Chapter 1
Alpha-1-Antitrypsin and the Serpins

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How α-1-Antitrypsin Became an Archetype

The identification in 1963 of the inherited deficiency of α-1-antitrypsin established its place as a model genetic disease [1]. This status as a model partly reflects the way in which this deficiency of a plasma protein results in both a loss of function, with the onset of emphysema [2], and a gain of function, in the form of progressive liver damage culminating in cirrhosis [3–5]. Interest in the deficiency was furthered by its common occurrence and from the realisation that the lung degeneration in α-1-antitrypsin deficiency resulted from a loss of lung elasticity exacerbated by tobacco smoking [6, 7]. This last focused attention on α-1-antitrypsin's action as a protease inhibitor and specifically as an antielastase [8–10]. Thus, early research addressed two prime questions. First and obviously, what was the molecular basis of this inherited deficiency? But it was a second subtler question that opened wider understandings. What was the special functional advantage of α-1-antitrypsin as compared to the apparently equal inhibitory efficiency of the much smaller protease inhibitors present in plants and other organisms?

An initial answer to these questions became apparent in the mid-1970s, with the amino acid sequencing of the terminal third of α-1-antitrypsin. This revealed not only the S and then the Z deficiency mutations [11, 12] but also identified the active centre of the molecule, the site of its cleavage by elastase and with that the clue to its inhibitory activity. The completion of this portion of sequence came with the bonus of the recognition in 1979 of its close homology with the sequence of another plasma protease inhibitor, the natural anticoagulant antithrombin [13, 14]. With that, α-1-antitrypsin took on a new and wider significance not just as a model for genetic disease but also as an archetype for a family of proteins controlling key functions of life [15].

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A. Wanner, R.S. Sandhaus (eds.), *Alpha-1 Antitrypsin*, Respiratory Medicine,
DOI 10.1007/978-3-319-23449-6_1

α-1-Antitrypsin and the New Superfamily

The alignment of the sequences of α-1-antitrypsin and antithrombin in 1979 indicated that we were looking at a new family of protease inhibitors, but the realisation that this was indeed a full-scale superfamily came from the subsequent alignment of a third family member, the egg-white protein ovalbumin [16]. The inclusion in the family of ovalbumin, a non-inhibitor, was an indication that this was indeed a protein superfamily with an ancient and diversified lineage—diversified in the functions of its members whilst still retaining a highly conserved framework structure. The recognition of this new superfamily at the commencement of the 1980s was timely, as it coincided with the introduction of cDNA sequencing. The availability of a simple and quick means of determining the amino acid sequence of previously unidentified proteins introduced a range of new members of the superfamily. These included the key functional proteins in human plasma, not only the protease inhibitors, antithrombin, C1 inhibitor, antichymotrypsin and the plasminogen activator inhibitor PAI-1, but also the non-inhibitory hormone carriers, thyroxine- and corticosteroid-binding globulins (TBG and CBG), and the source of the angiotensin vasopressors, angiotensinogen. Subsequently, manifold serpins have been identified as controlling vital intra- and extracellular functions in plants as well as in microbes and animals [17].

The breakthrough that tipped the balance in establishing α-1-antitrypsin as an archetype of the superfamily [18] was the solving in 1984 of its crystallographic structure [19], albeit in a modified physiologic form (Fig. 1.1). Much earlier experience with another protein family, the globins, had shown how the availability of the structure of just one member of a protein family allowed the accurate alignment of other members of the family, in a way that provides immediate insights into the specialist functions of each. Moreover, the study of clinical dysfunctions arising from genetic variations in any member of the family was seen to have implications for all [20]. Even more rewardingly, the lesson from the globins was that the identification of mutations in familial diseases can reveal previously unsuspected aspects, fundamental to the understanding of protein structure and function. The advance of science is overwhelmingly by vertical progression—the gradual building of one new increment in knowledge on another. But this stepwise approach narrows progress to preconceived concepts, which can become dogmas. The study of the consequences of mutations that result in the dysfunctions of disease opens lateral thought and provides insights to otherwise overlooked concepts. The lesson from the early work with haemoglobin is that individual genetic diseases are each unique experiments of nature, undertaken free of preconceptions. And similarly the story of the newly recognised α-1-antitrypsin superfamily is that of a series of advances in understanding, arising or authenticated by mutations that result in aberrant function and hence disease.

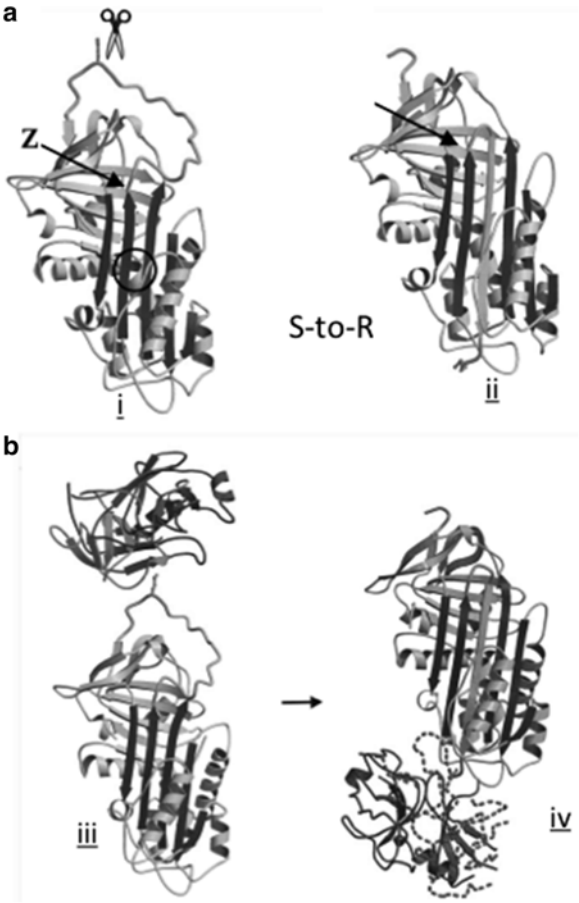

Fig 1.1 (**a**) The serpin mechanism. Cleavage of the reactive loop of α-1-antitrypsin triggers the archetypal serpin S-to-R transition: from a stressed active form (i) to a relaxed hyperstable form (ii) with inclusion of the cleaved loop as a middle strand in the main beta-sheet of the molecule. Mutations commonly causing intracellular aggregation in serpins occur at sites of hinge movements, as *arrowed* and *encircled*. (**b**) Serpins trap their target protease by exposing an ideal substrate (iii) with, on consequent cleavage, the spring-like S-to-R change flinging the entrapped protease to the other pole of the molecule (iv) with disruption of its activity

The Serpins as an Entity

By the mid-1980s, mutations in the plasma protein members of the family were known to result in multiple dysfunctions including thrombosis, haemorrhage and angioedema. Taken together, consideration of these mutations in terms of the template structure of α-1-antitrypsin promised new insights into the principles of protein structure and function. And so it turned out to be. There was first however a hurdle in encouraging the interest of the wider field of investigators needed to achieve the correlations of mutations and dysfunctions in terms of the family as a whole. A problem was an international confusion in nomenclature, between the medically based European researchers who had established the fundamentals of the inherited deficiency of *α-1-antitrypsin* [1, 15, 21] and the biochemically based researchers in the USA who were investigating the protease–antiprotease imbalances resulting from the inherited deficiency of the synonymous *α-1-proteinase*

inhibitor [8–10]. With time, the medical common-usage title of α-1-antitrypsin has become universally adopted, but a reminder of the controversy is still seen in the diplomatically chosen name of the major charity in the field—the Alpha-1 Foundation!

But there was also a real problem that hindered discussion and collective research on the family as a whole, due to its initial naming as the *ovalbumin–antithrombin-III–alpha-1-proteinase inhibitor superfamily of serine proteinase inhibitors* [16]. A title that if repeated twice was guaranteed to turn off not only audiences but also young investigators and funding bodies. Clearly, a simpler name was needed if the field was to progress. To meet this and with the precept of the globins in mind, the author proposed the name *serpins,* as an acronym for serine protease inhibitors. International acceptance of the new name was promptly assured in 1985 with the backing of the leading US investigator in the field, James Travis [22]. So, the stage was then set for what became a period of remarkable productivity in research. Now, 30 years later, some 60,000 serpin-based papers have been published with spin-off benefits to almost all branches of medicine and rewarding new insights in biology as a whole.

Z Mutation and Polymeric Aggregation

A satisfying feature of the studies of the serpin family is the way the findings in one member are frequently of direct relevance to all. This is especially true of the genetic dysfunctions in the protein family, as became apparent with the demonstration that the common Z mutation led to a misfolding and intracellular polymerisation of α-1-antitrypsin [23]. The consequent failure in secretion explained its plasma deficiency and the formation of large internal hepatocellular aggregates explained the accompanying liver degeneration. Several less common mutations of α-1-antitrypsin were also found to cause intracellular polymerisation and plasma deficiency. The significance of these mutations was demonstrated by alignments with other serpins [24]. These showed that the occurrence of identical mutations in other members of the family similarly resulted in intracellular aggregation, causing thrombosis when they occur in antithrombin, angioedema when they occur in C1 inhibitor and notably encephalopathy when they occur in the brain-specific serpin, neuroserpin.

The shared findings and pattern of dysfunctions, found in what came to be labelled together as the serpinopathies [25], strongly indicated that the causative mutations perturbed the conformational mechanism that is central to the serpin family. The realisation that this was so, motivated a switch in focus of the field in the 1990s, to the α-1-antitrypsin template and to the profound changes in conformation that enables the irreversible trapping of proteases. As illustrated in Fig. 1.1, it is the adaptation of this inherent ability of the template to spontaneously change its shape that explains not only the diversity of functions of individual serpins but also how their activity is modulated and the consequences of mutations affecting their structure.

How the Serpins Change Their Shape

α-1-Antitrypsin has a landmark place in molecular biology as the first protein to be shown to undergo a radical and functional change in shape. The serpins as a whole now demonstrate how this inherent change in conformation is not only central to the shared mechanism of the family but has also been adapted by individual serpins to meet their different roles. The first evidence of this conformational transformation came from the crystallographic structure solved by Huber and colleagues in Munich in 1984 [19]. Although they had commenced their study with intact α-1-antitrypsin (supplied by Laurell from Malmö), unbeknown to them, the protein had during the process of crystallisation been cleaved at its reactive site by a contaminating protease. The totally surprising feature of the solved structure was the finding that the reactive centre loop was not only cleaved but had become incorporated as a middle strand in the main beta-sheet of the molecule (Fig. 1.1a, ii). There was scepticism by many and even derision from some when it was proposed that this shift of some 70 Å resulted from a transition of a hyperstable stressed (S) native form, with an intact reactive loop, to a cleaved relaxed (R) form [26] and even more so with the suggestion that this spring-like S-to-R transition on cleavage of the reactive centre loop was central to the inhibitory mechanism of the serpins [27]. What was proposed was a molecular mousetrap. Nothing like it had been seen before. To settle the matter beyond all doubt required a series of crystallographic structures showing the different stages of the conformational change involved in the capture and trapping of a target protease. This daunting task took 15 years to complete, with the final frame of the complex, of α-1-antitrypsin with entrapped trypsin (Fig. 1.1b, iv), being solved in 2000 [28].

Inhibitory Mechanism The sequential changes involved in the transition in Fig. 1.1b provide a video depiction of the elegant though complex mechanism by which the serpins trap their target proteases. The exposed reactive centre loop contains a substrate sequence specific for the target protease, which forms a stabilised proteolytic intermediate with it. It is the subsequent changes that make the serpins unique amongst the many families of protease inhibitors. With the serpins, this initial binding with the protease moves beyond the reversible proteolytic intermediate to the formation of a covalent acyl-enzyme linkage. The consequent cleavage of the reactive site releases the energy of the stressed conformation, and the resulting spring-like S-to-R transition flings the bound protease to the other end of the serpin molecule, with effective irreversible destruction of the protease (Fig. 1.1b). This then is why the serpins have become the predominant protease inhibitors in higher organisms. The irreversibility of their action, as opposed to that of other inhibitors, is a vital advantage in tissue protection. But the video depiction of the S-to-R change reveals another factor that has ensured the success of the serpins. The S-to-R transition involves more than just the entry of the cleaved reactive loop into the main beta-sheet of the molecule; there is at the same time an overall conformational change as one pole of the molecule rotates about the other [29]. It is these concomitant changes in shape that have been adapted by evolution to give not only the specialist functions of the non-inhibitory serpin but also to allow the modulation of their activity by interactions with a range of ligands and receptors.

How α-1-Antitrypsin Became an Antithrombin

An intriguing report was published in 1978 of the finding in Pittsburgh, USA, of a severe bleeding disorder associated with the presence of a variant of α-1-antitrypsin [30]. Subsequent investigation [31] showed that the abnormality was due to the mutation of the methionine at the active centre of α-1-antitrypsin to an arginine, resulting in a complete change in its function, from being an inhibitor of elastase to that of a highly active inhibitor of thrombin (Fig. 1.2a).

This natural experiment in protein engineering, as well as showing the way in which a single mutation can completely change the function of a protein, also opened entirely new fields of understandings. The findings unequivocally settled controversies at the time as to the siting of the reactive centre of the serpins and elegantly illustrated how the inhibitory spectrum of the family could readily evolve from minor variations at the active site. Moreover, the demonstration that the variant Pittsburgh antitrypsin had an activity equivalent to that of heparin-activated

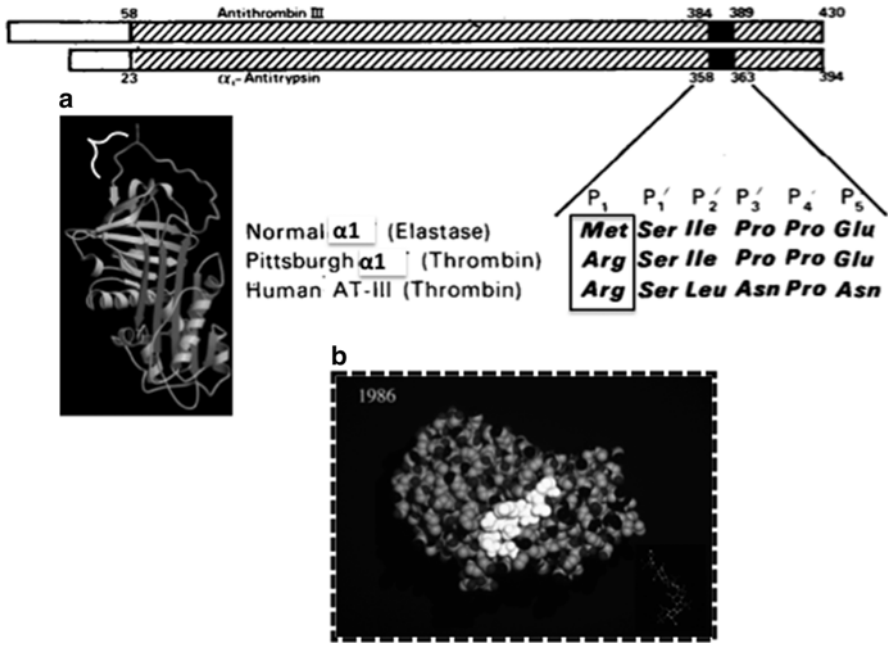

Fig 1.2 α-1-Antitrypsin as an archetype allowed the deduction of the mechanism of thrombin inhibition a decade prior to the solving of the structure of antithrombin. (**a**) At an early stage, when only the sequence but not the structure of α-1-antitrypsin was known, the finding of the mutation in the Pittsburgh variant (*boxed*) identified the active centre of both inhibitors and provided insights into the adaptive modulatory mechanism of antithrombin. (**b**) The heparin-binding site on antithrombin was unequivocally revealed a decade before the solving of the structure of antithrombin, by projection of conserved arginines and lysines onto the structure of antitrypsin. Heparin pentasaccharide, *shadowed bottom right*

antithrombin was the first evidence that heparin acts on antithrombin by releasing an otherwise suppressed inhibitory activity. This was a key to understanding how the anticoagulant function of blood is modulated by small movements of the reactive centre loop into and out of the main body of the antithrombin molecule, a mechanism that was found to be similarly utilised by other serpins to control a diversity of specialised functions. Yet another finding from this remarkable case opened a new field in biochemistry. The unexpected finding of proalbumin and other pro-proteins in the plasma of the Pittsburgh patient led to the first recognition of the nature of the propeptide-cleaving enzyme that has a key role in metabolism and endocrinology.

Ligand Activation of Serpins

Although not as yet known to be so with α-1-antitrypsin, the activities of many other serpins are affected by their interactions with ligands and receptors. Typically, the ligand binds to the serpin framework tightening its overall structure and hence affecting the movement of the intact reactive loop. In effect, the interaction with the ligand maintains the reactive centre 'protease bait' in an active exposed form.

PAI-1, Fibrinolysis and the Latent Transition The clearest example of this ligand-induced switching on-and-off activity is seen with the plasminogen activator inhibitor PAI-1, the serpin that regulates fibrinolysis by limiting the production of plasmin. The genetic deficiency of PAI-1 results in increased plasmin activity with consequent risk of haemorrhage, and its excess production results in decreased fibrinolysis with resulting thrombosis [32]. In normal function, circulating PAI-1 undergoes a ready transition to an inactive form, with the complete insertion of its intact reactive centre loop into the body of the molecule [33]. This conversion to an inactive latent conformation, which occurs spontaneously and reversibly in PAI-1, can also exceptionally occur, pathologically and irreversibly, in other serpins including α-1-antitrypsin. With PAI-1, however, the transition is reversible and so provides a physiological off-switch that maintains fibrinolytic activity in the circulation. When however the PAI-1 comes in contact with the extracellular matrix of the endothelium, it binds to vitronectin. The binding to this ligand expels the buried reactive loop and activates the plasmin-inhibitory activity of PAI-1 [34], with a consequent suppression of fibrinolysis.

Antithrombin Heparin and Thrombophilia A more subtle adjustment of activity occurs with antithrombin on its association with the complex polysaccharides that line the small vasculature and notably with their active component heparin. This interaction not only binds antithrombin to the endothelial surface but also activates it as an anticoagulant. Antithrombin differs from α-1-antitrypsin in that its reactive loop readily nudges in and out of the main beta-sheet of the molecule. In the circulating antithrombin, this equilibrated movement strongly favours partial insertion, with a consequently decreased inhibitory activity and hence an increased readiness

of coagulation. When however the antithrombin binds to the heparins lining the microvasculature, it changes to a full anticoagulant role, with the tightening of its structure and complete exposure of its reactive loop [35, 36]. The demonstration, now in video detail, of this activation by heparin fulfils the predictions decades before from the findings with the Pittsburgh variant of α-1-antitrypsin. In effect, heparin-activated antithrombin adopts the fully active conformation inherently present in α-1-antitrypsin and relevantly so in α-1-antitrypsin Pittsburgh.

Heparin is a heterogeneous mixture with its longer-chain forms containing sites that bind to and bridge both thrombin and antithrombin [36]. The shortest effective form however is a pentasaccharide. This binds to a highly specific site on antithrombin formed by the alignment on a surface helix of positively charged arginine and lysine side chains. Clinically, mutations at this site are a common cause of familial thrombophilia. The identification of this binding site on antithrombin further illustrates the critical part α-1-antitrypsin played in revealing features in other members of the serpin family. The binding of heparin occurs not only to antithrombin but also to three other heparin-binding serpins. Although in the 1980s the structure of antithrombin was unknown, the definitive recognition of the heparin-binding site was readily deducible from the precise alignment of the sequences of serpins, based on the template provided by the structure of α-1-antitrypsin. The alignment of serpin structures allowed the question: is there a conservation of arginine and lysines uniquely present in the four heparin-binding serpins? The result when projected on the framework structure of α-1-antitrypsin was stunning (Fig. 1.2b). It clearly defined the binding site of the heparin pentasaccharide, with unequivocal confirmation coming from mutations within the site identified in families with heparin-resistant thrombophilia [37].

Hormone Carriage: Serpins and the Modulation of Activity

When the initial listings of serpins in human plasma were made, three atypical members stood out. These were the non-inhibitory serpins: angiotensinogen and the two globulin carriers of thyroxine and corticosteroids, TBG and CBG. We now know that each of the three hormone carriers retains the serpin framework but, significantly, with inbuilt adaptations to allow the modulated control of hormone release. The findings raise the tantalising question, are there similar, as yet undetected, modulatory controls in α-1-antitrypsin?

TBG, CBG and the Mechanism of Modulation Thyroxine and corticosteroids bind to each of their carrier proteins in the circulation in a 1:1 ratio, and it was early realised that exposure to neutrophil proteases could trigger the S-to-R transition in both TBG and CBG, with the consequent massive release of the bound hormones [38]. The mechanism made sound physiological sense, as this would ensure hormone release at sites of greatest need, within the proteolytic milieu of loci of inflammation. And indeed this was demonstrably so. Yet this accelerated release in

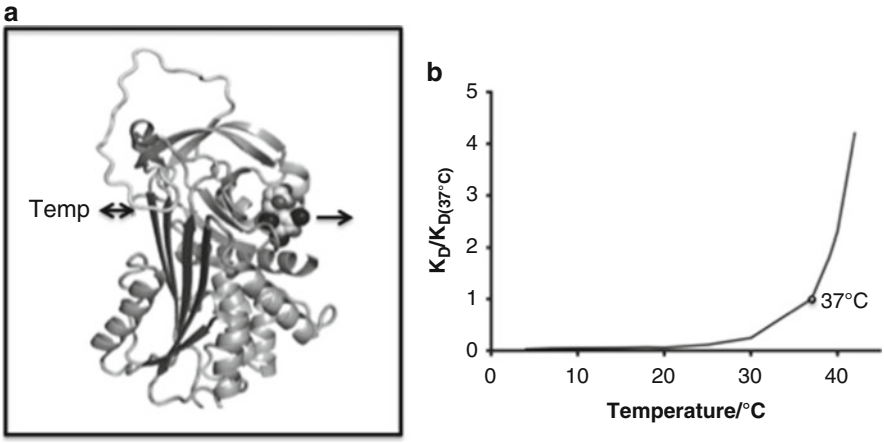

Fig 1.3 Hormone release from TBG and CBG, shown (**a**) in *side view. Upper left*, the exposed reactive loop nudging, as *arrowed*, in and out of the molecule and influencing the release of the bound hormone (space-filling depiction, *arrowed* on *right*). (**b**) The comparative decrease in binding affinity of CBG plotted against temperature shows the accelerated hormone release that will take place as the body temperature rises above 37 °C [42]

inflammation is an exception as for the most part the role of the circulating carriers is to supply hormones to healthy tissues. Until recently, it had been thought that such hormone release occurred passively, but the requirements of tissues inherently differ, between muscles and the brain, between being at exercise and rest and in hypothermia and fever.

An insight into the responsive way the hormone carriers meet these differential requirements came with the solving of the structures of TBG and CBG [39, 40]. The two structures are closely homologous, with identical external binding pockets on the exterior surface of each (Fig. 1.3a). The serpin framework in both TBG and CBG is strongly conserved and is identically superimposable on the framework of α-1-antitrypsin. Apart from the presence of the hormone-binding pocket, there is one other significant difference in the binding globulins as compared to α-1-antitrypsin, in that the main sheet of the molecule is partly opened. This is most markedly so in TBG, with the reactive loop being partially inserted into the sheet as is also seen in antithrombin. The striking similarity with antithrombin provided the clue to the mechanism influencing hormone binding and release in CBG as well as TBG, in that the mechanism is the converse of the interaction of heparin and antithrombin. With antithrombin, the binding of heparin tightens the molecule to favour the expulsion and hence activation of the reactive centre loop. TBG and CBG have a similarly conformed but inert loop, which, like that of antithrombin, can move in and out of the body of the molecule with an accompanying tightening and relaxing of the overall structure. But whereas in antithrombin this movement regulates inhibitory activity, in TBG and CBG, the accompanying tightening of structure modulates the release of the bound hormones. In this way, the flip-flop movement of the

loop in and out of TBG and CBG provides an equilibrated balance between higher and lower hormone-binding affinities. Such an elegant and balanced mechanism only makes sense in terms of a responsive modulation of hormone release to meet varying tissue or environmental needs.

Fever and a Serpin Thermocouple The tissue factors and receptors that influence differential hormone release from TBG and CBG are still unidentified. There is however one major mechanism that is well documented; this is the modulation in hormone-binding affinity that takes place with changes in body or tissue temperature. The affinity of binding of ligands to proteins in general decreases with increasing temperature, but this specifically occurs to a much greater extent with the hormone-binding globulins. In effect, TBG and CBG each act as protein thermocouples [39, 41–43]. The opening of the main sheet of the serpin molecule and the accompanying entry of the reactive loop is known to be temperature dependent. With TBG and CBG, this accelerated loop entry accompanying a rise in temperature will predictably result in a decreased affinity and hence an increased release of the bound hormone. Specifically, the structure of TBG shows how a triggering shift, as the loop enters the sheet, is transmitted directly to the binding site (Fig. 1.3a, b). Critically, a distinct drop in binding affinity will occur as the body temperature rises above 37 °C, such that a rise to 39 °C, as in fevers, will result with TBG in a 23 % increase in the free thyroxine concentration and with CBG in a doubling of the free cortisol concentration. This boosted release has clear physiological benefits, giving a raised release of cortisol in inflamed tissues and an increase in thyroxine delivery to meet the rise in metabolic activity that will accompany increases in body temperature.

Confirmation of the physiological significance of this thermally triggered increase in hormone release comes from two interactive mutations in TBG that have become established as a polymorphism [43]. The aboriginals of West Australia have lived for thousands of years in an environment where ambient temperatures consistently reach 40 °C and above. Under these conditions, the boost in thyroxine release as body temperatures reach above 39 °C, which in temperate climates is an advantageous response to inflammation, will historically for the aboriginal exacerbate the greater risk of heat exhaustion. The acquired polymorphisms in the aboriginal can be seen structurally to perturb the network of bonds that transmit the movement of the reactive loop to the thyroxine-binding site [39]. The effect of this is to recalibrate the protein thermocouple, with the boosted ‘fever’ release of thyroxine being cancelled, whilst the interactions of the two mutations otherwise maintain the physiological delivery of thyroxine.

Angiotensinogen and Redox Modulation Angiotensinogen, the source of the angiotensins that control blood pressure, is a plasma serpin that is not only a non-inhibitor but has also lost the ability to undergo the S-to-R conformational change. As with the other serpin hormone carriers, angiotensinogen also has an inbuilt mechanism to allow a responsive adjustment of hormone release. However, unlike the other carriers this modulation of activity is not dependent on reactive loop movements or other shifts in the serpin template but is a consequence of conformational adjustments in

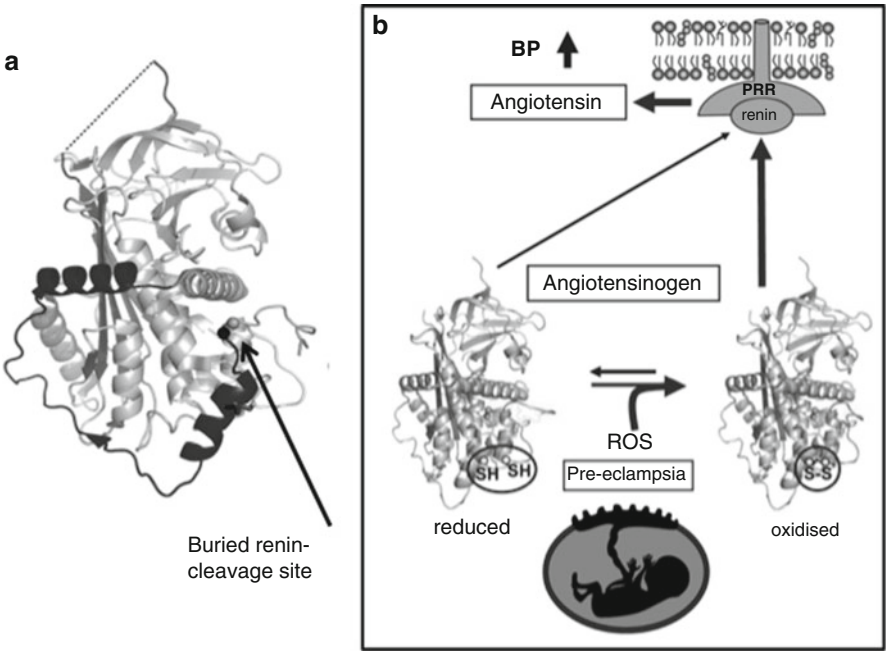

Fig 1.4 Angiotensinogen and the modulation of angiotensin release. (**a**) The amino-terminal tail of angiotensinogen, shown in *bold*, is firmly bound to the body of the molecule. The buried site that releases the terminal angiotensin I (*arrowed*) is revealed by a conformational change on binding to the enzyme renin. (**b**) Schematic, indicating how this renin-induced conformational change is affected by the oxidation of an S–S bridge, with a consequent effect on the efficiency of cleavage and hence the release of angiotensinogen. Although the modulating factor illustrated here is the release of reactive oxygen species ROS from the placenta, as in pre-eclampsia, other factors will influence the S–S bridging including nitrosylation by NO-releasing agents. *PRR* prorenin receptor

the amino-terminal tail of angiotensinogen [44]. This 61-residue extension contains the decapeptide angiotensin I, which is cleaved and released by a highly specific interaction with renin. Recent crystallographic structures show how the binding of the amino-terminal tail to the body of the circulating angiotensinogen inaccessibly obscures the angiotensin-cleavage site (Fig. 1.4). The interaction with renin, however, results in a major shift in the tail of angiotensinogen, to give the exposure of the otherwise buried site.

A key contribution to the coordination of this activating transition is provided by a disulphide bridge that links the amino-terminal tail of angiotensinogen to the body of the molecule. This disulphide bridge is labile, being readily broken by reduction, with some 60 % of the circulating angiotensinogen being in the bridged oxidised form and 40 % in the reduced unbridged form. The kinetics of cleavage of the two forms differs significantly, with the bridged oxidised form being a more effective substrate for cleavage and the release of angiotensin by renin. The proportions of

the bridged and unbridged forms vary however with tissue redox changes. In addition to this inbuilt ability of angiotensinogen to adjust its activity to oxidative changes, the susceptibility of the reduced bridge to nitrosylation will further allow the modulation of angiotensin release in individual tissues.

Evidence of the in vivo significance of such variations in activity comes from yet another experiment of nature, the finding of equivalent kinetic changes due to a mutation at the cleavage site in angiotensinogen [45]. The mutation was detected in five heterozygotes, each identified because of an association with hypertension and specifically with hypertension in pregnancy. Arising from this, the subsequent demonstration of markedly raised levels of oxidised angiotensinogen in pre-eclampsia now provides a persuasive explanation for the known association of hypertension in pregnancy with oxidative stress (schema: Fig. 1.4b).

Conclusion: Questions for α-1-Antitrypsin from the Serpins

Although the early studies of α-1-antitrypsin revealed its framework structure and unique inhibitory mechanism, subsequent studies of other serpins have shown the more subtle ways in which activity can be modified by tissue-specific receptors [46] and by changes in body temperature [42, 43]. Once again, key insights into these processes came from aberrations of function resulting in disease. The long list of disorders arising from mutations in the plasma serpins [24, 25] is now increasingly being added to by dysfunctions of the more recently characterised intracellular serpins, as with the collagen chaperone Hsp 47 and osteogenesis imperfecta and with the intracellular protease inhibitor SERPINB6A and familial hearing loss [47, 48].

Are there as yet undiscovered modulatory adaptations and dysfunctions in α-1-antitrypsin? Clues are likely to come from unexpected clinical leads. The non-committal label of the *α-1-antitrypsin syndrome* appropriately acknowledges that there is more to the ZZ clinical phenotype than just lung and liver degeneration [49]. The concentration of α-1-antitrypsin in the plasma at over a gram per litre is tenfold greater than that of any other plasma serpin and may rise several fold further in the acute phase of inflammation. What happens to the activity of α-1-antitrypsin as the body temperature increases in fever and pertinently does the acute phase response and a body temperature of 39 °C exacerbate the misfolding and intracellular aggregation of the variant Z that leads to liver cirrhosis? Another relatively unexplored part of the story of relevance to all the serpins is the catabolic pathway, with the turnover of α-1-antitrypsin notably being greater than a gram a day. What determines the senescence of the protein? What happens to it? Intriguing accounts in the earlier literature tell of high concentrations of the carboxy-terminal (leaving) peptide of α-1-antitrypsin in gallstones [50]. How could this happen? Examples of other challenges from the past that are yet to be followed through are the observations from Malmö of a 1:1 association of cholesterol with α-1-antitrypsin, putatively with the Z rather than M form [51], and of the studies in the USA of 30 years ago indicating the modulation of inhibitory activity by neutrophil oxidants [8, 10]. Overall, the lesson for α-1-antitrypsin from the serpins is that there is still much to be learnt.

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