Molecular medicine

Therapy by instant evolution

from Robin Carrell

EVOLUTION has modified and refined the structure and function of proteins. An understanding of how this has happened makes feasible the prospect of artificially inducing similar changes to create specifically designed variants of human proteins. As a starter, on page 77 of this issue¹, Rosenberg *et al.* (Chiron Research Laboratories) report the use of recombinant DNA technology and yeast to replace the amino acid at the reactive centre of human α_1 -antitrypsin, a natural inhibitor of protease enzymes. The mutant α_1 -antitrypsin retains the specificity of the natural inhibitor, but promises to provide more effective protection to tissues during inflammatory stress. Of particular interest is its potential to prevent the development of the degenerative lung disease, emphysema, but the independent production of related mutants of α_1 -antitrypsin by two other genetic engineering companies, Transgene, Strasbourg (M.A. Courtney *et al.* *Nature*, in the press) and Biogen, Cambridge, Massachusetts (D. Schindler, personal communication) attests to much wider therapeutic implications.

This focus of activity, follows from the realization² that α_1 -antitrypsin is one of a family of serine protease inhibitors, the serpins, each of whose function is critically determined by a single amino acid at the reactive centre. These protease inhibitors have specific roles in the control of the proteolytic cascades responsible for immune reactions, for coagulation, and for other responses to inflammation. Since it is the aberrant activation of these cascades that underlies many disease processes, there is great medical interest in the possibility of specific intervention by the use of engineered inhibitors.

α_1 -Antitrypsin is the archetype³ of the serpin family which includes the plasma proteins: antithrombin, α_1 -antichymotrypsin, C₁-inhibitor and α_2 -antiplasmin⁴. The structural homologies of the family, together with recent crystallographic studies⁵ of α_1 -antitrypsin, indicate that the serpins all share the same highly-ordered globular structure. A feature of this structure is the exposure of the reactive centre as a loop on the exterior of the molecule. This loop is believed to fix the reactive centre in a conformation that makes it an ideal substrate for the target protease. The inhibitor and its target protease thus form a tight 1:1 complex which can be rapidly recognized and removed from the circulation.

The specificity of each inhibitor is critically dependent on a putative cleavage site at the P₁ residue (see the figure) of the reactive centre of the inhibitor. This was initially indicated by comparing the reac-

tive centre of α_1 -antitrypsin, which has methionine at P₁ and is primarily an inhibitor of elastase, with that of antithrombin, an inhibitor of thrombin which has arginine at P₁. Unequivocal evidence that the P₁ residue was responsible for the specificity came from an unusual experiment of nature⁶: a child from Pittsburgh was found to have a bleeding disorder associated with a new genetic variant of α_1 -

Serpin	P ₂	P ₁	P ₁ '	P ₂ '	P ₃ '	P ₄ '	Target
α_1 -Antitrypsin	Pro-MET-Ser-Ile-Pro-Pro-						Elastase
Valine mutant	Pro-VAL-Ser-Ile-Pro-Pro-						Elastase
Pittsburgh mutant	Pro-ARG-Ser-Ile-Pro-Pro-						Thrombin
Antithrombin	Gly-ARG-Ser-Leu-Asn-Pro-						Thrombin
α_1 -Antichymotrypsin	Leu-LEU-Ser-Ala-Leu-Val						Chymase
Mouse contrapsin	Arg-LYS-Ala-Ile-Leu-Pro-						'Trypsin'
C ₁ -inhibitor	Ala-ARG-Thr-Leu-Leu-Val						Kallikrein

Reactive site sequences of natural human serine proteinase inhibitors (serpins) and two mutants of α_1 -antitrypsin. Cleavage putatively occurs at the P₁-P₁' bond with a specificity of inhibition that is clearly related to the P₁ residue (C₁-inhibitor sequence is a personal communication from J. Travis).

antitrypsin, in which the reactive centre methionine had been replaced by an arginine; as predicted, the mutant α_1 -antitrypsin had lost the ability to inhibit elastase but had become a highly effective inhibitor of thrombin.

The general applicability of this dependence on the P₁ residue was recently demonstrated by Hill and colleagues⁷, from a comparison of the amino acid sequences deduced from the cDNA of mouse and human serpins. Their most remarkable conclusion concerned human α_1 -antichymotrypsinogen and mouse contrapsin. These two proteins have a similar sequence but the human inhibitor has a leucine at P₁ and hence inhibits chymotryptic-like proteases, whereas the mouse inhibitor, with a P₁ lysine, has no antichymotryptic activity but predictably is as an efficient inhibitor of trypsin.

Evolution, in several experiments of her own, has thus demonstrated how to prepare specific inhibitors. Rosenberg *et al.*¹ have applied these lessons to the production of a mutant α_1 -antitrypsin in which the P₁ methionine has been replaced by a valine. Their choice follows from the fact that about one in a thousand northern Europeans has a severe genetic deficiency of α_1 -antitrypsin and is consequently susceptible to cumulative damage to the elastic tissue of the lungs, which results in emphysema. In principle, the defences of susceptible individuals could be improved by intermittent transfusions of α_1 -antitrypsin. Genetic engineers, anticipating this need, have taken the opportunity to produce a modified form of α_1 -antitrypsin that should inhibit elastase

and so provide improved protection.

The risk of damage to the lungs in both normal individuals and those deficient in α_1 -antitrypsin is heightened by the susceptibility of α_1 -antitrypsin to inactivation by oxidants released by leukocytes or present in cigarette smoke⁴. There is strong evidence that this is due to the oxidation of the P₁ methionine to methionine sulphoxide, with consequent loss of effectiveness as an inhibitor of leukocyte elastase. Improved protection might be provided by the replacement of the P₁ methionine of α_1 -antitrypsin with a residue resistant to oxidation^{3,4} — valine seems a logical choice⁸ for an inhibitor of leukocyte elastase. *In vitro* tests of the mutant that fits that bill support those predictions. Before the material is ready for therapeutic use, several questions will need an answer. Does it matter that the mutant protein, as produced by yeast, does not have oligosaccharide sidechains? Antigenicity will probably not be a problem, but how important is it that the mutant inhibitor is likely to be less soluble and long-lived than the natural form? This will be more of a problem if the material is to be administered by transfusions than if it can be directed to the lungs as an aerosol. A greater problem will lie in the assessment and marketing of an agent whose protective effect, with regards to emphysema, is measurable over years rather than days.

Other related mutants that are at the testing stage may have more immediate medical applications. It can be predicted that the P₁ arginine mutant^{1,6} will be an effective medium-term anticoagulant for individuals with either a genetic or functional deficiency of antithrombin. Here, a shortened half-life due to lack of glycosylation may prove an advantage by increasing precision in the day-by-day control of anticoagulation.

In a wider sense, the new papers underline the rapid growth in the practical applications of molecular biology. When we suggested the possible production of the valine α_1 -antitrypsin³ it was done so almost tongue-in-cheek. Yet 18 months later it has been achieved. The speed with which molecular biology is growing has turned protein chemistry into an adventure rather than threatening it, and has converted the academic exercises of correlating protein structure with function into matters of direct, practical importance. □

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