PROTEIN TRANSPORT

Z and the insoluble answer

Richard N. Sifers

ACCUMULATION in the liver of the Z variant of human α_1-antitrypsin can lead to cirrhosis, but the structural identity of the accumulated protein has been unknown. On page 605 of this issue[1], Lomas *et al.* unveil a totally unexpected finding — the Z variant forms insoluble homopolymers in the endoplasmic reticulum of the liver, and does so by a well-defined mechanism.

Human α_1-antitrypsin (AAT) is a single, folded polypeptide that inhibits the action of serine proteases. Although the macromolecule is one of the main components of serum, its predominant physiological role is to prevent degradation of elastin fibres in the lung. It accomplishes this task by forming a tight complex with elastase, a protease secreted from neutrophils in the lung, which ultimately inhibits its hydrolytic activity[2]. Heritable forms of AAT deficiency are most often associated with the synthesis of a variant macromolecule, which bears a mutation in the polypeptide[3] that somehow hinders its ultimate secretion from hepatocytes[4] (the principal site of synthesis). Decreased secretion lowers the level of serum AAT, and also the total elastase inhibitory activity in the lung. The resulting degradation of elastin fibres is implicated in emphysema[2].

A subset of AAT variants accumulates within the endoplasmic reticulum of the liver, and is associated with cirrhosis[5]. One such variant, designated Z, contains a glutamate-to-lysine substitution at residue 342 (ref. 2). Because of its frequency in the population, and the severity of its impaired secretion, the Z variant has served as a prototype for analysing intrahepatic protein retention and accumulation.

Discovery of the Z-variant accumulation mechanism resulted from analysis of a structural feature shared by this and other members of a class of serine protease inhibitors known as serpins[6]. Briefly, the reactive inhibitory site of serpins centres on two amino acids in a 14-amino-acid loop at the surface of the folded polypeptide[6,7]. For AAT and other inhibitory members of this family, amino-terminal peptides of the reactive centre loop are inserted into a gap in the major structural feature of the folded macromolecule, β-pleated sheet A (see Fig. 2 of the paper of Lomas *et al.*, page 606). This 'stressed' conformation is required for the reactive centre to function as a substrate to react with and inhibit targeted proteases. Indeed, the absence of inhibitory activity for some members of the serpin family may result from the substitution of amino acids within the reactive centre loop, thereby preventing its partial insertion into sheet A and the formation of the stressed conformation[7].

A clue to the mechanism of Z accumulation in liver was reported by Carrell *et al.*[8] last year, following their observation that under mild denaturing conditions the entire reactive centre-loop peptide of an intact inhibitor, such as AAT, could 'lock' into sheet A. This event is akin to the major structural rearrangement of AAT when the loop has been relaxed by proteolytic cleavage at its reactive inhibitory site and actually becomes strand 4 of the sheet. As described in the new report[1], incubation of the purified, secreted form of the normal M variant under these conditions led to its unexpected polymerization *in vitro*. Polymerization between macromolecules pre-bound with a synthetic peptide homologous to the reactive centre loop, which had inserted into sheet A, did not occur. This and subsequent analyses implied that polymerization under these conditions results from the stable insertion of the mobile reactive centre loop of one molecule into sheet A of another. Increased polymerization of the Z variant (that is, spontaneous polymerization under nondenaturing conditions) was then observed *in vitro*, as the authors had predicted. This prediction was based on the ability of the glutamate-to-lysine substitution at the hinge region of the reactive centre loop to prevent its insertion into sheet A, thereby leaving it in an 'opened' conformation ready to accept the appropriate loop from another Z molecule.

Confirmation that loop-sheep polymerization of the Z variant does occur in liver endoplasmic reticulum comes from the striking electron micrographs showing tangled polymers of the macromolecule purified from human liver biopsies (see Fig. 3, page 606). Furthermore, the morphology and physical characteristics of the liver-derived Z polymers are identical to those of the serum-derived protein polymerized *in vitro*. The authors did not test the effect of nonspecific peptides on *in vitro* polymerization of either the M or Z variant, but their hypothesis of loop-sheet polymerization is borne out by physical and biochemical analyses.

The tendency of the secreted Z variant to undergo spontaneous aggregation[9] led most, if not all, researchers in the field to conclude that a similar mechanism was responsible for its retention and accumulation in the liver endoplasmic reticulum. But this conclusion was never quite secure because it could not explain why the macromolecule isolated from liver inclusion bodies could exhibit full protease inhibition[10]. Conceivably, a randomly aggregated molecule would be grossly misfolded and therefore display little, if any, inhibitory activity after isolation. The evidence for the accumulation of polymerized Z protein now satisfies this discrepancy.

In terms of protein retention in the endoplasmic reticulum, it is reasonable to conclude that polymerization of the Z variant could dramatically hinder its export from that compartment, as the

Out of the frying pan

THE Saharan silver ant *Cataglyphis bombycina* treads a narrow line between heat exhaustion and being eaten by lizards. As R. Wehner *et al.* demonstrate on page 586 of this issue, the ants prefer to run the risk of the former than face the near-certainty of the latter. Most desert creatures, lizards included, seek shade when surface temperature exceed about 45 °C. In response, the ants emerge to forage only when temperatures rise above 46.5 °C, and can tolerate temperatures of up to about 53.6 °C. Confined to this narrow thermal 'window', they operate at the limits of their tolerance and adopt additional strategies to minimize the heat burden. The ant pictured here, for example, is resting on a stalk of grass where the ambient temperature is slightly less intense than that at ground level. H. G.