Hydrogen bonds shown to play 'conserved' role in protein folding

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By changing individual atoms in key places in proteins, Duke University chemists have found new evidence for the importance of comparatively weak "hydrogen bonds" in enabling stringlike proteins to fold into the maximally stable shape they need to assume their roles as biological workhorses. Such protein folding immediately after proteins are synthesized is central to their function in the cell.

Although they are much weaker than the preeminent "covalent" chemical bonds that bind atoms in biological molecules, hydrogen bonds are known to occur at key points along the central "backbone" structures of all folded proteins. The hydrogen bonds are created by attractions between adjacent hydrogen and oxygen atoms that are sandwiched into the molecular framework.

How big a role hydrogen bonds actually play in protein folding has been a controversial scientific question, according to Duke associate chemistry professor Michael Fitzgerald. "There's been an ongoing debate about the exact role of those hydrogen bonds," he said in an interview. "Are they really super-important, or are they really negligible?"

Fitzgerald, his graduate student Min Wang and his former graduate student Thomas Wales helped address that question in an effort that took years of work.

One by one, they slightly "mutated" the normal arrangement of atoms in proteins to effectively delete hydrogen bonds at five analogous positions

along the structural "backbones" of two different protein molecules that fold in the same pattern. Then they analyzed how each deletion affected the stability of the protein. "Stability" means how low energy, or "relaxed," the protein was.

"We deleted each hydrogen bond and then measured how relaxed the protein was afterwards," Fitzgerald said. "It turns out we destabilized the structure in each case. So the relaxed state was not so relaxed any more. The proteins were more stable with those hydrogen bonds.

"Those bonds seemed to clearly play a role in protein folding. And what we were able to uncover in this work is that this role may be highly conserved in a protein fold."

With Wang as the first author, the three chemists described their results in a paper published online on Friday, Feb. 10, 2006 in the journal Proceedings of the National Academy of Sciences. Their research was funded by the National Institutes of Health.

Their paper reported that deletions at each position on one folded protein, known as Arc, had the identical effect at the analogous position on the other protein, called CopG. "Remarkably, the five paired analogs with...mutations at structurally equivalent positions were destabilized to exactly the same degree," the authors wrote.

Obtaining equivalent results in five different places on two different molecules suggests that the thermodynamics, or energy, of such hydrogen bonding interactions "are conserved in a protein fold," their paper added. The word "conserved" means that those could be fundamental features of the folding state, Fitzgerald said.

The Proceedings of the National Academy of Sciences paper also notes that "the generality of our results to other protein folds remains to be

explored. However, the results of our studies on Arc and CopG suggest that the conservation of backbone hydrogen-bond thermodynamics in a protein fold may be an important general principle of protein folding reactions."

While scientists have well-characterized the three-dimensional structures of some 30,000 different kinds of protein molecules to date, they have also determined that those 30,000 proteins fold in a "remarkably" smaller 800 different ways, the Duke researchers wrote.

"We said, 'What is it about these 800 structures?'" Fitzgerald said. "The physical and chemical properties that define them are largely different except for one common feature: the hydrogen bonds in the polypeptide backbone which is a constant in all proteins."

When proteins fold, their architectures readjust in a way analogous to the way humans do when they're relaxed, he said. In the process most proteins change from a "spaghetti-like" form into something much more organized.

"Mother Nature has figured out the way to get proteins from bowls of spaghetti into the form of little biological machines," he quipped. "They like to be folded into well-defined three dimensional structures. That lets Mother Nature carry out all the reactions she needs to. And the energy of this folded form is much lower than the energy of the unfolded form.

"When we relax, our inclination is to be in a low-energy on-the-couch state. And proteins, with all their chemical functionalities, are also designed to hang in a certain way when folded.

"So we've uncovered at the level of individual chemical interactions what helps the protein hang, if you will, in its relaxed state."

The Duke researchers' experiments were "technically challenging," Fitzgerald said. As a first step, his former graduate student Wales had to make sure they could synthesize appropriately "unnatural" mutant forms of the Arc and CopG proteins in the test tube.

While scientists commonly use automated recombinant DNA technology to engineer proteins, that technique doesn't work very well to introduce unnatural mutant sequences that could selectively delete individual hydrogen bonds while keeping other parts of the architecture intact, Fitzgerald said.

These "single-atom" mutations were purposefully designed be subtle enough to measure effects on the proteins without totally unraveling their folded structures.

Any protein structural changes could be detected with an optical technique called far-ultraviolet circular dichroism spectroscopy. Protein stabilities were tested by measuring their responses to quanidinium chloride, a chemical used to make proteins unfold, or "denature."

After Wales graduated with a Ph.D. in 2003, it fell to Wang, now about to receive her own doctorate, to do the bulk for the laboratory work. "We asked a very simple question, but the experiments were not only very time-consuming but also required careful attention to analytical detail," said Fitzgerald. "And she got it done."

Source: Duke University