Straightening Out the Protein Folding Puzzle

A new paper suggests an old model is wrong—and the subject is too hot for protein chemists to touch

IF YOU'RE NOT A BIOCHEMIST, THEN YOU'RE probably not up on the latest details of protein folding. In fact, you might think the question of how a linear protein folds itself into the right three-dimensional shape is a bit arcane. If so, you may be surprised to find that it's such a sticky and controversial subject that, when a paper proposing a modification of the most important model of that process appears (see p. 1386 of this issue of *Science*), few in the field were willing to go on record with their opinions as to whether the new paper is correct.

Why are the stakes so high? One reason is that the payoff isn't merely theoretical. Of course, the scientific rewards are great: understanding how proteins function requires understanding how they assume their complex three-dimensional shapes. But beyond that, in the world of genetic engineering, protein folding is a key issue. "Biotechnology can produce almost any protein artificially, but often the protein does not fold up itself, and the biotechnicians find themselves in a mess," says Thomas Creighton of European Molecular Biological Laboratories in Heidelberg, Germany. "So suddenly protein folding has become a very important commercial concern."

Creighton is the researcher who, almost 20 years ago, provided one of the first breakthroughs in understanding protein folding—and it is his model that the current paper purports to modify in some important respects. Creighton didn't start out to study protein folding, he recalls; it wasn't then considered such a hot topic. On the contrary, he says, he was a crystallographer. "But I couldn't grow crystals. My career was disappearing, and I was desperate."

To overcome the frustrations of crystallography Creighton seized on the problem of understanding how the small protein called bovine pancreatic trypsin inhibitor (BPTI)—which blocks the protein-cleaving enzyme trypsin—folds itself into a functional, three-dimensional protein.

The folding of BPTI—and other proteins—isn't a simple thing. It may involve portions of the molecule coiling into helices or into an accordion-like pleated sheet; still other regions may contain loops, bends, or kinks. All of these configurations are joined by various types of bonds between the amino acids in the protein: hydrogen bonds, noncovalent interactions, and bridges between sulfur atoms in the amino acid cysteine. In spite of this complexity, protein chemists originally believed proteins took on their shapes more or less automatically. "We knew that proteins had to fold to be active, but we thought the newly synthesized protein just folded up by itself to its most stable conformation," says Creighton. "We now know that's a simplification."

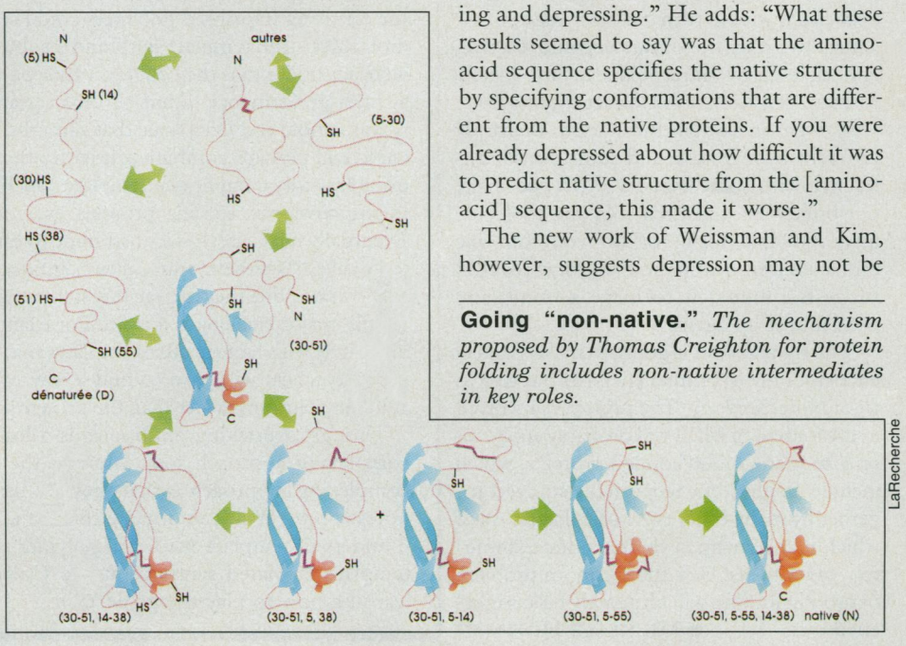
Creighton's work was an advance over that simplification on two levels. First, he showed that the process didn't "just happen." Creighton offered what biochemist David Goldenberg of the University of Utah, a former postdoc in Creighton's lab who continues to work on BPTI, calls "one of the first and clearest demonstrations that a specific pathway [for folding] did exist and that in folding, the protein passes through specific intermediates on its way to the final state." Second, Creighton showed what those intermediates were for BPTI.

And that's the starting point for the current controversy: Jonathan Weissman and Peter Kim of MIT and the Howard Hughes Medical Institute of the Whitehead Institute, authors of the *Science* paper, offer data indicating that the most common intermediates are different from those Creighton found. While Creighton agrees with the new data, he does not agree with the mechanism proposed by Kim and Weissman for protein folding. At issue is the relative importance of the various intermediates. The question is which of the two types of intermediates—the "native" type (whose pattern of bonds reflects the bonds in the final molecule) or the "non-native" type (containing unusual bonds that aren't present in the functioning protein)—ultimately directs the folding pattern.

Creighton's landmark work pointed toward the importance of the non-native molecules. Not that he hit on his conclusion directly; it required a clever strategy to find out what the intermediates were. Researchers who had tried to study protein folding in the test tube found molecules in one of two states: completely unfolded or completely folded. Creighton, however, devised strategies for "trapping" the intermediates. Before the folding could be completed, the intermediates were reacted with compounds that prevent further bond formation—and hence further folding.

When he analyzed the trapped intermediates, Creighton found results he calls "a bit surprising." BPTI, in its finished form, has three sulfur-sulfur bonds that help hold the molecule together. The simplest assumption would be that the bonds form sequentially to yield the final protein, but Creighton found that the bonds were not made "one, two, three—not in that order." Instead, of the intermediates with two sulfur bonds, there was an abundance of those with non-native bonds. That conclusion, says Goldenberg, was simultaneously "striking and depressing." He adds: "What these results seemed to say was that the amino-acid sequence specifies the native structure by specifying conformations that are different from the native proteins. If you were already depressed about how difficult it was to predict native structure from the [amino-acid] sequence, this made it worse."

The new work of Weissman and Kim, however, suggests depression may not be



Going "non-native." *The mechanism proposed by Thomas Creighton for protein folding includes non-native intermediates in key roles.*

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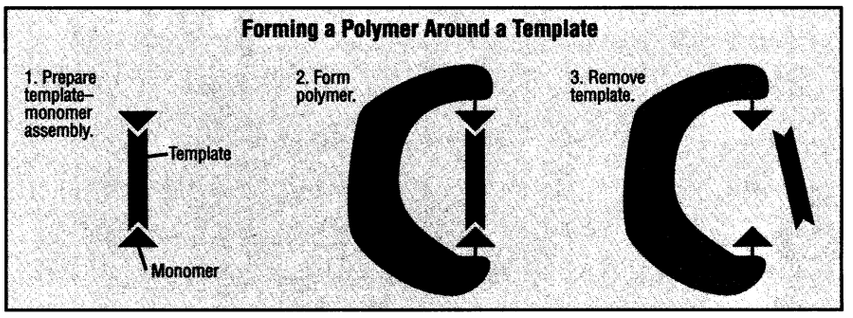
justified. Their findings suggest that during most of the folding process, intermediates stay closer to the native conformation than would have been predicted by Creighton's results. "The basic finding in our paper is that we have a predominance of native intermediates," says Kim. Using updated methods for separating the intermediates, the MIT team found both native and non-native versions of the intermediate containing two sulfur-sulfur bonds. But, in contrast to Creighton's work, the native version predominated.

Although Creighton and Kim disagree over how to interpret these new results, they do agree that the most difficult step in the process is going from the two-bond to the three-bond stage of the folded BPTI molecule. In Creighton's proposal, the native intermediate and its native conformation cause the difficulty in forming the final bond. As the intermediates approach the final configuration, he argues, they lose their flexibility—and the atoms that need to be juxtaposed to form the last bond can't reach each other, a situation Goldenberg compares to that of "two people [on a crowded bus] each facing the window but who would like to face each other to talk. The constraints all around them prohibit them from facing each other to interact more productively." Creighton argues that the non-native molecules are more flexible and "ideally suited for folding," making it possible to pass quickly into the final folded configuration.

Kim and Weissman don't think the problem is lack of flexibility. They believe the difficulty is that the sulfurs in the two-bond molecule are buried and hence nonreactive. "To proceed from there," says Kim, "we propose that you need to unfold the protein in such a way as to turn it almost inside out." Then, says Kim, the sulfurs become reactive, and non-native intermediates are briefly formed, but they don't direct folding.

Creighton says his mechanism is sharply different from Kim's, and sums up the difference with some asperity. "Do you make the bond first and then fold the molecule into the final form, or do you fold the molecule into nearly the final form [as Kim suggests], unfold it to make a bond, and then fold it up again?"

The right answer is going to make a difference not only to protein chemists but also to all of biotechnology. The principles involved in the folding of BPTI will probably apply not only to many sulfur-bonded proteins, which include insulin, but to proteins stitched together by many other types of linkages. All of which leaves many in the scientific community eager to see how the stubborn problem of protein folding will ultimately be straightened out. ■ MICHELLE HOFFMAN



Making Choosy Molecules

Shake and bake. Heat and beat. Mix and stir. The element of truth in these often-invoked caricatures of how chemists synthesize new molecules inspires many researchers to seek more precise control over molecular construction. For one community of chemists, the goal is to replace shake and bake with the more skilled-sounding "mold and cast" strategy (officially known as template-mediated synthesis), which proponents say could result in the production of agents for purifying chemicals and proteins or even detecting viruses. In the 11 September *Journal of the American Chemical Society*, polymer chemist Pradeep K. Dhal and chemical engineer Frances H. Arnold of the California Institute of Technology describe a promising new approach.

The general idea of template-mediated synthesis is to form polymer molecules around chemical templates, imprinting the polymers with precise chemical motifs that later will serve to bind, or recognize, target molecules that match the template. Progress has been slow, partly due to the need to find firm yet reversible ways of holding the polymer building blocks, called monomers, in place on the template as the polymer is synthesized.

Instead of depending on covalent bonds, hydrogen bonds, or electrostatic forces to hold the monomers in place on the templates—as other workers have done—Arnold and Dhal rely on the ability of certain metal atoms to form complexes with organic molecules. Critical to their imprinting tactic are copper-containing monomers and small template molecules that form complexes with the metal atoms. When the chemists mix these ingredients, the copper atoms serve as attachment points, binding a monomer to each end of a template molecule. Next, the researchers link these prearranged monomers to form a pocket around the template. They then cross-link the pockets and remove the template molecules from the resulting rigid blue (due to the copper) polymeric network.

"The molecular imprinting technique allows us to create a kind of mask, or cast, of the molecule we're interested in recognizing," says Arnold. She and Dhal found that the resulting imprinted polymers could selectively bind, and thereby distinguish, two molecules—the template itself and a subtle variation of it—whose chemical structures were so similar that they couldn't be separated by a powerful sorting technique known as high-performance liquid chromatography.

But Arnold and Dhal hope that's just the beginning. By honing their metal complexing tactic and perhaps combining it with other techniques, they hope to imprint polymers using far more complicated templates, such as protein molecules. The resulting polymers might recognize specific proteins even in a complex milieu such as blood serum. Eventually the Caltech scientists hope to build protein-binding polymers choosy enough to be called "synthetic antibodies." Antibodies remain the world champions of molecular recognition, but they are far less stable than synthetic polymers.

Still, major challenges remain. For example, imprinted polymers might intertwine so intimately with large protein templates that the templates would be hard to remove. Also, the researchers will have to find a way of doing their template-mediated synthesis in aqueous solutions rather than the protein-unfriendly organic solvents that they now use.

Though overtaking antibodies is a longshot, other workers agree that the metal complexing approach may improve the odds. "What is interesting in this [metal complexing] approach is that they are using a new type of binding site interaction," notes Günter Wulff, an organic chemist at the University of Düsseldorf and one of the founders of template-mediated polymer synthesis. Ken Shea, who has been pursuing template-mediated synthesis at the University of California, Irvine, agrees: "We're learning how to play the game." ■ IVAN AMATO