

Serpins, Serpinopathies, and Conformational Diseases

Note: The term **serpin**, denoting **serine protease inhibitor**, can be misleading when referring to proteins of the serpin family, because not all of them are serine protease inhibitors and not every serine protease inhibitor is a member of this family.

Serpins are mid-sized proteins (about 400 residues) whose tertiary structure consists of three γ -sheets (A, B, and C), nine α -helices, and an exposed reactive center loop (RCL) of about 25 residues tethered between γ -sheets A and C (Figure 1A). This tertiary fold traps the molecule in a metastable conformation, resulting mainly from the labile configuration of a pair of parallel γ -strands within the large central γ sheet-A (strands s3A and s5A in Figure 1A). As discussed later, this feature underlies the unique ability of serpins to act as irreversible (“suicidal”) protease inhibitors.

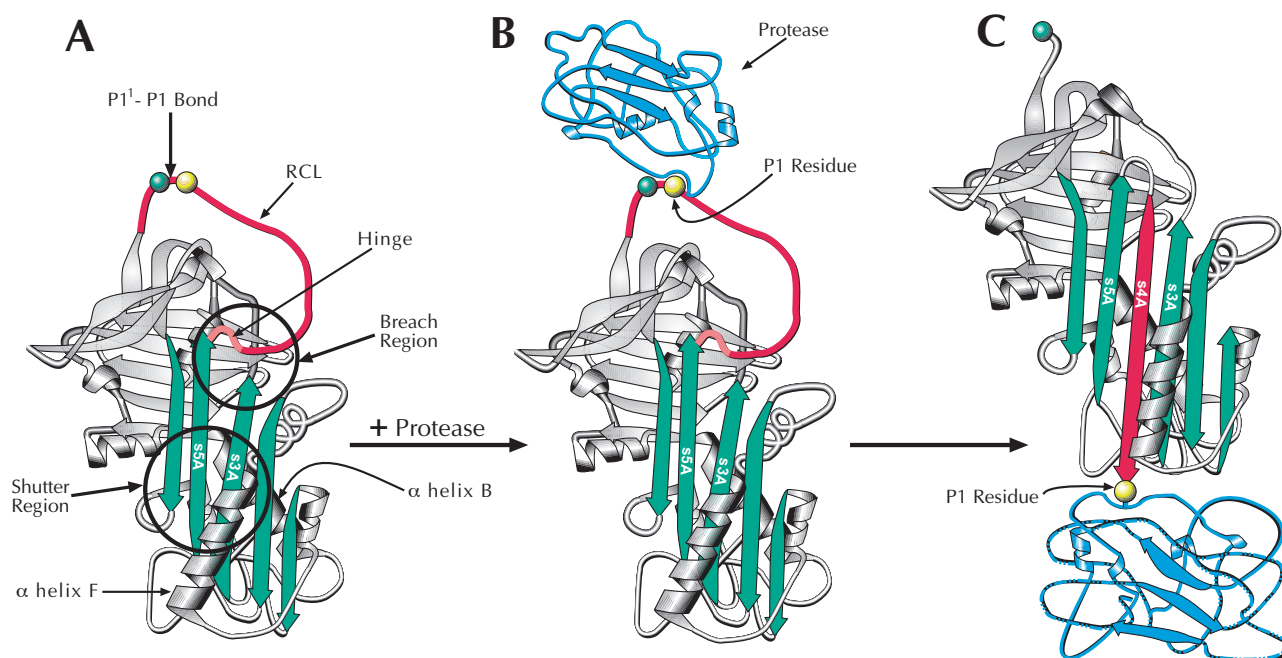
Serpins are distributed across all kingdoms of life including eukaryotes, prokaryotes, plants, and certain viruses. In humans, 36 serpins have been identified to date, of which 27 inhibit serine or cysteine proteases and the rest are a functionally diverse group referred to as “noninhibitory serpins”. However, since the molecular targets of most of the latter are still unknown it is possible that some of them may possess, as yet

unidentified, protease inhibitory activity. Serpins are omnipresent throughout the body, residing both intra- and extra-cellularly. Circulating serpins are variably glycosylated, but the carbohydrate side chains are not required for activity. The archetypal member of the family, α 1-antitrypsin (AAT), is the most abundant protease inhibitor in human plasma, comprising 1-2% of total serum proteins. It is produced and secreted by the liver and plays an important role in protecting lung and other tissues from proteolytic attack by elastase, a serine protease released from leukocytes during inflammatory responses. A similar protective role is played by another circulating serpin, α 1-antichymotrypsin, which neutralizes the activity of leukocyte-derived cathepsin G. Other inhibitory serpins are central in controlling proteolytic cascades in a number of fundamental biological pathways including blood coagulation, fibrinolysis, complement activation, and extracellular matrix (ECM) remodeling. Noninhibitory serpins, whose mechanism of action is largely unknown, exhibit a variety of biological activities functioning as hormone transporters, molecular chaperones, anti-angiogenic factors, sensitizers of insulin action, and tumor suppressors (Table 1).

Inhibitory serpins are distinguished from all

Figure 1: Schematic illustration of the inhibiting mechanism of Serpins

(A) Tertiary structure of the inhibitor (B) Serpin-Protease docking complex (C) Final Serpin-Protease complex



Serpins, Serpinopathies, and Conformational Diseases continued...

other protease inhibitors by their ability to set into motion a mousetrap-like mechanism that not only entraps the protease but also inactivates it. The protease recognition site (the “bait”) is contained within the exposed RCL in an accessible region for protease docking (Figure 1B). Cleavage of the reactive site bond (termed the P1-P1' bond) by the protease triggers a dramatic change in the conformation of both the serpin and the protease. The N-terminal portion of the cleaved RCL inserts as the fourth strand into the gap between strands s3A and s5A (referred to as the cleft) to yield a fully antiparallel γ -sheet A (Figure 1C). During this transition, termed the stressed to relaxed transition, the RCL transports the covalently linked protease (at its intermediate stage of the catalytic cycle) from one pole of the serpin molecule to the other (Figure 1C). Although the overall shape of the serpin molecule is not altered by this transition, the resulting conformation, in which the cleaved RCL is accommodated within γ -sheet A, is much more stable than the conformation of the native molecule. Most of the energy released by this transformation is used to severely distort the structure of the protease, as evident from the resolved crystal structures of several serpin-protease complexes. This distortion not only prevents the trapped protease from completing the proteolytic cycle, but also serves as a signal for clearing pathways to remove and degrade the inactivated serpin-protease complex.

The inhibitory mechanism outlined above is critically dependent upon the metastable conformation of the inhibitor and requires that the length and residues of the mobile part of the RCL be compatible with the length and residues of the cleft-forming strands so that a stable stretch of hydrogen bonds can be formed during the stressed to relaxed transition. A negative consequence of these requirements is that the inhibitory activity of serpins is susceptible to impairment by point mutations which may lead to both deficiency and degenerative disorders. Most serpin-related disorders or serpinopathies are caused by point mutations that, alone or in combination with other factors, disrupt the delicate conformation of the underlying serpin, rendering it vulnerable to self-association and tissue deposition. Accumulation of mutant serpin polymers in serpin-producing cells appears to trigger the premature apoptosis observed in degenerative serpinopathies, which are therefore classified as conformational diseases.

Mutant Serpins and Serpinopathies

Although rare, mutations that promote aberrant serpin-protease interactions have been identified. These include the Pittsburgh variant of ϵ 1-antitrypsin (Met358Arg) in which the substitution of the P1 residue from Met to Arg alters the specificity of the serpin, converting it into an effective thrombin inhibitor. The overabundance of antithrombin activity in the only identified heterozygous carrier of this variant resulted in a severe bleeding disorder and death in

childhood. An inherited autosomal recessive bleeding disorder is associated with the Enschede variant of ϵ 2-antiplasmin (ζ Ala366). Here, the presence of an extra alanine residue within the mobile segment of the RCL interferes with its insertion into γ -sheet-A, resulting in a protein that behaves as a substrate rather than an inhibitor of the protease (1). A more common cause of serpinopathies is point mutations that perturb the conformation of the pivotal γ -sheet A, rendering it promiscuous to aberrant loop insertions. In the Z variant of ϵ 1-antitrypsin (Z-AAT) the replacement of the negatively charged Glu-342 residue with a positively charged Lys residue (Glu342Lys) disrupts a salt bridge (with Lys-290) which normally narrows the opening at the top of the cleft of γ -sheet A. This opening, called the breach region, is the gate through which the cleaved RCL enters into γ -sheet A during the inhibitory process, and is always partially open in inhibitory serpins. The charge repulsion amongst the Lys residues in Z-AAT widens the breach of γ -sheet A and allows insertion of the uncleaved loop of another molecule to form a dimer. Such head-to-head self-association has been suggested to underlie the formation of dysfunctional forms of Z-AAT within the endoplasmic reticulum of hepatocytes (2). Since these self-associated forms do not present new epitopes that would elicit the cellular response to misfolded/unfolded proteins (e.g. degradation by proteasomes), they accumulate within the cells as insoluble inclusion bodies (also referred to as polymers). Z-AAT, especially in homozygous carriers of this variant, is a major cause of both early-onset liver cirrhosis and chronic obstructive pulmonary disease (emphysema). The former appears to result from cytotoxic effects of the retained polymer, whereas the latter is a consequence of diminished secretion of the inhibitor whose deficiency in the plasma exposes the lung to the deleterious effects of elastase.

Deficiency of inhibitory serpin resulting from self-association of the protein while in circulation is a rare phenomenon. Yet, this has been shown to be the case with a thermolabile variant of antithrombin (Phe229Leu) in which the substitution replaces a highly conserved and fully buried residue located within the breach region (3). Computer modeling, using X-ray structures of different forms of antithrombin, shows that replacing the side chain of Phe-229 with the smaller but more branched side chain of Leu creates a small internal cavity and imposes steric clashes with residues adjacent to the hinge segment of the RCL (Figure 1A). Such perturbations are predicated to trigger local conformational changes that could lead to destabilization of the variant and account for its tendency to undergo spontaneous polymerization at elevated body temperature (3). A homozygous carrier of this variant, the only son of parents with heterozygosity for the allele, suffered a severe venous thrombosis associated with fever and seizures at the age of 13 months. The first pregnancy of his mother ended with a fatal loss in the 7th month. During

Serpins, Serpinopathies, and Conformational Diseases continued...

Table 1: Function of Human Serpins

Serpin Name (localization)	Function
Alpha-2-antiplasmin (<i>Extracellular</i>)	Inhibits plasmin
Angiotensinogen (<i>Extracellular</i>)	Non-inhibitory, promotes angiogenesis
Antichymotrypsin (<i>Extracellular</i>)	Inhibits cathepsin G
Antithrombin (<i>Extracellular</i>)	Inhibits thrombin, factor Xa
Antitrypsin (<i>Extracellular</i>)	Inhibits neutrophil elastase
Antitrypsin-related protein	Unknown
Bomapin (<i>Intracellular</i>)	Inhibits thrombin, trypsin
C1 inhibitor	Inhibits C1 esterase
Centerin (<i>Extracellular</i>)	Non-inhibitory, maintenance of naïve B cells
Corticosteroid-binding globulin (<i>Extracellular</i>)	Non-inhibitory, cortisol binding
Cytoplasmic antiproteinase 8 (<i>Intracellular</i>)	Inhibits furin
Cytoplasmic antiproteinase 9 (<i>Intracellular</i>)	Inhibits Granzyme B
Epipin (<i>Intracellular</i>)	Unknown
Headpin (<i>Intracellular</i>)	Inhibits cathepsin L and K
Heat-shock protein	Non-inhibitory, chaperone for collagens
Heparin cofactor II (<i>Extracellular</i>)	Inhibits thrombin
Hs.512272	Unknown
Kallistatin (P14) (<i>Extracellular</i>)	Inhibits kallikrein
Maspin (<i>both Intra- and Extracellular</i>)	Non-inhibitory, tumor suppressor
Megsin (<i>Intracellular</i>)	Non-inhibitory, promotes megakaryocyte maturation
Monocyte neutrophil elastase inhibitor (<i>Intracellular</i>)	Inhibits neutrophil elastase
P114 (<i>Extracellular</i>)	Inhibits cancer metastasis
Neuroserpin (<i>Extracellular</i>)	Inhibits ECM-degrading protease (tPA?)
Pigment epithelium derived factor (<i>Extracellular</i>)	Non-inhibitory, potent anti-angiogenic factor
Plasminogen activator inhibitor-1 (PAI-1) (<i>Extracellular</i>)	Inhibits uPA, tPA, thrombin and plasmin
PAI-2 (<i>both Intra- and Extracellular</i>)	Inhibits uPA
Protease nexin I (<i>Extracellular</i>)	Inhibits thrombin, tPA
Protein Z-dependent proteinase inhibitor (<i>Extracellular</i>)	Inhibits factor Z and X1
Proteinase inhibitor-6 (<i>Intracellular</i>)	Inhibits cathepsin G
Protein C inhibitor (PAI-3) (<i>Extracellular</i>)	Inhibits active protein C
Squamous cell carcinoma antigen-1 (<i>Intracellular</i>)	Inhibits cathepsins L and V
Squamous cell carcinoma antigen-2 (<i>Intracellular</i>)	Inhibits cathepsin G and chymase
Thyroxine-binding globulin (<i>Extracellular</i>)	Non-inhibitory, hormone delivery
Vaspin	Non-inhibitory, insulin-sensitizing adipocytokine
XM_370772	Unknown
XP_170754.3	Unknown
Yukopin (<i>Intracellular</i>)	Inhibits trypsin

her (successful) second pregnancy she received heparin prophylaxis as a preventive measure against thrombosis. Her elder sister, a heterozygous carrier of the variant, had a pulmonary embolism at the age of 38 and has been put under lifelong anticoagulant treatment (3).

A more frequent cause of polymer formation is point mutations in the so-called shutter region of the inhibitor (Figure 1A). In this region, the central part of s3A and s5A and the N-terminal part of ϵ helix B are interconnected via a buried cluster of hydrogen bonds and form a shutter-like apparatus that blocks aberrant loop insertion into the lower part of the cleft of γ -sheet A. During the inhibitory process, the sliding movement of the cleaved RCL is involved with formation of a new set of hydrogen bonds along the upper half of the cleft, which favors disassembly of a key hydrogen bridge between s3A and s5A in the

center of γ -sheet A. This triggers a conformational change within the entire shutter region involving translocation of ϵ helices B and F and resulting in opening a space for the incoming new strand. Mutations in the shutter region not only affect RCL insertion and impair the inhibitory mechanism, but may also predispose the variant to polymerization via head-to-tail self-association. Such polymerization leads to formation of long-chain polymers and occurs more readily in variants whose substituted residue is incompatible with the hydrogen bonding network in this region, (e.g. residues with different charge, bulkiness, and/or rigidity). A large number of shutter-region mutations have been implicated in the pathogenesis of serpinopathies including, among others, mutations in ϵ 1-antichymotrysin and AAT that cause lung and liver disease and in C1-inhibitor and antithrombin that result in angioedema

Serpins, Serpinopathies, and Conformational Diseases continued...

and thrombosis, respectively. As discussed below, such mutations in the neuroserpin gene underlie a hereditary disorder called familial encephalopathy with neuroserpin inclusion bodies or FENIB.

FENIB and Conformational Diseases

Conformational diseases are a newly defined group of disorders whose underlying cause is the accumulation of pathogenic conformers of a host protein in the affected organ. These disorders usually progress slowly and only become evident late in life with failure of the organ. The progression of the disease involves a cumulative cell loss, which in organs with a high regenerative capacity results in successive fibrosis, as in the cirrhosis of the liver. In organs with a limited regenerative capacity, the severity of symptoms appears to be correlated with the accumulated amount of polymers/aggregates of the underlying protein, as in several neurodegenerative disorders including Alzheimer's disease, Huntington's chorea, prion disease, and FENIB. The latter is an autosomal dominant dementia caused by accumulation of mutant neuroserpin polymers in neurons of the cerebral cortex, hippocampus, and amygdala. To date, four neuroserpin variants have been identified to cause FENIB; all four harbor a substituted shutter-region residue. As summarized in Table 2, the severity of FENIB associated with these variants is correlated with their tendency to form polymers, which in turn is related to the predicted destabilizing effect of the substituted residue on the hydrogen bonding network in the shutter region. Thus, in individuals carrying the variants whose substituted residue has different charge and bulkiness (as in the Gly392Glu and Ser52Arg variants), the onset of symptoms occurs in the second or third decade of life and involves progressive dementia accompanied by epilepsy. On the other hand, in carriers of the variant with the less dramatic change (Ser49Pro) FENIB is manifested several decades later and is associated with milder symptoms of cognitive decline.

Despite the apparent correlation between the pathogenesis of FENIB and the buildup of neuroserpin polymers within neurons, the contribution of the accompanying neuroserpin deficiency to development of the disease should also be taken into consideration. Neuroserpin is one of two inhibitory serpins expressed predominantly in the nervous system. The other is protease nexin-1 (PN-1), a serine protease inhibitor whose physiological target in the brain is thrombin, but can also inhibit tissue-type plasminogen activator (tPA). Although the identity of the physiological target(s) of neuroserpin is still unclear, cumulative evidence suggests that it plays an important role in controlling proteolytic degradation of extracellular matrix (ECM) during the development of neuronal plasticity. In the adult brain neuroserpin is secreted from the growth cones of neurons in areas where synaptic changes are associated with learning and memory. The delicate ECM remodeling

Table 2: Effects of point mutations in the neuroserpin gene on the Pathogenesis of FENIB

Mutant	Predicted instability of the mutant	Tendency of mutant to form Polymers	Age at onset of FENIB
Ser49Pro	+	+	48
Ser52Arg	++	++	24
His338Arg	+++	+++	15
Gly39Glu	++++	++++	13

needed for these changes requires a fine balance between the actions of ECM-degrading proteases and their cognate inhibitors. Overpowering action by either group may lead to a neurological disorder.

ECM-degrading proteases are a large group of specialized enzymes including various matrix metalloproteinases (e.g. collagenases and gelatinases) and a number of proteases of the plasminogen activator (PA) system (e.g. plasmin and thrombin). Typically, these proteases are synthesized and secreted as zymogens (pro-enzymes), which are activated *in-situ* by serine proteases such as tPA and urokinase-type plasminogen activator (uPA). This activation usually triggers a cascade of proteolytic events with multiple protease activation/inhibition steps. Because neuroserpin and tPA are co-expressed in neurons and the release of the former occurs in a time delayed manner following the release of tPA, it has been suggested that tPA is the target protease of neuroserpin. However, the finding that the acyl-enzyme complexes between tPA and neuroserpin are atypically short-lived *in vitro* suggests that the physiological target of neuroserpin may be another, as yet unidentified, serine protease (5). Moreover, kinetic experiments showed that neuroserpin is a poor inhibitor of tPA even at a thousand-fold molar excess over tPA, which is quite opposite to the effective inhibition of tPA by plasminogen activator inhibitor-1 (PAI-1) (5). Recent studies suggest that neuroserpin is involved in controlling both tPA-dependent and tPA-independent proteolytic degradation of ECM during axonal outgrowth and synaptic formation (reviewed in reference 4). $1^{TM1}TM1^{TM1}TM1^{TM1}TM1^{TM1}TM1^{TM1}TM1^{TM1}TM1^{TM1}$ Since the survival of neurons is dependent on contacts with the ECM, uncontrolled degradation of the ECM may result in premature cell death. The neuroprotective role of neuroserpin has been demonstrated in transgenic mice lacking neuroserpin expression. The deficiency of neuroserpin in these mice was associated with a motor neuron disease characterized by axonal degradation (6). In another study, genetically modified mice lacking and over-expressing neuroserpin were generated. Both groups showed an impaired explorative behavior and neophobia (7). Taken together, these findings suggest that neuroserpin is an important regulator of ECM remodeling during the development of neuronal plasticity. Lack of such

Serpins, Serpinopathies, and Conformational Diseases continued...

plasticity is likely to impair learning and memory and could lead to neurologic disorders, including FENIB. That an abnormal level of inhibitory serpin may trigger pathological processes in the brain was also demonstrated in mice lacking or overexpressing PN-1. The deficiency of this serpin in PN-1 knock-out mice was associated with an increased epileptic activity (8), whereas overexpression of PN-1 resulted in progressive neuronal and motor dysfunction (9).

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