Molecular mousetraps and the serpinopathies


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Abstract

Members of the serine proteinase inhibitor or serpin superfamily inhibit their target proteinases by a remarkable conformational transition that involves the enzyme being translocated more than 70 Å (1 Å = 10⁻¹⁰ m) from the upper to the lower pole of the inhibitor. This elegant mechanism is subverted by point mutations to form ordered polymers that are retained within the endoplasmic reticulum of secretory cells. The accumulation of polymers underlies the retention of mutants of α₁-antitrypsin and neuroserpin within hepatocytes and neurons to cause cirrhosis and dementia respectively. The formation of polymers results in the failure to secrete mutants of other members of the serpin superfamily: antithrombin, C1 inhibitor, antithrombin and plasminogen activator inhibitor-1, which play an important role in the control of proteinases involved in the inflammatory, complement, coagulation and fibrinolytic pathways respectively [1]. The family is characterized by more than 30% sequence homology with α₁-antitrypsin and conservation of tertiary structure. Crystal structures have demonstrated that serpins are composed of three β-sheets (A–C) and an exposed mobile reactive loop that presents a peptide sequence as a pseudosubstrate for the target proteinase [2–8]. The critical amino acids within this loop are the P1–P1' residues, which act as a ‘bait’ for the target enzyme [9]. After docking, the enzyme cleaves the P1–P1' peptide bond of the serpin [10] and the proteinase is inactivated by a mousetrap action that swings it 70 Å (1 Å = 10⁻¹⁰ m) from the upper to the lower pole of the protein in association with the insertion of the reactive loop as an extra strand (s4A) in β-sheet A (Figure 1a) [11–15]. This altered conformation of α₁-antitrypsin bound to its target enzyme is then recognized by hepatic receptors and cleared from the circulation [16–18]. The remarkable mousetrap action of α₁-antitrypsin is central to its role as an effective inhibitor of serine proteinases. Paradoxically, it is also its ‘Achilles heel’ since point mutations in these mobile domains make the molecule vulnerable to aberrant conformational transitions that underlie the retention of the serpin within the cell of synthesis. This gives rise to clinical conditions that result from either protein overload and cell death or plasma deficiency. These can manifest as diseases as diverse as cirrhosis, thrombosis, angio-oedema, emphysema and dementia. In view of the common mechanism underlying these diseases, we have grouped these conditions as the serpinopathies. We review in this paper the molecular and structural basis of the serpinopathies and show how this has allowed the development of specific agents to block the polymerization that underlies disease.

The serpin superfamily and the serpin inhibitory mechanism

Members of the serine proteinase inhibitor or serpin superfamily play an important role in the regulation of enzymes involved in proteolytic cascades. They are typified by α₁-antitrypsin that controls proteinases of the inflammatory cascade. Other members include α₁-antichymotrypsin, C1 inhibitor, antithrombin and plasminogen activator inhibitor-1, which play an important role in the control of proteinases involved in the inflammatory, complement, coagulation and fibrinolytic pathways respectively [1]. The family is characterized by more than 30% sequence homology with α₁-antitrypsin and conservation of tertiary structure. Crystal structures have demonstrated that serpins are composed of three β-sheets (A–C) and an exposed mobile reactive loop (Figure 1a) that presents a peptide sequence as a pseudosubstrate for the target proteinase [2–8]. The critical amino acids within this loop are the P1–P1' residues, which act as a ‘bait’ for the target enzyme [9]. After docking, the enzyme cleaves the P1–P1' peptide bond of the serpin [10] and the proteinase is inactivated by a mousetrap action that swings it 70 Å (1 Å = 10⁻¹⁰ m) from the upper to the lower pole of the protein in association with the insertion of the reactive loop as an extra strand (s4A) in β-sheet A (Figure 1a) [11–15]. This altered conformation of α₁-antitrypsin bound to its target enzyme is then recognized by hepatic receptors and cleared from the circulation [16–18]. The remarkable mousetrap action of α₁-antitrypsin is central to its role as an effective inhibitor of serine proteinases. Paradoxically, it is also its ‘Achilles heel’ since point mutations in these mobile domains make the molecule vulnerable to aberrant conformational transitions that underlie the retention of the serpin within the cell of synthesis. This gives rise to clinical conditions that result from either protein overload and cell death or plasma deficiency. These can manifest as diseases as diverse as cirrhosis, thrombosis, angio-oedema, emphysema and dementia. In view of the common mechanism underlying these diseases, we have grouped them...
Figure 1 | (a) Inhibition of neutrophil elastase by α₁-antitrypsin and (b) the structural basis of polymerization

(a) After docking (left), the neutrophil elastase (grey) is inactivated by movement from the upper to the lower pole of the protein (right). This is associated with the insertion of the reactive loop (red) as an extra strand into β-sheet A (green). Part (a) of the Figure is reproduced from [19] with permission (original publication in Nature Reviews Genetics). (b) The structure of α₁-antitrypsin is centred on β-sheet A (green) and the mobile reactive centre loop (red). Polymer formation results from the Z variant of α₁-antitrypsin (Glu342 → Lys at P₁₇, indicated by arrow) or mutations in the shutter domain (blue circle) that open β-sheet A to favour partial loop insertion and the formation of an unstable intermediate (M*). The patent β-sheet A then accepts the loop of another molecule to form a dimer (D), which then extends into polymers (P). The individual molecules of α₁-antitrypsin within the polymer, although identical, are coloured red, yellow and blue for clarity. Adapted with permission from [80] © 2000 Proceedings of the National Academy of Sciences of the United States of America.
together as the serpinopathies [19–21]. In the present study, we explain the molecular and structural basis of the serpinopathies and show how an understanding of this mechanism can provide new strategies to prevent disease.

**Mutants of α₁-antitrypsin form polymers that are retained in the liver to cause cirrhosis and plasma deficiency**

α₁-Antitrypsin is the archetypal member of the serpin superfamily. It is an acute-phase glycoprotein that is synthesized by the liver and macrophages and present in the plasma at a concentration of 1.5–3.5 g/l. It functions as an inhibitor of a range of proteolytic enzymes, but its primary role is to inhibit the enzyme neutrophil elastase. α₁-Antitrypsin is subject to genetic variation resulting from mutations in the 12.2 kb, seven-exon gene at q31-31.2 on chromosome 14 [22]. More than 75 allelic variants have been reported and classified using the PI nomenclature that assesses α₁-antitrypsin mobility in isoelectric focusing analysis [23]. Normal α₁-antitrypsin migrates in the middle (M), and variants are designated A–L if they migrate faster than M or N–Z if they migrate more slowly. The most clinically relevant variants are the S (Glu264→Val) and Z (Glu342→Lys) alleles and the uncommon Null alleles that result from the point mutations that introduce premature stop codons.

The highest frequency of the S allele is within the Iberian Peninsula and gradually decreases in the direction of south to north and from west to east [24]. Heterozygotes for the S allele of α₁-antitrypsin (PI*MOS) are found in up to 28% of Southern Europeans and, although homozygotes have plasma α₁-antitrypsin levels that are 60% of the M allele, the deficiency is not associated with any clinical sequel. In contrast, the Z allele is very common in northwest Europe, with frequencies decreasing from west to east and from north to south [24]. Approximately 4% of Northern Europeans are heterozygous for the Z allele (PI*MZ) with 1 in 1700 being homozygotes (PI*ZZ). The Z allele results in plasma levels that are 10–15% of the M allele. The retention of Z α₁-antitrypsin within hepatocytes causes protein overload that is manifest as neonatal and adult liver disease. The lack of circulating α₁-antitrypsin leaves the lungs exposed to enzymatic damage that predisposes to early onset of emphysema in adults (see below).

The severe Z mutation (Glu342→Lys) of α₁-antitrypsin distorts the relationship between the reactive centre loop and β-sheet A (Figure 1b). The consequent perturbation in structure allows the reactive centre loop of one α₁-molecule to lock into the A sheet of a second to form a dimer, which then extends to form chains of loop–sheet polymers [25–28]. These polymers are then degraded [29] or they accumulate within the endoplasmic reticulum of hepatocytes to form the PAS (periodic acid–Schiff)-positive inclusions (Figures 2a–2c) that are the hallmark of Z α₁-antitrypsin liver disease [30] (Figures 2d and 2e). Although many α₁-antitrypsin deficiency variants have been described, only two other mutants of α₁-antitrypsin have similarly been associated with profound plasma deficiency and hepatic inclusions: α₁-antitrypsin Siiyama (Ser53→Phe) and Mmalton (deletion of phenylalanine at position 52). α₁-Antitrypsin Siiyama is the most common cause of α₁-antitrypsin deficiency in Japan [31,32] and Mmalton [33], which is also known as Mnichinan [34] and Mcagliari [35], is the most common cause of α₁-antitrypsin deficiency in Sardinia. Both these mutants are in the shutter domain underlying the bifurcation of strands 3 and 5 of β-sheet A (Figure 1b). The mutations disrupt a hydrogen bond network that is based on His354 and bridges strands 3 and 5 of the A sheet [36], causing it to part and thus allow the formation of folding intermediates [37,38] and loop–sheet polymers in vivo [39,40].

Polymerization also underlies the mild plasma deficiency of other variants that perturb the shutter domain: S (Glu264→Val) and I (Arg39→Cys) α₁-antitrypsin [41,42]. These point mutations cause less disruption to β-sheet A when compared with the Z variant. Thus the rates of polymer formation are much slower compared with that of Z α₁-antitrypsin [27] and this results in less retention of protein within hepatocytes, milder plasma deficiency and the lack of a clinical phenotype. However, if a mild, slowly polymerizing I or S variant of α₁-antitrypsin is inherited with a rapidly polymerizing Z variant, then the two can interact to form heteropolymers within hepatocytes, leading to inclusions and finally cirrhosis [42–44]. Thus the severity of retention of mutants of α₁-antitrypsin within hepatocytes can be explained by the rate of polymer formation. Those mutants that cause the most rapid polymerization cause the maximum retention of α₁-antitrypsin within the liver. This in turn correlates with the greatest risk of liver damage and cirrhosis, and the most severe plasma deficiency (Table 1).

**Polymerization of Z α₁-antitrypsin within the lungs provides a novel pathway for the pathogenesis of emphysema**

The retention of α₁-antitrypsin within hepatocytes in the Z homozygote decreases the plasma level of α₁-antitrypsin to 10–15% of the normal. This in turn markedly decreases the α₁-antitrypsin that is available to protect the lungs against proteolytic attack [45]. The situation is exacerbated as the Z mutation decreases the rate of association between α₁-antitrypsin and neutrophil elastase approx. 5-fold [46–49]. Thus the α₁-antitrypsin available within the lungs is not as effective as the normal M protein. The combination of α₁-antitrypsin deficiency and cigarette smoking can have a devastating effect on lung function [50,51], probably by allowing the unopposed action of proteolytic enzymes. The inhibitory activity of Z α₁-antitrypsin can be further decreased as the Z mutation favours the spontaneous formation of α₁-antitrypsin loop–sheet polymers within the lungs [52]. This conformational transition inactivates α₁-antitrypsin as a protease inhibitor, thereby further decreasing the already depleted levels of α₁-antitrypsin that are available to protect the alveoli (see [20] for a recent review). Moreover, the conversion of α₁-antitrypsin from a monomer into a polymer
Figure 2 | Z α₁-antitrypsin is retained within hepatocytes as intracellular inclusions

These inclusions are PAS-positive and diastase-resistant (a) and are associated with neonatal hepatitis and hepatocellular carcinoma. (b) Electron microscopy of a hepatocyte from the liver of a patient with Z α₁-antitrypsin deficiency shows the accumulation of α₁-antitrypsin within the rough endoplasmic reticulum (arrow). These inclusions are composed of chains of α₁-antitrypsin polymers shown here from the plasma of a Siiyama α₁-antitrypsin homozygote (c). More recently, polymers have been identified within PAS-positive inclusions with a monoclonal anti-polymer α₁-antitrypsin antibody [26,30]. (d, e) Immunohistochemistry of the liver from an individual with Z α₁-antitrypsin deficiency, showing staining with an anti-α₁-antitrypsin polyclonal antibody (d, arrow) and a monoclonal anti-polymer α₁-antitrypsin antibody (e, arrow). It is these intracellular inclusions of polymers that are associated with neonatal hepatitis and hepatocellular carcinoma. Figure part (b) reproduced from [25] with permission (original publication in Nature); part (c) reproduced from [39] with permission © 1993 The American Society for Biochemistry & Molecular Biology; parts (d) and (e) reproduced with permission from Hepatology [30] © 2004 American Association for the Study of Liver Diseases.

converts it into a chemoattractant for human neutrophils [53,54]. The magnitude of the effect is similar to that of the chemoattractant C5a (Figure 3) and is present over a range of physiological concentrations (EC₅₀ = 4.5 ± 2 µg/ml). Polymers also induce neutrophil shape change and stimulate myeloperoxidase release and neutrophil adhesion [53]. The chemoattractant properties of α₁-antitrypsin polymers may explain the excess number of neutrophils in bronchoalveolar lavage [55] and in tissue sections of lung parenchyma [20] from individuals with Z α₁-antitrypsin deficiency. Moreover, polymers may contribute to the excess inflammation that is apparent even in individuals with Z α₁-antitrypsin deficiency with very early lung disease [56]. Any pro-inflammatory effect of polymers is likely to be exacerbated by inflammatory cytokines, cleaved or complexed α₁-antitrypsin [57], elastin degradation products [58] and cigarette smoke that also cause neutrophil recruitment. Thus our understanding of the biological properties of α₁-antitrypsin provides novel pathways for the pathogenesis of emphysema in individuals who are homozygous for the Z mutation (Figure 4).
Table 1 | Correlation between the rate of polymerization of mutants of α\(_1\)-antitrypsin and the severity of the accompanying plasma deficiency

The Table is based on data from [27]. There is a striking genotype–phenotype correlation that is explicable by the rate of polymer formation.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Rate of polymerization</th>
<th>Plasma deficiency</th>
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<tbody>
<tr>
<td>Z</td>
<td>Glu(^{342}) → Lys</td>
<td>++++</td>
</tr>
<tr>
<td>Siyama</td>
<td>Ser(^{63}) → Phe</td>
<td>++++</td>
</tr>
<tr>
<td>Mmalton</td>
<td>ΔPhe(^{62})</td>
<td>++++</td>
</tr>
<tr>
<td>S</td>
<td>Glu(^{364}) → Val</td>
<td>++</td>
</tr>
<tr>
<td>I</td>
<td>Arg(^{39}) → Cys</td>
<td>+</td>
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Figure 3 | Chemotactic properties of conformers of α\(_1\)-antitrypsin for human neutrophils

The horizontal axis indicates the conditions present in the lower well of the chemotaxis chamber and the vertical axis the number of cells that have successfully migrated into the lower chamber. Ovalbumin (5 mg/ml), lipopolysaccharide (8 ng/ml) and PBS were the negative controls and CsA (10\(^{-7}\) M) was the positive control. Different concentrations of polymeric α\(_1\)-antitrypsin were used to construct a concentration-response curve [53]. Reproduced from with permission from J.S. Parmar, R. Mahadeva, B.J. Reed, N. Farahi, K.A. Cadwallader, M.T. Keogan, D. Bilton, E.R. Chilvers and D.A. Lomas (2002), “Polymers of alpha(1)-antitrypsin are chemotactic for human neutrophils: a new paradigm for the pathogenesis of emphysema”, American Journal of Respiratory Cell and Molecular Biology, vol. 26, pp. 723–730, Official Journal of the American Thoracic Society © American Thoracic Society.

Polymerization of mutants of antithrombin, C1 inhibitor and α\(_1\)-antichymotrypsin cause plasma deficiency associated with thrombosis, angio-oedema and emphysema respectively

The phenomenon of loop–sheet polymerization is not restricted to α\(_1\)-antitrypsin and has now been reported to cause disease in mutants of other members of the serpin superfamily. Mutants of C1 inhibitor (Phe\(^{52}\) → Ser, Pro\(^{54}\) → Thr, Val\(^{66}\) → Met, Phe\(^{370}\) → Ser, Pro\(^{391}\) → Ser), antithrombin (Pro\(^{34}\) → Thr, Asn\(^{158}\) → Asp) and α\(_1\)-antichymotrypsin (Leu\(^{55}\) → Pro, Pro\(^{228}\) → Ala) can also destabilize the protein architecture to form inactive polymers that are retained within hepatocytes. The associated plasma deficiency results in uncontrolled activation of proteolytic cascades and angio-oedema, thrombosis and chronic obstructive pulmonary disease (see [19–21] for reviews). More recently, a mutation in heparin cofactor II (Glu\(^{428}\) → Lys) has been associated with plasma deficiency [59]. This is of particular interest since the mutation is the same as the Z allele that causes polymerization and deficiency of α\(_1\)-antitrypsin. The same mutation also causes polymerization and inactivation of the Dro sophila serpin, Necrotic [60]. However, to date, plasma deficiency of heparin cofactor II has not been associated with any clinical phenotype [59].

Figure 4 | Proposed model for the pathogenesis of emphysema in patients with Z α\(_1\)-antitrypsin deficiency

The plasma deficiency and decreased inhibitory activity of Z α\(_1\)-antitrypsin may be exacerbated by smoking (which can oxidize α\(_1\)-antitrypsin) and by the polymerization of α\(_1\)-antitrypsin within the lungs. These processes inactivate the inhibitor, thereby further decreasing the antiproteinase screen. α\(_1\)-Antitrypsin polymers may also act as a pro-inflammatory stimulus to attract and activate neutrophils, thereby increasing tissue damage. The Figure is reproduced from [20] with permission.

Mutants of neuroserpin form polymers that are retained in the brain to cause the dementia FENIB (familial encephalopathy with neuroserpin inclusion bodies)

The process of disease-related polymerization is most strikingly displayed by the inclusion body dementia, FENIB [61–63]. This is an autosomal dominant dementia characterized by eosinophilic neuronal inclusions of neuroserpin (Collins’ bodies) in the deeper layers of the cerebral cortex and the substantia nigra. The inclusions are PAS-positive and diastase-resistant and bear a striking resemblance to those of Z α\(_1\)-antitrypsin that form within the liver (Figure 5a). The finding that FENIB is associated with a mutation,
**Figure 5** | Mutant neuroserpin is retained within neurons as intracellular inclusions

These inclusions stain positive with PAS (a) and can be seen within the endoplasmic reticulum on electron microscopy (b). Electron microscopy of the isolated inclusions confirms that the mutant neuroserpin forms bead-like polymers identical with those of Z α1-antitrypsin (c). Figure parts (a) and (b) reproduced from [21] with permission © 2002 Massachusetts Medical Society; part (c) from [62] with permission (original publication in Nature).

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**Table 2** | Correlation between the rate of polymerization of mutants of neuroserpin, the number of inclusions and the severity of the associated dementia

The Table is based on data from [62,64-67]. There is a striking genotype–phenotype correlation that is explicable by the rate of polymer formation.

<table>
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<tr>
<th>Mutation</th>
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<th>Inclusions</th>
<th>Age of onset (years)</th>
<th>Clinical features</th>
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<td>Ser49→Pro</td>
<td>++</td>
<td>+</td>
<td>45–63</td>
<td>Dementia, seizures</td>
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<tr>
<td>Ser52→Arg</td>
<td>+++</td>
<td>+++</td>
<td>20–40</td>
<td>Dementia, myoclonus</td>
</tr>
<tr>
<td>His338→Arg</td>
<td>++++</td>
<td>++++</td>
<td>15</td>
<td>Progressive myoclonus epilepsy</td>
</tr>
<tr>
<td>Gly392→Glu</td>
<td>+++++</td>
<td>+++++</td>
<td>13</td>
<td>Progressive myoclonus epilepsy, chorea</td>
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</table>

Ser49→Pro, in the neuroserpin gene that is homologous with the one in α1-antitrypsin that causes cirrhosis (Ser53→Phe) [39] strongly indicated a common molecular mechanism. This was confirmed by the finding that the neuronal inclusion bodies of FENIB were formed by entangled polymers of neuroserpin with identical morphology when compared with those present in hepatocytes from a child with α1-antitrypsin deficiency-related cirrhosis (compare Figures 5b and 5c with Figure 2) [62].

A direct relationship between the magnitude of the intracellular accumulation of neuroserpin and the severity of disease is clearly shown by the identification of other mutations of neuroserpin in families with FENIB [64]. In the original family with Ser49→Pro neuroserpin (neuroserpin Syracuse), the affected family members had diffuse small intraneuronal inclusions of neuroserpin with an onset of dementia between the ages of 45 and 60 years [61–63]. However, in a second family, with a conformationally more severe mutation (neuroserpin Portland; Ser52→Arg) and larger inclusions, the onset of dementia was in early adulthood; and in a third family, with yet another mutation (His338→Arg), there were more inclusions and the onset of dementia in adolescence (Table 2). The most striking example was the family with the most ‘polymerogenic’ mutations of neuroserpin,

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**Figure 6** | Mutant Syracuse and Portland neuroserpin aggregate within transfected COS-7 cells

(a) Immunocytochemistry with an anti-neuroserpin antibody showing the distribution of wild-type (a, d, g, j), Syracuse (b, e, h, k) and Portland (c, f, i, l) neuroserpin in transfected COS-7 cells. The nucleus appears blue due to DNA staining with DAPI (4,6-diamidino-2-phenylindole). During a 3 day period, wild-type neuroserpin shows a normal endoplasmic reticulum staining pattern, whereas the neuroserpin mutants form distinct protein aggregates after 24 h of expression that persist for the 3 days of the experiment. (b) Intracellular localization of wild-type, Syracuse and Portland neuroserpin in transfected COS-7 cells. Confocal microscopy of cells that were cultured for 24 h after transfection and stained for neuroserpin (labelled with Texas Red) and an endoplasmic reticulum-resident protein (ER), namely calreticulin (labelled with fluorescein). The merged image (yellow) shows that the mutant protein is retained within the endoplasmic reticulum. The nucleus appears blue due to DNA staining with DAPI. Parts (a) and (b) reproduced from [67] with permission © 2004 The American Society for Biochemistry & Molecular Biology.
Figure 6 | For legend see facing page

(a) 12 hours  24 hours  48 hours  72 hours

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(b)

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| Syracuse |    |     |
|          |    |     |

| Portland |    |     |
|          |    |     |
Figure 7 | Design of a selective inhibitor to block the polymerization of Z α₁-antitrypsin

The Z mutation (ringed in blue) allows partial insertion of the reactive centre loop (red) into β-sheet A (green) to form the intermediate M* (see Figure 1b). This opens the lower part of β-sheet A, thereby favouring the incorporation of the reactive loop of another molecule and hence polymerization (upper pathway). Understanding the configuration of the reactive loop has allowed the design of a 6-mer peptide (yellow) that specifically binds to Z α₁-antitrypsin and so prevents polymer formation [75].

Gly₃⁹² → Glu. This replacement of a consistently conserved residue in the shutter region resulted in large inclusions, with affected family members dying by the age of 20 years [64].

The role of polymerization in disease is supported by our demonstration that recombinant Ser⁴⁹ → Pro neuroserpin has a fast rate of polymerization when compared with the wild-type protein [65,66] and that Ser⁵² → Arg, which causes a more severe clinical phenotype, polymerizes even more rapidly [66]. The cellular handling of neuroserpin has been assessed by transiently transfecting COS cells with wild-type neuroserpin and mutants of neuroserpin that cause FENIB (Figure 6). The most striking feature of the cell model is the retention of Syracuse (Ser⁴⁹ → Pro) and Portland (Ser⁵² → Arg) neuroserpin as intracellular aggregates composed of polymers of mutant neuroserpin, similar to the loop-sheet polymers of mutant neuroserpin that can be isolated from the brains of individuals affected by FENIB [67]. Moreover, (i) N-glycan digestion of metabolically labelled neuroserpin and (ii) co-staining with antibodies against neuroserpin and the endoplasmic reticulum chaperone calreticulin demonstrated that neuroserpin aggregates are contained within the endoplasmic reticulum. This is in keeping with the localization of mutant neuroserpin within the brains of individuals with FENIB. Once again, Portland (Ser⁵² → Arg) neuroserpin accumulates more rapidly than the Syracuse (Ser⁴⁹ → Pro) mutant, in keeping with the more severe clinical phenotype. Thus FENIB shows a clear genotype–phenotype correlation,
with the severity of disease correlating closely with the propensity of the mutated neuroserpin to form polymers (Table 2).

**Prevention of polymer formation**

There is substantial evidence that polymers of α1-antitrypsin, and indeed all other serpins, are formed by an aberrant linkage between the reactive centre loop of one molecule and β-sheet A of another [2,25,28,67–71]. This has allowed the development of new strategies to attenuate polymerization and thereby treat the associated disease. We have shown previously that the polymerization of Z α1-antitrypsin can be blocked by the annealing of reactive loop peptides to β-sheet A [2,25,27]. These peptides were 11–13 residues in length and could bind to other members of the serpin superfamily [72,73]. This was most clearly demonstrated by the finding that the reactive loop peptide of antithrombin is inserted more readily into β-sheet A of α1-antitrypsin and vice versa [74]. These peptides, although useful in establishing the mechanism of polymerization, are too long and too promiscuous to be suitable for rational drug design. More recently, we have designed a 6-mer peptide that specifically anneals to Z α1-antitrypsin alone and blocks polymerization [75,76]. This was based on our understanding of the M* intermediate on the polymerization pathway (Figure 7). Indeed, other small peptides have been developed that will also anneal to a patent β-sheet A of the serpins to block polymerization [36,77]. The aim now is to convert these peptides into small drugs that can be used in vivo.

A second strategy comes from the identification of a hydrophobic pocket in α1-antitrypsin that is bounded by strand 2A and helices D and E [5,78]. The cavity is patent in the native protein but is filled as β-sheet A accepts an exogenous reactive loop peptide during polymerization [5]. We have shown that introducing mutations into this pocket retards the polymerization of M α1-antitrypsin and increases the secretion of Z in α1-antitrypsin from a Xenopus oocyte expression system [79]. Therefore this cavity is an ideal target for the development of drugs that will stabilize β-sheet A and so ameliorate polymer formation.

**Conclusion**

The molecular basis of the serpinopathies has now been elucidated with biochemical, cellular and structural studies. The next goal must be to develop therapeutic strategies to block polymerization in vivo and thereby treat the associated disease.

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**References**


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