BIO/TECHNOLOGY CONFERENCE

THE REAL PROBLEMS WITH PROTEIN DESIGN

NEW ORLEANS—The protein design session at this year's *Bio/Technology* conference made clear the serious gap between theory and data in relating amino acid sequence to protein folding, conformation, and activity. As a result, except in a very limited number of cases, predictability does not yet exist. To truly tap the potential of protein engineering will require bridging this gap.

Jonathan King (MIT, Cambridge, MA), Lila Gierasch (University of Delaware, Newark), and Rainier Jaenicke (University of Regensburg, F.R.G.) focused on the dynamic properties of polypeptide chains in achieving their final conformation. Gierasch, making ingenious use of classical physical chemical techniques, has analyzed the actual behavior of synthetic signal peptides at aqueous: membrane interfaces. The take-home lesson from her work is that these sequences do not encode the static property of maintaining a particular conformation. Rather, they confer the ability to switch between a subset of conformations in different environments.

King emphasized the need to identify the residue-residue interactions critical in determining folding pathways. These residues, his work shows, are often different from those specifying catalytic sites. Two interesting practical lessons emerged from his experiments. One is that protein aggregation and the formation of inclusion bodies can occur as off-pathway dead-ends in the native folding pathway. The second is that this can often be prevented by simply lowering the temperature a few degrees.

Jaenicke, one of the few people who has succeeded in refolding oligomeric proteins, showed that oligomer formation and refolding are generally competing pathways. Thus, success is largely a matter of whether the rate limiting step is refolding, chain association, or later conformational changes—again an area in which theory falls far short of data.

In the case of modifying the properties of a native protein by judicious amino acid substitutions, results have been somewhat more encouraging—particularly for proteins whose crystal structures are known. But even here surprises are not uncommon. Charles Craik (University of California, San Francisco) likened some of his results to replacing a round tire with a square one and discovering the car now runs backwards. His group has been studying the very well characterized enzyme trypsin, which catalyzes the hydrolysis of lysine or arginine amides and esters. The proposed catalytic mechanism involves a triad of residues—His^{57}, Asp^{102}, and Ser^{195}. When Craik replaced Asp^{102} by an asparagine, the mutant enzyme had less than 0.01 percent activity at pH 7.0.

However, at pH 10 the mutant trypsin gave significant catalysis. The crystal structures of the two molecules are nearly identical, except that His^{57} occurs in two conformations in the mutant. Thus Asp^{102} is required both for catalytic activity at physiological pH, and also to correctly position His^{57}. Perhaps one reason catalysis is so difficult to model accurately is that it involves multiple states of the enzyme, and substitutions may affect these states differently.

—**Harvey Bialy**