Factors with conformational effects on haemostatic serpins: Implications in thrombosis

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Summary
Serpins are key actors of systems involving proteolytic reactions, such as the haemostatic system, as they are irreversible suicide inhibitors of serine proteases. The structural flexibility and physical properties of serpins that are required for their efficient inhibitory mechanism also make them especially vulnerable to even minor factors that induce conformational changes in the native form of these molecules, leading to a number of inactive conformations, such as latent, cleaved or polymers. Increasing numbers of conformational mutations affecting haemostatic serpins, mainly antithrombin, the main endogenous anticoagulant, have been described. These mutations cause circulating deficiencies of the molecules, in most cases due to intracellular retention, which may be associated with a hypercoagulable state. Indeed, conformational mutations in antithrombin have been identified in patients with severe venous thrombosis, which has led to the hypothesis that these disorders might be included in the group of conformational diseases. Moreover, we have recently demonstrated that other factors, including both drugs, such as the treatment with L-asparaginase, or environmental factors, such as high temperatures or hyperlipidemia, may also have conformational consequences on hepatic antithrombin, thus resulting in intracellular aggregation and plasma deficiency, which may increase the risk of thrombosis. In this study, we review the causes of deficiency of haemostatic serpins that may be explained by conformational mechanisms, and their association with an increased risk of venous thrombosis.

Keywords
Antithrombin, SERPINs, venous thrombosis, liver

Introduction
Serine protease inhibitors (Serpins) are the largest family of protease inhibitors that have been identified to date (over 1,500 members have been described), with members broadly distributed in animals, plants, bacteria, archaea, as well as certain viruses (1, 2). The reason for such numbers and functional relevance is their unusual, but efficient, mechanism of inhibition of serine proteases that has provided serpins with a central role in controlling many important proteolytic cascades. Serpins are large globular molecules with sizes ranging from 300–500 amino acids folded into three β-sheets (A-C) and eight to nine α-helices (A-I), presenting at the top of the molecule an exposed and mobile peptide loop containing the reactive centre of the inhibitor, the reactive centre loop (RCL). All the members of the family share a tightly conserved structure with more than 30% sequence identity and a common framework tertiary structure (3). The structural data available so far (over 70 serpin structures have been determined) and biochemical and biophysical information, reveal a significant structural flexibility. This feature allows inhibitory serpins to act as “suicide” or “single-use” inhibitors. The mechanism of inhibition involves a dramatic conformational rearrangement of the molecule, called the “stressed” to “relaxed” (S to R) transition, which is triggered by the proteolytic action of the target protease to the P1-P1’ bond at the RCL, that is also accompanied by a change in topology. During this rearrangement, the RCL moves from an exposed position to form an extra strand in the centre of the A β-sheet. This conformational change is directly responsible for the inhibition of the target protease which is covalently attached to the serpin via an acyl bond (4), and is translocated to the opposite pole of the serpin with its consequent distortion into a partially unfolded state. This efficient mechanism of inhibition has favoured the selection of serpins to play a key role in the control of processes as diverse as DNA binding and chromatin condensation (5), embryo development (6, 7), immunoregulation (8) and control of apoptosis (9). Nevertheless, most inhibitory serpins are key regulatory molecules of proteolytic cascades such as the inflammation, complement, and of course, the coagulation and fibrinolysis cascades of the haemostatic system (10).
In this review we will summarise the conformational mechanisms that might impair serpin function and which can result in disease, with particular regard to the relationship between haemostatic serpins and thrombosis.

Control of the haemostatic system by serpins

The haemostatic system of humans has evolved to achieve a rapid and strong response to the ever-present risk of fatal haemorrhage that vascular injury might have in a closed and high-pressure circulation system. Such an efficient response is achieved without protein synthesis by a serial proteolytic activation of huge amounts of inactive circulating proteins that exponentially amplify the initial signal: the coagulation cascade (11). However, a precise regulation of blood coagulation is essential in order to avoid a generalised activation of the system and massive fibrin deposition. To be effective, the haemostatic system must confine clotting to the local site of vascular injury and remain active for only a sufficient period of time to produce enough fibrin to seal the wound. Two systems carry out such functions: the anticoagulant and fibrinolytic systems, which dominate the haemostatic equilibrium and maintain the blood as a fluid. Because many of the procoagulant molecules of the coagulation cascade are serine proteases, serpins have been selected as key elements for the regulation of both, the anticoagulant and the fibrinolytic systems (Table 1). Apart from their efficient inhibitory mechanism, serpins have an additional advantage over other protease inhibitors. The inhibitory activity of serpins can be exquisitely controlled by specific cofactors, which is particularly relevant within the haemostatic system, where the control of the balanced equilibrium between coagulation and anticoagulation (haemorrhage and thrombosis) is vital for human health.

The main haemostatic serpin is antithrombin (SERPINC1). This molecule has a wide range of inhibitory activities of procoagulant enzymes, including the final serine proteases of the coagulation cascade: thrombin and factor (F) Xa. Additionally, this serpin also inhibits FIXa, FXIa, F XIIa, F VIIa, plasmin and kallikrein (12). Antithrombin has an additional feature that explains why it has been selected as the most important endogenous anticoagulant: by itself it is a relatively poor inhibitor of thrombin and FXa, but it is conformationally activated by the binding of glycosaminoglycans (GAG) (13). The GAG-dependent control of the action of antithrombin (and other haemostatic serpins such as heparin cofactor II, or protein C inhibitor) is probably essential as GAGs allow these serpins to significantly enhance their inhibitory activity and restrict it to the site of the vascular injury, thereby delaying the shutting off of the clotting process (14). Because antithrombin plays an essential role in the control of coagulation activity, even modest deficiencies in the levels or activity of this serpin (below 60–70%) significantly increase the risk of thrombosis, and complete antithrombin deficiency is lethal (15).

There are other serpins that are able to inhibit coagulation enzymes, such as heparin cofactor II (HCII) (SERPIND1), which also inhibits thrombin, protease nexin I (SERPINE2), and C1-inhibitor (SERPING1) (16). Recently, a new serpin was identified to inhibit procoagulant enzymes. The protein Z-dependent inhibitor (ZPI) (SERPIN A10) potently inhibits FXa in a process that requires the presence of protein Z (PZ) as a cofactor, calcium ions, and cephalin (17). Moreover, this serpin also may inhibit FXIIa (18) and FIXa (19) with no PZ requirement in both cases. Interestingly, the mechanism of inhibition of ZPI does not follow the typical serpin-protease interactions as the ZPI-protease complex is not stable and dissociates rapidly, releasing the cleaved ZPI (18, 19). Although deficiency of some of these serpins (HCII, ZPI) has been associated with cases of venous thrombosis (20–23), the relevance of these serpins in thrombosis is controversial, which most likely reflects a secondary role in the control of the coagulation (24–26). However, although probably redundant in their functions, the association with other prothrombotic risk factors may dramatically increase the thrombotic phenotype, supporting the thrombotic potential of deficiency states affecting these molecules (23–25).

There are also serpins that belong to the fibrinolytic system. The best known serpin of this system is the plasminogen activator inhibitor 1 (PAI-1) (SERPINE1), but other examples of ser-

<table>
<thead>
<tr>
<th>Serpin</th>
<th>Haemostatic inhibition</th>
<th>Cofactor</th>
<th>Clinical effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT</td>
<td>FXa, thrombin (FXa, FXIa, FXIIa, FVIIa, plasmin, kallikrein)</td>
<td>GAG</td>
<td>Thrombosis</td>
</tr>
<tr>
<td>HCII</td>
<td>Thrombin</td>
<td>GAG</td>
<td>Thrombosis??</td>
</tr>
<tr>
<td>ZPI</td>
<td>FXa, (FXa, FIIa)</td>
<td>PZ</td>
<td>Thrombosis??</td>
</tr>
<tr>
<td>Protease Nexin I</td>
<td>Thrombin, urokinase</td>
<td>GAG/Collagen IV</td>
<td>Stroke, thrombosis??</td>
</tr>
<tr>
<td>C1-inhibitor</td>
<td>(FXIa, FIIa, kallikrein, tPA, plasmin)</td>
<td></td>
<td>Angioedema</td>
</tr>
<tr>
<td>α2-AP</td>
<td>Plasmin</td>
<td></td>
<td>Bleeding</td>
</tr>
<tr>
<td>PAI-1</td>
<td>tPA, uPA</td>
<td>Vitronecin</td>
<td>Thrombosis</td>
</tr>
<tr>
<td>PAI-2</td>
<td>tPA, uPA</td>
<td></td>
<td>Thrombosis??</td>
</tr>
<tr>
<td>PCI</td>
<td>APC (kallikrein, FXIa, thrombin, uPA, tPA, αPA)</td>
<td></td>
<td>Thrombosis??</td>
</tr>
</tbody>
</table>

Table 1: Haemostatic serpins, inhibitory functions on the haemostatic system, and clinical consequences of impaired function or deficiency.
pins that inhibit proteases involved in the generation and control of plasmin are PAI-2 (SERPINB2) and α2-antiplasmin (SERPINF2) (27).

Finally, protein C inhibitor (PCI) (SERPINA5) not only inhibits the activated protein C but also a number of arginine-specific serine proteases. These include procoagulant proteases, such as kallikrein, FXIa and thrombin, and fibrinolytic enzymes, such as urokinase-type plasminogen activator (uPA), or tissue plasminogen activator (tPA) (28).

Serpipathies

The structural flexibility of serpins renders them particularly susceptible to any insult that affects their conformational flexibility. Thus, missense mutations affecting mobile regions or different destabilizing conditions, may result in protein misfolding and formation of pathogenic conformers. Under these conditions, serpins can adopt metastable conformations as occurs in polymer formation, with the insertion of the RCL of another to form a loop-sheet linkage (29,30). Serpin polymerization can be pathologic at least by two different mechanisms. First, polymers are retained inside the cell and may be resistant to proteasomal degradation, and after usually relatively long periods of time, they become toxic to the cell that synthesises the serpin, causing its death and tissue destruction. Hence, many serpins have been involved in the so-called conformational diseases (31). Indeed, a general category of conformational diseases caused by serpins has been proposed: the serpinopathies (32,33). The second pathological consequence of conformational changes in serpins is the loss of function associated to abnormal conformers. Serpin polymers can no longer inhibit proteases. In addition to this loss of function associated with the abnormal conformers, formation of polymers reduces the secretion of extracellular serpins, resulting in a significant deficiency that almost certainly will have significant consequences on the control of the proteases inhibited by the affected serpin.

Serpins that have been identified to date include: cirrhosis and emphysema associated with α1-antitrypsin (34), neuroserpin-caused dementia (35), chronic obstructive bronchitis associated with antichymotrypsin (36), angioedema caused by conformational mutations of the C-1 inhibitor (37), and thrombosis that might be associated with conformational changes in different haemostatic serpins, mainly antithrombin (38,39). Other serpin-related diseases are caused by null mutations or mutations that affect key functional regions of the serpin and significantly impair the inhibitory capacity of the molecule (40).

Conformational mutations in haemostatic serpins and thrombosis

For the past few decades, the recognition of the association of a gene with a disease depended on the finding of significant mutations that caused either deficiency or inactivation of the encoded protein. As expected, mutations resulting in premature stop codons (nonsense mutations and frameshift mutations), and those affecting relevant functional domains readily explained a number of genetic disorders. However, it was difficult in many cases to explain the severe effects of single missense mutations that primarily affected neither the expression nor the function of the protein. This generally describes the study of antithrombin deficiencies. The worldwide genetic analysis of patients with a congenital deficiency of antithrombin has identified up to 184 different mutations in the antithrombin gene (47–49). Many of these mutations have an obvious effect that explains the associated phenotype. Thus, non-sense mutations and insertions or deletions causing a frameshift explain the associated type I deficiencies. Moreover, missense mutations affecting the heparin-binding site or the reactive centre disturb these functional domains, explaining the associated type II deficiencies. However, there was no explanation for 40 different missense mutations associated with type I and type II (pleiotropic) deficiencies. The conformational consequences of some of these mutations have clearly been identified. The first identified factor involved in venous thrombosis was a serpin. In 1965, Egeberg described a thrombophilic family with a common haemostatic phenotype in the affected members: deficiency of antithrombin (46). Since then, up to 184 different mutations in the antithrombin gene have been described in patients with venous thromboembolism (47–49), indicating that deficiency of this relevant anticoagulant serpin is probably the single most potent risk factor that increases the risk of venous thrombosis. Indeed, the fact that heterozygous mutants, which have about 50% of the normal concentrations of antithrombin, are predisposed towards thrombotic disorders, suggests that venous thrombosis might be a monogenic disorder. However, during the last decade numerous studies have clearly demonstrated that venous thrombosis is a complex polygenic and multifactorial disease (50). Unfortunately, despite considerable efforts made in determining the molecular basis of thrombophilia during the last 40 years, 60% of cases with venous thrombosis are due to so-far unidentified risk factors. Two reasons that explain such frustrating results are the restriction of the search to a reduced number of candidate genes, and also the existence of unknown mechanisms leading to thrombosis. In this framework, serpins, as key elements of the haemostatic system, emerge as candidates needing to be investigated. Firstly, new haemostatic serpins that might play a role on venous thrombosis may be identified, such as ZPI (51). Other serpins might have new functions affecting the formation of the blood clot that have yet to be investigated. Finally, these molecules are susceptible to conformational changes with pathological consequences that have hardly been investigated in the context of thrombosis and which warrant further investigation. Details of current knowledge in this field will be outlined later.
recently been described and they certainly explain the observed deficiency and clinical severity (Table 2). Some of these mutations occurred in the mobile regions of antithrombin, mainly at the hinges of the RCL, or in the region involved in the shutter-like opening of the main β-sheet of the molecule required for insertion of the reactive loop into the sheet (38). The modification of just a single amino acid in these sensitive regions can disturb the network of interactions in the whole molecule, resulting in overall conformational changes that can affect all of the functions of the molecule, including its inhibitory activity and its heparin binding affinity. But most importantly, such conformational changes can result in a loss of stability and facilitate the formation of intermolecular linkages, leading to formation of oligomers or transformation to the latent conformation. These variants lose the anticoagulant properties of the native conformation, as their reactive loops are buried (by insertion into their own or another molecule), which explains per se the thrombotic risk. Moreover, particularly for mutations inducing oligomerization, secretion of these variants is impaired, which results in a circulating deficiency. However, the clinical severity of these phenotypes is greater than expected from that of a mere loss of function or inefficient secretion. It seems then, that conformational mutations of antithrombin may have an additional pathological effect which increases the severity and risk of venous thrombosis. Some explanations have been proposed but the exact mechanism remains to be elucidated. In the case of mutations that facilitate the transition to the latent conformation, there is an additional loss of anticoagulant activity, as the abnormal latent molecule impairs the function of the most active anticoagulant form of antithrombin – the β-antithrombin glycoform by forming reversible dimers (52). An additional explanation could be that abnormal conformers might acquire a new prothrombotic function. Indeed, there is also increasing evidence showing that the latent conformation of antithrombin, as well as causing a loss of anticoagulant activity, also gains a function as a significant antiangiogenic agent (53, 54). Again, in a way that is not yet fully understood, the putative gain-of-function of these conformational variants may be exacerbated by specific situations, such as pregnancy or infection, frequently observed to be associated with thrombosis in individuals carrying some of these anti-thrombin variants (55–58).

Examples of conformational mutations have been described for other haemostatic serpins (Table 2). Two interesting examples have recently been described for the HCII. In one variant, a missense mutation (P443L) in the reactive loop of HCII appears to have key structural consequences, as the mutant variant

<table>
<thead>
<tr>
<th>Serpin</th>
<th>Mutation</th>
<th>Region</th>
<th>Conformational effect</th>
<th>Functional effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT</td>
<td>P80S</td>
<td>Shutter</td>
<td>Disulphide dimer</td>
<td>Circulating deficiency</td>
<td>(55)</td>
</tr>
<tr>
<td></td>
<td>S82N</td>
<td>Shutter</td>
<td>Intracellular retention</td>
<td>Circulating deficiency</td>
<td>(60)</td>
</tr>
<tr>
<td>T8SM/K</td>
<td>Shutter</td>
<td>Latent/Polymer</td>
<td>Decreased function</td>
<td>(58)</td>
<td></td>
</tr>
<tr>
<td>C95R</td>
<td>hC</td>
<td>Disulphide dimer</td>
<td>Circulating deficiency</td>
<td>(61)</td>
<td></td>
</tr>
<tr>
<td>CI28Y</td>
<td>hD</td>
<td>Complexes</td>
<td>No inhibitory function</td>
<td>(62)</td>
<td></td>
</tr>
<tr>
<td>N187K/D</td>
<td>hF</td>
<td>Latent/Polymer</td>
<td>Decreased function</td>
<td>(57,63)</td>
<td></td>
</tr>
<tr>
<td>F229L</td>
<td>s3A</td>
<td>Polymer</td>
<td>Circulating deficiency</td>
<td>(56)</td>
<td></td>
</tr>
<tr>
<td>A382T</td>
<td>s5B</td>
<td>Trimer</td>
<td>No inhibitory function</td>
<td>(64)</td>
<td></td>
</tr>
<tr>
<td>G424R</td>
<td>Shutter</td>
<td>Disulphide dimer</td>
<td>Circulating deficiency</td>
<td>(55)</td>
<td></td>
</tr>
<tr>
<td>HCII</td>
<td>E428K</td>
<td>RCL</td>
<td>Polymer</td>
<td>Circulating deficiency</td>
<td>(26)</td>
</tr>
<tr>
<td></td>
<td>P443L</td>
<td>RCL</td>
<td>Intracellular retention</td>
<td>Circulating deficiency</td>
<td>(59)</td>
</tr>
<tr>
<td>CI inhibitor</td>
<td>F147S</td>
<td>Shutter</td>
<td>?</td>
<td>Circulating deficiency</td>
<td>(65)</td>
</tr>
<tr>
<td></td>
<td>P149L</td>
<td>Shutter</td>
<td>?</td>
<td>Circulating deficiency</td>
<td>(65)</td>
</tr>
<tr>
<td>V432E</td>
<td>RCL</td>
<td>RCL inserted in sheet A</td>
<td>No inhibitory function</td>
<td>(66)</td>
<td></td>
</tr>
<tr>
<td>A434E</td>
<td>RCL</td>
<td>RCL inserted in sheet A</td>
<td>No inhibitory function</td>
<td>(67)</td>
<td></td>
</tr>
<tr>
<td>A436V</td>
<td>RCL</td>
<td>RCL inserted in sheet A</td>
<td>No inhibitory function</td>
<td>(68)</td>
<td></td>
</tr>
<tr>
<td>A436T</td>
<td>RCL</td>
<td>Polymers</td>
<td>Circulating deficiency</td>
<td>(69)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V451M/E/G</td>
<td>s1C</td>
<td>Polymers/Not secreted</td>
<td>Circulating deficiency</td>
<td>(70–72)</td>
</tr>
<tr>
<td></td>
<td>F455S</td>
<td>s1C</td>
<td>Polymers</td>
<td>Circulating deficiency</td>
<td>(70)</td>
</tr>
<tr>
<td>L459R/P</td>
<td>s4B</td>
<td>Not secreted</td>
<td>Circulating deficiency</td>
<td>(70)</td>
<td></td>
</tr>
<tr>
<td>P467R</td>
<td>s5B</td>
<td>Not secreted</td>
<td>Circulating deficiency</td>
<td>(70)</td>
<td></td>
</tr>
<tr>
<td>P476S</td>
<td>C-term</td>
<td>Polymers/Not secreted</td>
<td>Circulating deficiency</td>
<td>(70)</td>
<td></td>
</tr>
<tr>
<td>α2-AP</td>
<td>del E137</td>
<td>hE</td>
<td>Intracellular retention</td>
<td>Circulating deficiency</td>
<td>(73)</td>
</tr>
</tbody>
</table>

AT, antithrombin; HCII, heparin cofactor II; α2-AP, α2-antiplasmin; h, helix; RCL, reactive centre loop; s, sheet.
is retained inside the cell associated with the GRP78/BiP chaperone, one element of the quality control system of the cell, and results in a heterozygous deficiency (59). Another conformational mutation of HCII is the missense change (E428K) identified in a family with homozygous deficiency for this serpin (25). This mutation is identical to the archetypal example of a conformational disease, the deficiency of the plasma serpin α1-antitrypsin commonly present in people of European descent and responsible for the Z-antitrypsin (34). This mutation affects a conserved glutamate at the critical hinge of a mobile peptide loop (P17). Its mutation causes instability of folding such that the RCL of one molecule can insert into a β-sheet of another to give sequentially the formation of long bead-like polymers, thereby resulting in a grossly reduced secretion of the protein and hence in the plasma deficiency.

Table 2 summarizes mutations affecting haemostatic serpins with demonstrated conformational consequences (25, 55–73) available at the serpin mutation database (74) and other serpin mutation databases (48, 49). The search for other conformational mutations or polymorphisms on all haemostatic serpins is encouraged, particularly in serpins recently described, such as the ZPI.

Other agents with a possible conformational effect on haemostatic serpins

In the genomic era, most studies were addressed to identify genetic changes that cause impaired function of a protein. However, there are other factors that may affect the function of a protein. This is particularly evident for serpins and their ability to undergo controlled conformational changes also renders these molecules susceptible to conformational rearrangements under certain conditions and, even spontaneously (75). Thus, the transformation of native antithrombin to the monomeric latent structure (featured by the insertion of the intact reactive centre loop into the A β-sheet) takes place spontaneously under physiological conditions with some 3% of the total circulating antithrombin changing to the latent form each day (76, 77). PAI-1 uses spontaneous conformational changes to control its inhibitory activity, and in the absence of its cofactor vitronectin, native PAI-1 rapidly converts to a latent inactive form (78). Moreover, heat and low pH also induce transition of native antithrombin to the latent or polymeric forms (76–77, 79). Therefore, it might be reasonable to hypothesise that pathophysiological conditions or drugs might have conformational consequences on serpins, particularly on antithrombin, with thrombotic consequences. Indeed, different acquired antithrombin deficiency states have been described, producing an increase in the risk of thrombosis. We have recently obtained evidence that supports the notion that intracellular antithrombin is particularly sensitive to different environmental conditions that cause acquired conformational modifications of the molecule, leading to its aggregation and intracellular retention.

L-asparaginase is an antineoplastic agent commonly used in the treatment of acute lymphoblastic leukemia that acts via depleting the intracellular pool of asparagine that is essential for the survival of actively dividing lymphoblasts. Unfortunately, this treatment causes a severe deficiency of antithrombin that might explain the associated high incidence of venous thromboembolism (as high as 36.7% in some series) (80). Our group showed the mechanism that contributes to the circulating deficiency of this serpin under this treatment. Thus, L-asparaginase-treatment produces the intracellular retention of antithrombin into inclusion-like bodies within the endoplasmic reticulum from hepatocytes that resemble those described for the Z form of α1-antitrypsin, the archetypal conformational disease (81). Interestingly, intracellular antithrombin from L-asparaginase-treated cells differed in its pI, suggesting that L-asparaginase could interfere with the accurate glycosylation and folding of the molecule within the lumen of the endoplasmic reticulum. Remarkably, this mechanism was not exclusive to antithrombin, but also affected α1-antitrypsin and other unknown proteins within other conformationally-sensitive organs, such as the brain and pancreas (81).

Non-alcoholic liver steatosis (NALS) is a condition normally linked to a hypercoagulable status that is associated with a decrease in the circulating levels of antithrombin (82). This decrease is particularly remarkable in cases of acute fatty liver of pregnancy (83). Our group has recently described in a chick model of NALS how high circulating levels of lipids that produce NALS have similar effects to those of L-asparaginase, i.e., the cytoplasmic retention of hepatic antithrombin and heparin cofactor II within inclusion-like bodies with an accompanying deficiency of circulating antithrombin (84). Interestingly, the levels and sizes of intracellular antithrombin aggregates were inversely correlated with the levels of circulating antithrombin (84).

Finally, we have recently shown in different mice models that hyperthermic stimuli are able to produce a moderate deficiency of circulating antithrombin and a slight increase in its latent form (85). Moreover, hyperthermia caused intracellular retention of antithrombin into aggregates within the lumen of the endoplasmic reticulum of hepatocytes (85). In this case, the intracellular retention and subsequent type I deficiency was co-related with the intensity and time of exposition to the heat stimulus. Moreover, circulating antithrombin did not reach normal levels and intracellular antithrombin aggregates had not disappeared even after returning mice for 90 minutes at room temperature after a mild heatstroke (85). All these hyperthermia-induced effects may contribute to the prolonged hypercoagulable state that associates with hyperthermia, particularly with that seen in heatstroke.

In all of these three situations, the first consequence of intracellular aggregation of antithrombin is the loss of function derived from its impaired secretion, the consequent circulating deficiency, and the lower anticoagulant capacity. However, we did not observe a significant gain of function that could contribute to a possible hepatic toxicity. Since antithrombin is synthesised in the liver at concentrations that are only a fraction of those of α1-antitrypsin, it is not surprising that the misfolding and accumulation of antithrombin within hepatocytes has no apparent effects on liver functionality.

The elevated percentage of cases with so far idiopathic venous thrombosis encourages the search for additional factors that may share similar acquired conformational mechanisms and consequences.
Overall conclusions and future directions

It is now acknowledged, and well documented, that thrombophilia is usually due to multifactorial genetic deficiencies, but there has also been an unvoiced realisation that external factors also make critical contributions. Herein, we show recent work providing clear evidential examples of how this can occur in serpins, key molecules in the haemostatic system with extraordinary structural sensitivity. These two features explain why both genetic and environmental factors may cause conformational changes in these molecules with loss of their inhibitory function that may be involved in venous thrombosis. Many examples of disease-associated mutations affecting serpins have been described, some of them in haemostatic serpins identified in patients with venous thrombosis. These results encourage the search for further conformational mutations affecting haemostatic serpins as new genetic risk factors for thrombosis. This is particularly interesting as new serpins with a relevant role in haemostasis are being described. Moreover, it has been suggested that conformational changes of serpins might also have a gain-of-function over the loss of their inhibitory activity. Indeed, some abnormal conformers may also have other physiological effects.

Finally, we have identified other acquired factors able to induce intracellular conformational effects on antithrombin that cause a severe plasma deficiency of this anticoagulant and an increased risk of thrombosis.

The search for new haemostatic serpins, the identification of further conformational mutations, and new environmental conditions, drugs or post-translational modifications that might have conformational consequences on haemostatic serpins may help to identify new thrombotic risk factors. Additionally, it would be interesting to characterise the biological function of abnormal conformers of haemostatic serpins, and to elucidate their possible use as markers for characterisation of disease states. Elucidation of the biological activities of non-inhibitory forms of serpins may provide useful insights into the pathogenesis of diseases and suggest new therapeutic strategies.

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