VIRGINIA W. CORNISH

PROBING ENZYME COMPLEXITY

Biochemistry is moving beyond individual enzymes to tackle more global phenomena

THE PANTHEON STOOD IN ROME for almost 1,300 years before a structure its equal was built on Roman soil. The knowledge required to erect such an enormous dome without interior support was lost with the fall of the Roman Empire. During the Renaissance, architects studied the ancient Roman buildings in their midst and were inspired to imitate and even surpass them. Bramante designed and began construction of St. Peter's Basilica, and Michelangelo completed the famous dome.

At the turn of the 20th century, scientists developed techniques for purifying proteins from cells and began characterizing proteins structurally and mechanistically. Proteins were shown to be responsible for cellular metabolism. A low-resolution structure of hemoglobin was constructed from X-ray diffraction data.

Now, as we start the 21st century, we take all of this for granted. The 3-D structures of more than 10,000 proteins have been determined to high resolution. Soon, it will be possible to predict the amino acid sequence of every human protein using the DNA sequence information generated by the Human Genome Project. The mechanisms of several enzymes have been characterized in exquisite detail. This work has defined how we talk about proteins and has provided the framework for rational drug design in the pharmaceutical industry.

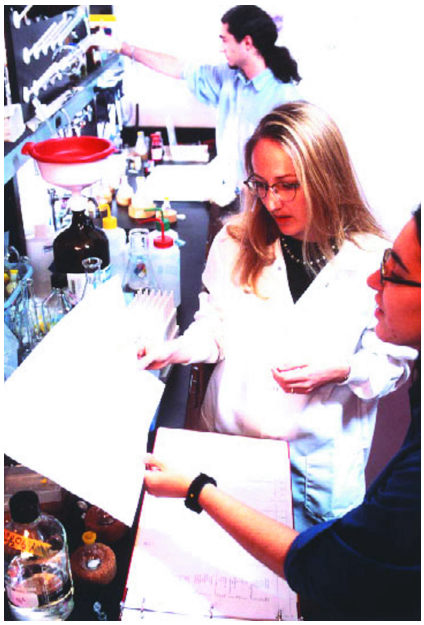
Initially, it was hoped that protein function could be explained largely based on the amino acids lining the active site, which directly contact the substrate. The ability to mutate any amino acid in the protein sequence has shown, however, that protein function is much more complex. For example, a comparison of the 3-D structures of lactate and malate dehydrogenase would seem to suggest that the substrate specificities of these two enzymes can be rationalized by a key active site residue that interacts with the substrate—Gln^{102} and Arg^{81}, respectively. Converting Gln^{102} to Arg in lactate dehydrogenase, in fact, swaps the substrate specificity. However, the opposite mutation in malate dehydrogenase simply results in an enzyme with poor substrate specificity. This and similar experiments with other enzymes suggest that the protein is not merely a rigid scaffold; rather, the entire protein is actively involved in catalysis.

For the most part, enzyme function has been analyzed using transition-state theory and our knowledge of the reactivity of organic small molecules. As our understanding of enzyme function improves, however, we may find that these theories do not tell the whole story. Recently, there has been a move to consider how quantum mechanics and dynamics affect protein activity, and new experimental approaches are being developed to address these hypotheses. Proteins are being studied at the single-molecule level. Crystallography is being adapted to observe intermediates along a reaction pathway, and methods are being developed to measure protein dynamics using nuclear magnetic resonance spectroscopy.

Seeing the power of natural enzymes, our impulse is to try to design enzymes with novel functions. These design efforts serve as a test of our understanding of enzyme mechanism and provide reagents for the chemical industry and for research. As suggested by the dehydrogenase example, it has proven difficult to modify specificity, much less function, by making one or two changes in the amino acid sequence. Advances in molecular biology have made it possible to generate billions of protein variants simultaneously. Now the challenge is to design selections for molecular recognition and catalysis that will allow us to "evolve" enzymes with novel properties.

Mechanistic studies began with small, single-domain proteins that utilize acid-base chemistry. More recently, progress has been made studying enzymes that catalyze reactions using radical and organometallic chemistry. We are beginning to tackle proteins that historically have proven difficult, such as membrane proteins and large protein complexes. Recently, for example, the first high-resolution structure of an ion channel protein was reported. As structural and mechanistic studies improve our understanding of protein function and genetics, and genome-sequencing projects allow us to piece together entire biochemical pathways, the question will increasingly change from how a protein functions in isolation to how proteins function as ensembles inside the cell, what is necessary for a robust signal-transduction cascade, which factors control the resilience of a genetic switch, and how the cell cycle is coordinated.

Just as basic chemical principles shaped our understanding of protein function, these same principles should help us understand the importance of redundancy, compartmentalization, and other factors to the integrity of biochemical pathways. Biochemistry will move from the study of local interactions to more global phenomena. Having begun to master the building, we will tackle the city.



WADING THROUGH **Cornish and her students use a combination of synthetic chemistry and molecular genetics to develop selection strategies for identifying enzymes from large pools of proteins.**

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