Emphysema is a chronic progressive lung disease characterised by abnormal permanent enlargement of airspaces as a result of destruction of alveolar walls. It most patients develop emphysema as a consequence of smoking but 1–2% of patients with emphysema develop the condition as a result of a genetic deficiency of the plasma proteinase inhibitor \( \alpha_1 \)-antitrypsin. The two common deficiency variants of \( \alpha_1 \)-antitrypsin, S and Z, result from point mutations in the \( \alpha_1 \)-antitrypsin gene and are named on the basis of their slower electrophoretic mobility on isoelectric focusing analysis compared with the normal M allele. S \( \alpha_1 \)-antitrypsin (264Glu→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 Z variant (342Glu→Lys) results in a more severe deficiency that is characterised, in the homozygote, by plasma \( \alpha_1 \)-antitrypsin levels of 10% of the normal M allele and by levels of 60% in the MZ heterozygote (50% from the M allele and 10% from the Z allele). The Z mutation results in the accumulation of \( \alpha_1 \)-antitrypsin in the rough endoplasmic reticulum of the liver (fig 1A) and predisposes the homozygote to juvenile hepatitis, cirrhosis, and hepatocellular carcinoma. Z \( \alpha_1 \)-antitrypsin inclusions are associated with abnormal liver function tests in over 90% of Z homozygotes in the first year of life but only 10–15% of these develop the prolonged cholestatic jaundice that can progress to cirrhosis and the requirement for hepatic transplantation.

The role of \( \alpha_1 \)-antitrypsin is to protect the tissues against enzymatic digestion by neutrophil elastase. The low circulating levels are unable to inhibit this proteinase and predispose the Z homozygote to early onset panlobular emphysema, bronchiectasis, and vasculitis. Alpha1-antitrypsin deficiency related emphysema is predominantly panlobular and basal compared with the centrilobular upper lobe disease seen in smokers. Patients usually present with increasing dyspnoea and weight loss, with cor pulmonale and polycythaemia occurring late in the course of the disease. Chest radiographs and high resolution CT scans typically show bilateral basal emphysema with paucity and pruning of the basal pulmonary vessels (fig 1B). Upper lobe vascularisation is relatively normal and abnormal perfusion radionuclide scans and angiography also show abnormalities with a lower zone distribution. Lung function tests are typical for emphysema with a reduced FEV1/FVC ratio, evidence of air trapping and a low gas transfer factor.

**Structure and function of \( \alpha_1 \)-antitrypsin**

Alpha1-antitrypsin is the archetypal member of the serine proteinase inhibitor or serpin superfamily. Members of the family have
more than 30% sequence homology with 
\( \alpha_1 \)-antitrypsin and share a similar molecular 
architecture\(^{14}\) based on a dominant A \( \beta \)-pleated 
sheet and nine \( \alpha \)-helices. This structure 
supports an exposed, mobile, reactive loop 
peptide that acts as a pseudosubstrate to inhibit 
the target proteinase. In the case of \( \alpha_1 \)- 
antitrypsin the loop presents a methionine 
residue at the P\( \_1 \) site to interact with the 
substrate binding pocket of neutrophil elastase. 
After binding the proteinase is inactivated by 
complex formation and cleared from the 
circulation.\(^{16}\) Crystallographic analysis of the 
serpins has yielded a series of structures which 
illustrate the striking mobility of the reactive 
centre loop, allowing its function to be likened 
to that of a mousetrap (fig 2A). More recently 
our crystal structures of intact 
\( \alpha_1 \)-antitrypsin\(^{17,18}\) have revealed the conformation 
of the loop that is ideal for docking with 
neutrophil elastase (fig 2B) before it is locked 
into a stable complex by the mousetrap mecha-
nism. This mobility of the loop is essential for

Figure 2  (A) The conformational transition of the serine proteinase 
inhibitors (serpins) modelled on antithrombin by J Whisstock and A Lesh 
(Department of Haematology, University of Cambridge). The mechanism 
by which the reactive loop moves to inhibit its target enzyme may be 
 likened to the function of a mousetrap. The top frame shows the active 
circulating serpin identified in the crystal structure of antithrombin\(^{16}\). 
Following binding to the target proteinase (not shown), the loop (shown in 
pink) is presumed to insert into the dominant A \( \beta \)-pleated sheet (shown in 
purple) to form the locking conformation (middle frame) that irreversibly 
binds and inactivates the enzyme. If antithrombin or \( \alpha_1 \)-antitrypsin are 
heated to high temperatures in the presence of stabilising agents, the loop is 
able to pass around the C-sheet spur (in blue) to become fully incorporated 
into the molecule\(^{60}\) to form the latent conformation (lower frame). This 
conformation can be restored to the active moiety by refolding from 
denaturants.\(^{22}\) The position of the Z mutation (P\(_{17}\)) of \( \alpha_1 \)-antitrypsin is 
shown. (B) \( \alpha_1 \)-Antitrypsin (yellow) traps the enzyme neutrophil elastase 
(white) to form an irreversible complex which is then cleared from the 
circulation. Such a complex protects the lungs against uncontrolled 
proteolytic degradation by neutrophil elastase (reproduced from ref 17 with 
permission).
A inhibitory function but may allow aberrant conformations by insertion into the A β-sheet of a second α1-antitrypsin molecule to form a dimer which then extends to form a chain of loop-sheet polymers. Such polymers form following treatment with mild denaturants and upon heating α1-antitrypsin and other serpins at high temperatures.

**Loop-sheet polymers and liver disease**

The Z mutation of α1-antitrypsin is located at residue Phe51 (17 residues proximal to the P reactive centre) at the head of strand 5 of the A β-sheet and the base of the mobile reactive centre loop (fig 2). We predicted that the mutation would act to open the A β-sheet thereby favouring spontaneous reactive loop into β-sheet polymerisation. Indeed, functional studies showed that Z α1-antitrypsin was more unstable and able to polymerise at 37°C under physiological conditions. The rate was accelerated by raising the temperature to 41°C and could be blocked by exogenous reactive loop peptides that compete with the loop for a β-sheet annealing. The role of this phenomenon in vivo was clarified when it was found that polymers with identical microscopic appearance could be isolated from the liver of a Z α1-antitrypsin homozygote. 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 Siiyama ("Ser→Phe")27 and α1-antitrypsin Mnalton ("Phe deleted"). Both of these mutants also formed spontaneous loop-sheet polymers in vivo (fig 3A). The temperature and concentration dependence of polymerisation, along with genetic factors, may account for the heterogeneity in clinical features. As α1-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 hepatic inclusions and the associated hepato-cellular damage.

The phenomenon of loop-sheet polymerisation is not restricted to α1-antitrypsin and has now been reported to account for the deficiency of mutants of C1 inhibitor and antithrombin in patients with angio-oedema and thrombosis, respectively, and has been linked to a deficiency of α1-antichymotrypsin in patients with liver disease and emphysema. It also underlies the plasma deficiency associated with common S variant of α1-antitrypsin. The rate of polymerisation of S α1-antitrypsin is slower than that associated with the Z variant which is compatible with the milder clinical phenotype. The precise linkage underlying the formation of polymers in vivo has been probed by site directed mutagenesis and protein expression in *Xenopus* oocyte system. A point mutation in the region of opening of the A sheet ("Phe→Leu") stabilises the α1-antitrypsin molecule and attenuates the effect of the Z mutation in vitro and in vivo. Moreover, this mutation completely prevents the accumulation of α1-antitrypsin Siiyama in a *Xenopus* oocyte expression system. Recombinant α1-antitrypsin with and without the "Phe→Leu" mutation was expressed in *E coli* and purified to homogeneity. The proteins function normally as inhibitors of neutrophil elastase and were used for crystallographic analysis. Crystals of recombinant protein were grown and two structures of α1-antitrypsin solved to 2.9 Å. These structures and that of Ryu et al provide an explanation for the ready formation of loop-sheet polymers as the reactive loop strand configuration fits easily into the A β-pleated sheet of α1-antitrypsin to form a dimer that can then extend as trimers, tetramers, and so on, to as many as 20 molecules in length (fig 3B). It is these filaments which then tangle within the endoplasmic reticulum of the hepatocyte to form the insoluble inclusions that are characteristic of Z α1-antitrypsin deficiency.

**Loop-sheet polymers and emphysema**

α1-antitrypsin deficiency related emphysema is believed to result from the reduced levels of circulating protease inhibitor being unable to protect the lungs against proteolytic attack. The Z α1-antitrypsin that does escape from the liver into the circulation is less efficient at protecting the tissues from enzyme...

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**Figure 3** (A) Loop-sheet polymers of α1-antitrypsin isolated from the plasma of a Siiyama α1-antitrypsin homozygote. This point mutation favours the formation of long chains of polymers as well as circles (inset). These polymers tangle in the liver to form insoluble inclusions that predispose to liver damage (reproduced from ref 29 with permission). (B) Our recent crystal structure of α1-antitrypsin provides strong support for the loop-sheet linkage being between the β-pleated reactive loop centre and the A β-pleated sheet of the next. These dimers extend to form long chains shown here as alternating yellow, blue and red α1-antitrypsin molecules (reproduced from ref 17 with permission). The prevention of this linkage would block polymerisation and so attenuate the liver disease associated with the Z mutation.
may be inactivated by oxidation of the P1 methionine residue. The demonstration that Z α₁-antitrypsin can undergo a spontaneous conformational transition in association with liver disease raised the possibility that this may also occur within the lung. Indeed, we have detected polymers in broncholavector lavage fluid in two out of five patients with Z α₁-antitrypsin deficiency but not in lavage fluid from 13 MZ, MS, or M α₁-antitrypsin controls. This may have important implications for the pathogenesis of disease as polymerisation obscures the reactive loop of α₁-antitrypsin, rendering the protein inactive as an inhibitor of proteolytic enzymes. Thus, the spontaneous polymerisation of α₁-antitrypsin within the lung will exacerbate the already reduced antiprotease screen, thereby increasing the susceptibility of the tissues to proteolytic attack. The relationship of α₁-antitrypsin polymers to smoking, infection, and rate of decline in lung function remains to be established and other studies are required to determine their effects on neutrophils which are found in increased numbers in the lungs of patients with α₁-antitrypsin deficiency. 10

Treatment

This new understanding of the structural basis of α₁-antitrypsin deficiency provides a platform for rational drug design to block polymerisation in vivo and so attenuate the associated liver disease. Any treatment that improves secretion on the physical and inhibitory properties of α₁-antitrypsin with the P1 methionine mutated to a valine residue. The demonstration that Z α₁-antitrypsin lacks the target areas which are most suitable for resection.

Conclusion

Z α₁-antitrypsin deficiency results from the accumulation of loop-sheet polymers within the endoplasmic reticulum of the hepatocyte. These polymers also form within the lung where they inactivate α₁-antitrypsin, thereby further reducing the antiprotease screen that protects the tissues against proteolytic attack. Recent advances in understanding the transition from monomeric to polymeric protein raises the prospect of rational drug design to treat the deficiency and provides a useful paradigm for other conformational diseases such as the prion diseases, amyloidosis and Alzheimer’s disease. 10

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