Abstract

$\alpha_1$-Antitrypsin functions as a ‘mousetrap’ to inhibit its target proteinase, neutrophil elastase. The common severe $Z$ deficiency variant (Glu$^{A22}$ → Lys) destabilizes the mousetrap to allow a sequential protein–protein interaction between the reactive-centre loop of one molecule and $\beta$-sheet A of another. These loop–sheet polymers accumulate within hepatocytes to form inclusion bodies that are associated with juvenile cirrhosis and hepatocellular carcinoma. The lack of circulating protein predisposes the $Z \alpha_1$-antitrypsin homozygote to emphysema. Loop–sheet polymerization is now recognized to underlie deficiency variants of other members of the serine proteinase inhibitor (serpin) superfamily, i.e. antithrombin, $C1$ esterase inhibitor and $\alpha_1$-antichymotrypsin, which are associated with thrombosis, angio-oedema and emphysema respectively. Moreover, we have shown recently that the same process in a neuron-specific protein, neuroserpin, underlies a novel inclusion-body dementia, known as familial encephalopathy with neuroserpin inclusion bodies. Our understanding of the structural basis of polymerization has allowed the development of strategies to prevent the aberrant protein–protein interaction in vitro. This must now be achieved in vivo if we are to treat the associated clinical syndromes.

Clinical features

$\alpha_1$-Antitrypsin deficiency was first described as a clinical entity in 1963 by Laurell and Eriksson [1], who noted the absence of the $\alpha_1$ band on serum protein electrophoresis. Over 70 naturally occurring variants have been described and characterized by their migration on isoelectric focusing gels [2]. The two most common deficiency variants, $S$ and $Z$, result from point mutations in the $\alpha_1$-antitrypsin gene [3,4] which make the protein migrate more slowly than normal $M \alpha_1$-antitrypsin. S $\alpha_1$-antitrypsin (Glu$^{A22}$ → Val) is found in up to 28% of Southern Europeans, and although it results in plasma $\alpha_1$-antitrypsin levels that are 60% of those in subjects with $M \alpha_1$-antitrypsin, it is not associated with any clinical sequelae. The $Z$ mutation results in the accumulation of $\alpha_1$-antitrypsin as inclusions in the rough endoplasmic reticulum of the liver. These inclusions predispose the homozygote to juvenile hepatitis, cirrhosis [5] and hepatocellular carcinoma [6]. The major function of $\alpha_1$-antitrypsin is to protect the tissues against the enzyme neutrophil elastase [7], and the lack of
circulating α1-antitrypsin results in early-onset panlobular emphysema [8].

**Molecular pathology of α1-antitrypsin deficiency**

α1-Antitrypsin is the archetypal member of the serine proteinase inhibitor, or serpin, superfamily. This family includes members such as α1-antichymotrypsin, C1 esterase 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 cascades [9]. The family is characterized by > 30% sequence identity with α1-antitrypsin and conservation of tertiary structure [10]. Consequently, physiological and pathological processes that affect one member may be extrapolated to another. The structure of the serpins is based on three β-sheets (A–C) and nine α-helices. This structure supports an exposed, mobile, reactive loop that presents a peptide sequence as a pseudosubstrate for the target proteinase (Figure 1, left panel). In the case of α1-antitrypsin the loop presents the P1–P1′ residues methionine–serine as a ‘bait’ for neutrophil elastase [11]. After docking, the proteinase is inactivated by a ‘mousetrap’ action that swings it from the top to the bottom of the protein in association with the insertion of an extra strand in β-sheet A [12]. This six-stranded protein bound to its target enzyme is then recognized by hepatic receptors and cleared from the circulation [13].

The structure of the serpins is very much a dual-edged sword, in that it is central to their role as effective antiproteinases but also renders them liable to undergo conformational changes in association with disease. Point mutations can destabilize β-sheet A to allow incorporation of the loop of another serpin molecule (Figure 1, middle panel). Sequential loop insertion results in chains of polymers that are retained within the cell of synthesis (Figure 1, right panel). This process is best characterized for the severe Z deficiency variant of α1-antitrypsin, which results in protein retention in hepatocytes in association with cirrhosis [14, 15]. The Z mutation of α1-antitrypsin is

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**Figure 1**

Structure of α1-antitrypsin is centred on β-sheet A (green) and the mobile reactive-centre loop (red). Polymer formation is best characterized for the Z variant of α1-antitrypsin (Glu342 → Lys at P17; left panel), which opens β-sheet A to favour the insertion of the reactive loop of another molecule (middle panel). The resulting chain of polymers (right panel) is retained within hepatocytes as intracellular inclusions that are associated with neonatal hepatitis and hepatocellular carcinoma. Each α1-antitrypsin molecule in the polymer is shown in a different colour [16].

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at residue P17 (i.e. 17 residues proximal to the P1 reactive centre) at the head of strand 5 of β-sheet A and the base of the mobile reactive loop. The mutation opens β-sheet A, thereby favouring the insertion of the reactive loop of a second α1-antitrypsin molecule to form a dimer (Figure 1). This can then extend to result in the formation of polymers that tangle in the endoplasmic reticulum of the liver to form inclusion bodies [16,17]. Support for this scenario comes from the demonstration that Z α1-antitrypsin formed chains of polymers when incubated under physiological conditions [14,15]. The rate was accelerated by raising the temperature to 41 °C and could be blocked by peptides that compete with the loop for annealing to β-sheet A [14,18]. The role of polymerization in vivo was demonstrated by the finding of α1-antitrypsin polymers in inclusion bodies from the livers of subjects homozygous for Z α1-antitrypsin.

Although many α1-antitrypsin deficiency variants have been described, only two other mutants of α1-antitrypsin have similarly been associated with plasma deficiency and hepatic inclusions: α1-antitrypsin Siyama (Ser39 → Phe; [19]) and α1-antitrypsin Mmalton (Phe62 deleted; [20]). Both of these mutants also destabilize β-sheet A to allow the formation of loop-sheet polymers in vivo [21,22]. The temperature- and concentration-dependence of polymerization [23], along with genetic factors [24,25], may account for the heterogeneity in liver disease among individuals who are homozygous for the Z mutation. As α1-antitrypsin is an acute-phase protein, its concentration will rise during episodes of inflammation. At these times the formation of polymers is likely to overwhelm the degradative pathway, thereby exacerbating the formation of hepatic inclusions and the associated hepato-cellular damage. There is anecdotal evidence to support this hypothesis from the prospective study of Sveger in Sweden [26]. They screened 200000 newborn babies and identified 120 Z α1-antitrypsin homozygotes, whom they have followed into late adolescence. Two of these patients developed progressive jaundice during the course of the study; this followed appendicitis in one and severe pneumonia in the other.

Investigations have shown that polymerization also underlies the mild plasma deficiency associated with the S and I variants of α1-antitrypsin [27,28]. The point mutations that are responsible for these variants have less effect on β-sheet A than does the Z variant. Thus the rates of polymer formation are much lower than for Z α1-antitrypsin, which results in less retention of protein within hepatocytes, milder plasma deficiency and the lack of a clinical phenotype [23]. However, if a mild, slowly polymerizing S or I variant of α1-antitrypsin is inherited along with a rapidly polymerizing Z variant, then the two can interact to form heteropolymers within hepatocytes and inclusions, leading to cirrhosis [28].

The serpinopathies: serpin polymerization in angio-oedema, thrombosis, emphysema and dementia

The phenomenon of loop-sheet polymerization is not restricted to α1-antitrypsin, and has now been reported in other serpin variants to cause disease. Mutants of C1 esterase inhibitor, antithrombin and α1-antichymotrypsin can also destabilize the serpin architecture to form inactive polymers that are associated with angio-oedema, thrombosis and emphysema respectively [29]. The process is most striking in a recently described inclusion body dementia, familial encephalopathy with neuroserpin inclusion bodies (FENIB) [30], that results from polymerization of the neuron-specific serpin, neuroserpin [31]. The dementia has been described in two Caucasian families in the U.S.A. In the first family, 95% of affected individuals presented with dementia between the ages of 45 and 56 years. The second family had an earlier age of onset of symptoms, with epilepsy and progressive decline in cognitive function occurring in the second and third decades of life. Both were characterized by eosinophilic neuronal inclusion bodies in the deeper layers of the cerebral cortex and the substantia nigra. The inclusions were periodic acid-Schiff-positive and diastase-resistant, but were distinctly different from any previously described entity, including Lewy bodies, Pick bodies and Lafora bodies. The inclusion bodies had a striking resemblance to those of Z α1-antitrypsin in the hepatocytes of homozygote subjects with cirrhosis. Biochemical analysis revealed that the inclusions were formed of neuroserpin, and that affected individuals carried point mutations that would destabilize the protein to form polymers. Indeed, one of the mutations was in the same position as in the Siyama variant of α1-antitrypsin, which causes hepatic inclusions and profound plasma deficiency [21]. Structural analysis showed that the mutant neuroserpin had
indeed formed intraneuronal polymers that were identical with those of Z α1-antitrypsin [31].

**Prospects for therapy**

We have shown previously that the polymerization of Z α1-antitrypsin can be blocked by annealing of reactive-loop peptides to β-sheet A [14,18]. Such peptides were 11–13 residues in length and could anneal to other members of the serpin superfamily [18,32]. We have now designed smaller peptides that anneal specifically to Z α1-antitrypsin and block polymerization in vitro. The challenge is to deliver these agents to the endoplasmic reticulum of hepatocytes in order to block the polymerization of Z α1-antitrypsin in vitro and so ameliorate the hepatic inclusions and associated liver disease.

**References**


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