Loop-sheet polymerization: the mechanism of \( \alpha_1 \)-antitrypsin deficiency

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\( \alpha_1 \)-antitrypsin deficiency results from point mutations that distort the structure of the protein to allow a unique protein–protein interaction that we have termed loop-sheet polymerization. Polymers of \( \alpha_1 \)-antitrypsin accumulate within hepatocytes to form inclusion bodies that are associated with juvenile cirrhosis and hepatocellular carcinoma. The lack of circulating protein predisposes the \( \alpha_1 \)-antitrypsin homozygote to emphysema. This polymerization process also occurs in variants of other members of the serine proteinase inhibitor (serpin) superfamily, antithrombin, C1-inhibitor and \( \alpha_1 \)-antichymotrypsin in association with thrombosis, angioedema and chronic obstructive pulmonary disease respectively, and we have recently shown that it underlies a novel inclusion body dementia. Understanding this mechanism of polymerization allows rational drug design to block the protein–protein linkage and so ameliorate the associated disease.

Key words: serine proteinase inhibitor; serpin; loop-sheet polymerization.

Clinical features of \( \alpha_1 \)-antitrypsin deficiency

\( \alpha_1 \)-antitrypsin deficiency was first described as a clinical entity in 1963 by Laurell and Eriksson who noted an absence of the \( \alpha_1 \) band on serum protein electrophoresis. The major function of \( \alpha_1 \)-antitrypsin is to protect the tissues against the enzyme neutrophil elastase (1). Its role in protecting the lungs against proteolytic attack is underscored by the association of plasma deficiency with early onset panlobular emphysema, asthma, bronchiectasis and Wegener’s granulomatosis. Over 70 naturally occurring variants have been described and characterized by their migration on isoelectric focusing gels. The two most common deficiency variants, S and Z, result from point mutations in the \( \alpha_1 \)-antitrypsin gene and make the protein migrate more slowly than normal \( \alpha_1 \)-antitrypsin on isoelectric focusing. \( \alpha_1 \)-antitrypsin (264Glu\( \rightarrow \)Val) is found in up to 28% of Southern Europeans and although it results in plasma \( \alpha_1 \)-antitrypsin levels that are 60% of the M allele it is not associated with any pulmonary sequelae. The \( \alpha_1 \)-antitrypsin deficiency 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 and hepatocellular carcinoma (2).

Alpha\( \alpha_1 \)-antitrypsin deficiency and polymerization

Alpha\( \alpha_1 \)-antitrypsin is the archetypal member of the serine proteinase inhibitor or serpin superfamily. This family includes proteins such as \( \alpha_1 \)-antichymotrypsin, C1 esterase inhibitor, antithrombin and plasminogen activator inhibitor-1 which play important roles in the control of proteinases involved in the inflammatory, complement, coagulation and fibrinolytic cascades (3). Members of the family have a similar structure characterized by three \( \beta \)-sheets (A–C) and nine \( \alpha \)-helices. This scaffold supports an exposed mobile reactive loop that presents a peptide sequence as a pseudosubstrate for the target proteinase. 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 \( \beta \)-sheet A (4). This six-stranded protein bound to its target enzyme is then recognized by hepatic receptors and cleared from the circulation.

The structure of the serpins is very much a dual edged sword in that it is central to their role as effective anti-proteinases but also renders them liable to undergo
conformational change in association with disease. Point mutations can destabilize $\beta$-sheet A to allow incorporation of the loop of another serpin molecule. Sequential loop insertion results in chains of polymers that are retained within the cell of synthesis. This process is best characterized for the severe Z deficiency variant of $\alpha_1$-antitrypsin that results in protein retention in hepatocytes in association with cirrhosis (5). The Z mutation of $\alpha_1$-antitrypsin is at residue P17 (17 residues proximal to the P1 reactive centre) at the head of strand 5 of $\beta$-sheet A and the base of the mobile reactive loop [Fig. 1(a)]. The mutation opens $\beta$-sheet A, thereby favouring the insertion of the reactive loop of a second $\alpha_1$-antitrypsin molecule to form a dimer [Fig. 1(b)]. This can then extend to form polymers [Fig. 1(c)] that tangle in the endoplasmic reticulum of the liver to form inclusion bodies (6). Support for this comes from the
demonstration that Z z1-antitrypsin formed chains of polymers when incubated under physiological conditions. 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 (5). The role of polymerization *in vivo* was clarified by the finding of z1-antitrypsin polymers in inclusion bodies from the livers of Z z1-antitrypsin homozygotes.

Although many z1-antitrypsin deficiency variants have been described, only two other mutants of z1-antitrypsin have similarly been associated with plasma deficiency and hepatic inclusions: z1-antitrypsin Siyama (53Ser->Phe) and z1-antitrypsin Mmalton (52Phe deleted). Both of these mutants also destabilize β-sheet A to allow the formation of loop-sheet polymers *in vivo* (7). The temperature and concentration dependence of polymerization, along with genetic factors (8), may account for the heterogeneity in liver disease amongst individuals who are homozygous for the Z mutation. As z1-antitrypsin is an acute phase protein the 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 hepatocellular damage.

Recent investigations have shown that polymerization also underlies the mild plasma deficiency of the S and I variants of z1-antitrypsin (9). 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 slower than that of Z z1-antitrypsin which 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 z1-antitrypsin is inherited with a rapidly polymerizing Z variant then the two can interact to form heteropolymers within hepatocytes, inclusions and cirrhosis (9).

**Polymerization of serpins underlies chronic obstructive pulmonary disease, thrombosis, angio-oedema and dementia**

The single most important factor in the development of emphysema in patients with z1-antitrypsin deficiency is smoking (10). The combination of anti-proteinase deficiency and cigarette smoke can have a devastating effect on lung function. We have shown that the anti-proteinase screen within the lung can be further reduced in Z z1-antitrypsin homozygotes by the spontaneous formation of loop-sheet polymers (11). This conformational transition inactivates the protein, thereby further reducing the already depleted levels of z1-antitrypsin that are available to protect the lungs. The relationship of intra-pulmonary Z z1-antitrypsin polymers to smoking, infection and rate of decline in lung function in Z homozygotes requires further evaluation in prospective studies.

The phenomenon of loop-sheet polymerization is not restricted to z1-antitrypsin and has now been reported in other serpin variants to cause disease. Mutants of Cl-inhibitor, antithrombin and z1-antichymotrypsin can also destabilize the serpin architecture to form inactive polymers that are associated with angio-oedema, thrombosis and chronic obstructive pulmonary disease respectively (12). The process is most striking in a recently described inclusion body dementia, familial encephalopathy with neuroserpin inclusion bodies (FENIB), that results from polymerization of the neurone-specific serpin, neuroserpin (13). The dementia has been described in two Caucasian families in the United States. In the first family, 95% of affected individuals presented with dementia between the ages of 45 and 56. 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 PAS-positive and diastase-resistant and had a striking resemblance to those of Z z1-antitrypsin in the hepatocytes of homozygotes with cirrhosis. Biochemical analysis revealed that the inclusions were formed of neuroserpin and that affected individuals carried point mutations in a region that was critical to protein function. Structural analysis showed that the mutant neuroserpin had formed intraneuronal polymers that were identical to those of Z z1-antitrypsin (13). Thus therapies that attenuate serpin polymerization may be useful in a whole range of diseases.

**Treatment of patients with z1-antitrypsin deficiency**

This new understanding of the structural basis of z1-antitrypsin deficiency provides a platform for rational drug design to block polymerization *in vivo* and so attenuate the associated liver disease (14). Any therapy that improves secretion from the liver will raise the circulating levels of z1-antitrypsin and so enhance the anti-proteinase protection within the lung. Until that time the prevention of emphysema is better than cure and there is good evidence that many Z z1-antitrypsin homozygotes would develop only mild lung disease if they abstain from smoking (10). The genetic deficiency in the anti-elastase screen may be rectified biochemically by intravenous infusions of z1-antitrypsin. There is registry data to suggest that this therapy may slow the rate of decline in lung function in patients with an FEV1 of 35–49% predicted but this has yet to be proven in randomized, controlled trails (15).

In the meantime, patients with z1-antitrypsin deficiency related emphysema should receive conventional therapy with advice on smoking cessation, trials of bronchodilators and inhaled corticosteroids and, where appropriate, assessment for long-term oxygen therapy and single lung transplantation. The role of lung volume reduction surgery in this group is unclear.
References


