ATIII also exhibits a latent state [10].

2.4. Intact antithrombin III

Schreuder et al. [9] and Carrell et al. [10] have reported 3 Å resolution structures of dimers of antithrombin III (ATIII). The authors disagree about the form of one monomer in the dimer (whether it is cleaved or, more likely, intact but latent), and Schreuder et al. [9] report that the binding loop of the other monomer is partially inserted into sheet A and involved in crystal contacts, consistent with suggestions from some experiments (see below). Carrell et al. [10] report fragmented electron density at the region of putative partial insertion. This form has a conformation more suited to docking with a serine proteinase but still lacking a canonical conformation [11] at the binding site, further evidence of the flexibility of the binding loop.

3. Conformations of the 'inhibitory state'

The inhibitory state, that is, a non-latent state of an inhibitory serpin, may involve a variety of conformations. The serpin binding loop has occcurred in several different conformations in X-ray structures, and it is not possible at present to determine whether different stages of protease inhibition or binding involve unique conformations. This is in contrast to the small serine proteinase inhibitors [11], which, apart from small local changes in cleaved forms, are unchanged upon complexation. Crystallographic and other experimental evidence for serpins indicates rather a picture of an inhibitor with a flexible binding loop which may be induced to adopt a canonical conformation either by complexation or prior to it, although it is not clear whether a canonical conformation must be retained after initial binding. In addition, partial insertion of the loop into sheet A must be possible for inhibitory activity (see below).

3.1. Experimental clues to conformational properties

The occurrence of the S → R transition [39] upon cleavage is accompanied by both a dramatic increase in thermal stability, from 58°C to 85°C for ATIII and α1PI, and also increased resistance against guanidine hydrochloride induced unfolding [12]. This has been seen in all inhibitory serpins tested as well as in the non-inhibitory TBG and CBG [44]. Ovalbumin and angiotensinogen notably lack this transition [38].

The increase in thermal stability parallels effects observable with fluorescence emission, CD, NMR, and FT-IR spectroscopy. These can thus be used to characterize conformations induced by experiment and compare them with naturally occurring conformations. In CD experiments, cleavage increases the negative ellipticity around 220 nm, attributed to an increase in secondary structure [12]. FT-IR studies indicate an increase in antiparallel β-structure [45]. NMR studies also show greater stability due to increased antiparallel β-sheet interactions [46]. Ovalbumin and angiotensinogen also lack these features [47], and ovalbumin shows characteristics intermediate between those of typical inhibitory serpins [38,47].

Perhaps the most revealing and unique experiments involve the complexation of short polypeptides with intact serpins [12], often referred to as peptide annealing experiments (see below). These peptides, designed to mimic lengths of strand s4A, can assume the role of strand s4A in the local refolding of sheet A. Accessibility of sites in the binding loop to proteolytic cleavage [15] or antibody binding [48] have also been used to evaluate models of conformations of the inhibitory state. Similarly, differing inhibitory properties of strand s4A mutants suggest requirements for inhibitor models. For example, the replacement of Thr^{345} with Arg in α1PI [13,49], as found in ovalbumin, renders α1PI a substrate. Based on studies of naturally occurring mutants of the C1 inhibitor, Skriver et al. [50] showed further effects also involving the P12 and P10 sites. Finally, since a major pathway for aggregation involves the complexation of strand s4A of one molecule in sheet A of another, aggregation studies also provide structural information [12,15,48,49] and additionally demonstrate the mechanism of a type of serpin deficiency mutant syndrome.

Several states of serpins may be distinguished, for example (i) native, capable of inhibition, (ii) substrate, when distinguishable from an inhibitory state, (iii) cleaved and (iv) latent states, the latter as in PAI-1 [7,43]. These may be well defined conformations (Fig. 2) but may also represent sets of interconverting flexible states. If the latter case obtains, the native serpin would be flexible, and the binding loop may adopt a canonical conformation only upon complexation, accompanied by partial insertion of strand s4A into sheet A at some stage of the inhibition. This is consistent with a variety of results, including the failure to observe a canonical conformation in the crystal structure of intact AChy [8] and with the occurrence of partial insertion in the crystal structures of dimeric ATIII [9.10].

3.2. Peptide annealing experiments

The first peptide annealing experiment involved the complexation of the serpin with a synthetic peptide mimicking strand s4A [12]. This induced a state in α1PI which no longer had inhibitory properties, but instead led to substrate-like cleavage at the reactive site by trypsin. The complex shows CD spectra and denaturation stability similar to that of the cleaved form, indicating that the peptide binds in the position of strand s4A as seen in the X-ray structure of α1PI*. Similar results were obtained for ATIII [17], which shows reduced heparin affinity when complexed with an annealed peptide. Conversely, incubation of ATIII with high affinity heparin and the tetradecamer peptide retards peptide–ATIII complex formation.

Complexation with the tetradecameric peptide thus prevented a transition of α1PI to an inhibitory form. This suggested an explanation for the conservation of small hydrophobic residues at position 345 and for the results of the mutant studies, namely, that strand s4A must be at least partially inserted into β-sheet A for inhibition. The peptide, like the P14 mutant ($Thr^{345} \rightarrow Arg$), prevents this insertion. The extent of insertion is significantly narrowed by further peptide annealing experiments [14]. Beginning with the full length tetradecamer peptide, peptides progressively shortened at the N-terminus were synthesized and complexed with intact α1PI. The resulting complexes were tested for inhibitory activity, with the hypothesis that inhibition should occur when the complexed peptide is short enough to allow the necessary degree of insertion. Based on the observed onset of inhibition with a unadecamer peptide blocked with an acetyl protecting group, a model including insertion of Thr^{345} and maximally part of residue 346 is supported (Fig. 3).

3.3. Models for the inhibitory form(s)

Detailed models require a number of assumptions in addition to the experimental constraints. First, the conformation of the binding loop in the serpin–inhibitor complex most likely adopts the canonical conformation [11] of standard mechanism inhibitors [51] at binding. This assumption is quite likely a good one, since all X-ray structures of the small serine protease inhibitors show this conformation with only small deviations, although the loop in the isolated inhibitor may be flexible, like that of eglin c [52,9]. Second, the partial insertion of strand s4A in the inhibitory form is assumed to include only P14(Thr^{345}), as indicated by the peptide annealing results. This requirement need not be fulfilled simultaneously with the occurrence of a canonical conformation, since the partial insertion may occur after esterification of the scissile peptide bond in the enzyme. If simultaneous, however, a third assumption, that sheet C is unchanged in the inhibitory form, greatly reduces the possible conformations. This third hypothesis is rather weakly

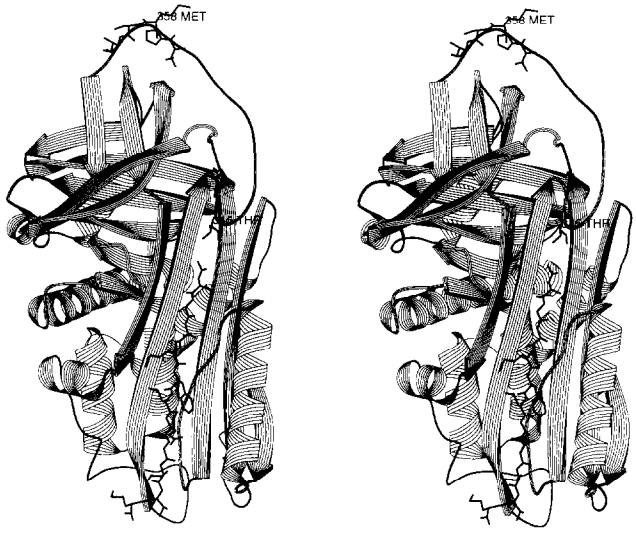

Fig. 3. One possible model for an inhibitory complex of α1PI and a blocked unadecamer peptide. A completely inserted synthetic peptide would only allow partial insertion, including P14 (Thr^{345}) and possibly part of P13 (Glu^{346}).

supported by the observation that all X-ray structures of cleaved serpins have very similar structures and conserved hydrogen bonding patterns in the sheet C region. An appropriate model would then represent the conformation of the inhibitor in the serpin–enzyme encounter complex, and so is further restricted by the requirment that it must fit into the active site of the enzyme. For α1PI, it must be possible to dock a model to its target, leukocyte elastase. Further, it must be possible to dock a model of the Pittsburgh variant ($Met^{358} \rightarrow Arg$) to the structure of thrombin, the more restricted active site of which provides stricter restraints.

3.4. Sheet A

Löbermann et al. [1] speculated that β-sheet A is destabilized in the intact inhibitor to allow strand insertion. The structure of PLAK [5] as well as molecular dynamics simulations [53] suggested a five-stranded β-sheet structure for an intact form. An inhibitory complex with a peptide (s4A mimic) bound serpin (Fig. 3), however, most likely has a six-stranded β-sheet structure, where the peptide adopts the conformation of strand s4A as seen in α1PI*. This, along with the inability to form peptide complexes with ovalbumin, suggests that ovalbumin is not conclusive as a model for β-sheet A of an intact inhibitory serpin, and that the destabilization of sheet A may involve deviations from the ideality of the ovalbumin A sheet. The structure of the sheet in intact AChy [8] is, however, not obviously destabilized (D. Christianson, personal communication), and sheet A in ATIII [9] is consistent with models of a 'zipper' like insertion [53] whereby sheet A is open below the insertion point and intact elsewhere.

3.5. Implications of inhibitory models for the inhibitory mechanism

Models of inhibitory serpin structures are based on the assumption that serpins bind to their target enzymes much as the standard mechanism protein inhibitors do [11,51], that is, that at least in an encounter complex the binding loop adopts the canonical conformation. Whether such a conformation exists prior to binding or is induced by binding is unknown. Three descriptions are discussed: (i) a folding model, which classifies the inhibitory state as an intermediate folding state; (ii) a pre-equilibrium model [33,50]; and (iii) an induced fit model. The latter two models describe the succession of steps leading to a canonical conformation, and are applicable independent of considerations of the folding model.

In the folding model the serpin is folded and secreted from the cell in a metastable conformation which, if not readily bound to its target proteinase, proceeds to a stable inactive form, perhaps latent or cleaved. The fact that latent and cleaved forms are significantly more stable than active conformations, and that there is no known physiological mechanism to reactivate latent PAI-1 by an energetically expensive refolding, support such a folding model. An active inhibitor, then, is considered an intermediate folding state, which is either identical to an encounter complex conformation, or may adopt the conformation upon binding.

The two models which describe binding to a protease are: (i) the 'pre-equilibrium' model, whereby the inhibitor adopts an inhibitory conformation prior to complexation with its target, and (ii) the 'induced fit' model, whereby complexation with the protease induces conformational change or rigidification leading to an inhibitory complex. The two models are not mutually exclusive, and differ primarily in the degree to which the enzyme influences adoption of a bound conformation. Considering the models of an inhibitory form which require a partial insertion of strand s4A in β-sheet A simultaneously with a canonical conformation, an induced fit model involves an initial complex, perhaps as a typical protein substrate, followed by partial insertion and generation of a tightly bound and therefore inhibitory complex. A pre-equilibrium model, on the other hand, postulates a set of equilibrated, interconverting conformations which exist prior to complexation, whereby only a distinct subset of conformations are able to form complexes with the proteinase. Mutating residue 345 or peptide annealing experiments would disrupt inhibition in either model, and cannot be used to discriminate between them. Heparin modulation of serpin reactivity by induced conformational change was interpreted to support a pre-equilibrium model [17], as was the temperature dependence of the cleavage of the reactive site loop of α2AP by trypsin and chymotrypsin [54]. Recent studies showed identical immunoreactivity to antibodies, raised against cleaved ATIII, of either partially denatured, protease-cleaved or peptide-complexed ATIII [55], whereas the native form was not recognized. Heparin binding was excluded as a cause of the relevant change. The epitopes are likely to be at sites which in the native state are either internal, not tightly packed, or in a different conformation [55]. The authors argue that a native form which has a 'pre-inserted' binding loop should be recognized along with the cleaved or latent forms. However, the cleaved and latent forms have different degrees of insertion. PAI-1 exhibits epitopes in the cleaved and latent form which are not seen in the active species [29]. These lie mostly on the rear side and the upper left part of sheet A and the whole structure (Fig. 1). HF, hE and s1A are equally accessible in all conformations, suggesting that these regions are almost unaffected by structural changes. One particularly significant result is that α2AP inhibits trypsin and chymotrypsin at adjacent reactive sites [54], supporting the view that the enzyme plays an important role in inducing conformational changes in serpins.

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