NOTICE 86

PROTEASE AND ANTI-PROTEASE ENGINEERING

LONDON—A protein engineering workshop at Biotech 86 here, chaired by Brian S. Hartley of London's Centre for Biotechnology at Imperial College, offered particular insight into the strides made recently with two related classes of proteins—the proteases and their inhibitors.

Hartley himself offered an overview of significant recent work in engineering industrial enzymes—increasing oxidation resistance, substrate range, and pH stability.

The alkaline proteases or subtilisins are the most economically significant of industrial enzymes, with a world market of more than 500 metric tons a year worth more than \$150 million—mainly for enzyme detergents and food processing. These compounds are prey to oxidation of methionine at position 222, near the active site. J. A. Wells and his colleagues (in *Design, Construction and Properties of Novel Protein Molecules*, D.M. Blow ed., The Royal Society, 1986) showed that substitution of Ala-222, Ser-222, and Leu-222 all provide complete resistance to 1 M hydrogen peroxide, with specific activities ranging from 0.3 percent (Lys-222) to 50 percent (Ala-222) of the wild type's. (These activities, said Hartley, "would have been difficult to predict by modeling.") An oxidation-resistant subtilisin mutant raises the possibility of a new line of commercial products, including a bleaching enzyme detergent.

Inserting disulfide bridges is the key to increasing temperature and pH stability. Hartley drew some lessons from his own work—especially techniques for inserting stabilizing bridges without distorting the molecule's three-dimensional structure. For example, he suggested substituting cysteines for pairs of hydrogen-bonded serines (or for some methionines whose methyl group touches a glycine α -carbon). Hartley also cited recent work by L. J. Perry and R. Wetzel (*Biochemistry* 25:733, 1986), who inserted disulfide bridges in T4 lysozyme, increasing its half-life at 67°C and pH 8 from 11 minutes to over 6 hours.

While speakers like Peter Murray-Rust (Glaxo Group Research Ltd.) insisted on the primacy of X-ray crystallography in protein engineering, others, like Barry Robson of the Victoria University of Manchester's Epsilon protein engineering project, maintained that engineers can go far by drawing analogies from the actions of compounds with similar first-order

The protein engineering cycle, adapted from Steffen Petersen, manager of Novo Industri's Department of Protein Engineering. Getting through the cycle takes about 6.3 man-years.

structures.

Michael Courtney, assistant scientific director of Transgene, illustrated this point with a report on the opposite side of the protease coin—specifically on engineering of α_1 -antitrypsin, an inhibitor of serine proteases, especially the neutrophil elastase found in the lungs. Hereditary α_1 -antitrypsin deficiency is responsible for about 1 percent of emphysema cases. Courtney estimated that there are about 50,000 victims of such a deficiency in the U.S. alone. Treating them would require about a metric ton of α_1 -antitrypsin a year.

The other 99 percent of emphysemas are overwhelmingly linked to cigarette smoking, apparently because activated neutrophils in smokers' lungs release reactive oxygen species, which combine with the Met-358 at the center of the active site. Thus, an oxidation-resistant α_1 -antitrypsin could be therapeutic for smoking-induced emphysema.

Courtney and co-workers produced a series of substitutions at that

position. They found that Leu-358 was 82 percent as active as the natural form and, like Wells's substituted subtilisin, strongly resisted oxidation.

Other substitutions produced interesting results. While both the natural Met-358 and the engineered Leu-358 inhibitors act against both elastase and another protease, cathepsin G, the Phe-358 mutant bound only to the latter.

The Arg-358 α_1 -antitrypsin shows no inhibition of neutrophil elastase, but it does act potently against thrombin—and, unlike antitrypsin III, it requires no cofactors. This mutant could thus be useful as an anticoagulant. The Arg-358 mutant also inhibits contact-phase proteases like kallikrein and factor XII, which are involved in coagulation, kinin formation, and inflammation.

Courtney pointed out that Transgene's *Escherichia coli* production system produces its α_1 -antitrypsin mutants in an unglycosylated form, which decreases its half-life by a factor of 2 or 3. —Douglas McCormick