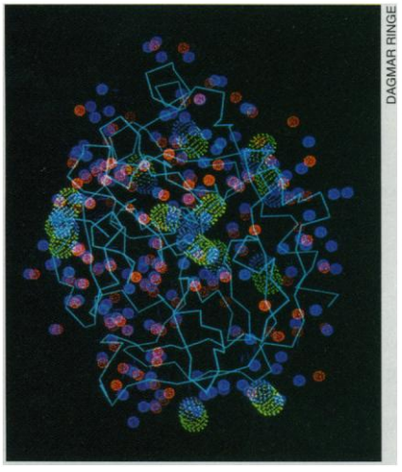
X-RAY CRYSTALLOGRAPHY

Proteins and Organic Solvents Make an Eye-Opening Mix

Protein chemists can't be blamed for thinking that proteins and organic solvents are like oil and water. They simply don't mix well. Most proteins can't dissolve in organic solvents like ethanol and acetone—and thus can't form crystals in such liquids—and the few that do dissolve are usually biologically inactive because the solvents disrupt the protein's native three-dimensional structure. "The lore in biochemistry is that organic solvents are harmful," explains Brandeis biochemist Dagmar Ringe.

It's not surprising then that investigators trying to resolve a protein's structure by x-ray crystallography do so by irradiating crystals mounted in a water solution. Now, however, Ringe and her colleagues have shown for the first time that valid structures can indeed be obtained from crystals residing in organic solvents. What's more, their data appear to provide a variety of useful information about proteins that cannot be obtained from crystals looked at in water.



DAGMAR RINGE

Crystal clear. In this elastase structure, water molecules from a water medium are in blue, while those from an organic medium are red. The yellow/blue balls are acetonitrile molecules.

Among its benefits, the new technique provides a better look at the location of the water molecules that determine a protein's shape. Moreover, the experiments show where individual solvent molecules bind to the crystal, a clue to regions on the protein that could be useful in the rational design of more effective drugs that work by binding to and inhibiting the action of proteins. Potential targets include, for example, the clotting protein thrombin and elastase, an enzyme whose excessive activity contributes to the lung degeneration of emphysema and cystic fibrosis. The location of bound solvent molecules also provides, for the first time, an experimental check on recently developed computational methods that predict how regions of a protein will react to other probe molecules.

The research, which was partially described in the 15 September *Proceedings of the National Academy of Science* and detailed more recently by Ringe at a structural biology meeting,* has already prompted excitement, mixed with wariness, among researchers who know how difficult rational drug design is. "It allows you to intelligently look outside the active region of a protein. It will provide a very valuable tool to understand the target surface which you want drugs to bind to," says Joseph Rosa, director of protein engineering at Biogen Inc. of Cambridge, Massachusetts. "It's a fair step from here to drug design," cautions Tony Kossiakoff, Rosa's counterpart at Genentech in South San Francisco, "but it's important data to have."

Ringe was inspired to undertake the x-ray crystallography studies by recent work performed by Alexander Klibanov, a chemist at the Massachusetts Institute of Technology and a co-worker on this research. Klibanov and a few other chemists have in the past few years started a small new field called nonaqueous enzymology in which they study the properties, often unusual, of enzymes that can survive in solutions of organic solvent. Such enzymes might prove useful in industry and so Klibanov wanted to understand why proteins in organic solvents behave differently. The obvious question was whether they fold into a different shape depending on the medium, and so he turned to Ringe's lab to attempt the x-ray crystallography.

In their first endeavor, Ringe and Klibanov tackled subtilisin Carlsberg, a protein-splitting enzyme. They first had to grow crystals of the enzyme in an aqueous solution. They then removed the crystals and washed them repeatedly in acetonitrile, an organic solvent commonly used in laundry detergents, to get rid of all the water. Finally they took a single crystal and mounted it in a quartz tube containing acetonitrile and took x-ray diffraction data. To their surprise, images of that crystal revealed almost no structural changes, indicating there must be other explanations for the different enzymatic properties observed in organic solvents.

The group then began looking at what their data said about water. Water associated with crystallized proteins is normally of two types: "structural water," consisting of individual molecules that attach firmly to protein molecules in a crystal and a more loosely ordered surrounding shell. Even though they had chosen acetonitrile for its ability to displace water, the solvent did not dislodge all of it; structural analysis of the data showed nearly 100 water molecules were still affixed firmly to the protein molecule, only 20 fewer than found in a crystal mounted in water.

Other solvents also displaced some structural water, often different molecules for each solvent, but there always remained a core group of water molecules that seemed impossible to dislodge. And that has intrigued many protein experts. "It certainly is extremely interesting to see the water molecules that persist when the protein is placed in organic solvents," says Irwin Kuntz of the University of California, San Francisco. The reason is that the results suggest that not all structural water is created equal; certain molecules appear more crucial than others to the protein's overall shape.

In contrast to the basic scientists, the biotech industry may be more concerned with those few water molecules that were displaced by solvent molecules rather than the many that stayed put. For example, the initial experiments showed that 12 molecules of acetonitrile bound to the enzyme, four of them displacing structural water molecules. And a series of experiments involving different solvents and elastase disclosed that each solvent displaces its own distinct pattern of water molecules.

Such information might one day help lead to the construction of new drugs that inhibit elastase, suggests Ringe. By analyzing the structural features that determine where each type of solvent molecule sticks, drug designers could synthesize small molecules that bind tightly to the same sites. (That's how some of the current computational methods are being used.) And by linking these molecules, including one that inhibits the protein's biologically active region, drug designers might build so-called hydra-headed drugs that are more specific—and thus have fewer side-effects—than traditional single-target drugs.

That at least is the hope—and it's not unreasonable. Through other research methods, Biogen has already synthesized a two-headed drug. It's an anticlotting agent based on a protein found in leeches called hirudin. If the company had tried to look at thrombin in organic solvents, speculates Rosa, they might have developed their drug more quickly. But, he says, "it was just the sort of experiment no one believed could work." Good thing Klibanov and Ringe thought otherwise.

–John Travis

*The symposium, "Structural Biology: The Shape of Things to Come," was sponsored by *Nature* and held in Boston on 11 and 12 November.