Material Peptide

A piece of yeast protein becomes a building block for scientists

By ELIZABETH PENNISI

Four years ago, Shuguang Zhang was a typical molecular biologist. Questions about the replication of genetic material occupied most of his waking hours and all of his lab work at the Massachusetts Institute of Technology.

Now, thanks to his son's Lego set, he finds himself more and more drawn to materials science.

Those plastic building blocks provide never-ending entertainment for children. Pegs jutting out of each block's top fit into indentations on the undersides of other blocks, making possible the construction of towers and structures of all sorts.

Zhang and his MIT colleagues recently discovered that nature has created its own set of molecular Legos and hidden them away in a peculiar yeast protein. Each of these "Legos" consists of a string of 16 amino acids, which together form a type of molecule known as a peptide. Peptides serve as the building blocks for proteins.

From the biologist's viewpoint, the newly identified peptide may provide clues about certain disease processes, says Zhang. These peptide molecules interlock with each other to create structures resembling the proteinaceous plaques found in the brains of people with Alzheimer's disease and in the livers of people with cirrhosis.

Peptides that readily fit together to form bigger structures might also help explain how life originated. Perhaps, Zhang speculates, collections of peptides like these assembled into primordial, cell-like compartments.

But the Lego peptide also shows potential in disciplines traditionally far removed from biology. Increasingly, materials scientists seek molecules that they can piece into ever more complex and useful entities. This approach leads to new materials for all sorts of structural, mechanical, and biomedical applications.

"The overall objective is to do things from the molecular scale up," explains Stephen A. Fossey, a materials engineer at the U.S. Army Natick (Mass.) Research, Development, and Engineering Center. Some materials researchers have begun to eye biological molecules in their quest for easy-to-use building blocks and for guidance on how these starting molecules should fit together (SN: 2/23/91, p.119; 4/20/91, p.246; 5/16/92, p.328).

Like most biologists, however, Zhang had focused on how proteins and other molecules function in living cells. Several times during the past four years, he found himself at apparent dead ends in his investigations of the yeast protein and its unusual peptide fragment. But when viewed instead from the perspective of materials science, those same efforts represent an exciting success story.

"It's a very interesting observation," comments David A. Tirrell, a polymer chemist at the University of Massachusetts at Amherst. He, too, studies peptides, but primarily with the goal of making new materials. "By our standards, this [peptide] is a relatively complicated polypeptide chain. It has relevance both to the design of synthetic materials that self-assemble and to [biological questions]."

With the newly discovered peptide or other molecules like it, scientists might one day build "natural" drug-delivery platforms or other implantable devices whose degradation would leave no foreign matter behind in the body, suggests Alexander Rich, a biophysicist at MIT.

Zhang's divergence from the field of molecular biology began with the discovery of the protein that contains this amino acid sequence. Working with Rich, he was looking for proteins that bind to a special form of DNA, called Z-DNA.

Z-DNA twists in the opposite direction from the typical, right-handed double helix, known as B-DNA. The left-handed conformation requires quite a bit of extra energy to keep from reverting to a right-handed twist, notes Zhang.

The discovery of left-handed DNA some 14 years ago puzzled scientists, who until then thought DNA had only one conformation. Soon afterward, researchers noticed that certain peptides and metal ions readily convert B-DNA to the left-handed form.

Then, in 1987 and again in 1989, scientists reported that the weirdly twisted DNA might play a role when genetic material switches places on chromosomes. Observations that several proteins seem to prefer to bind to this odd DNA instead of normal DNA bolstered this notion. Why would these proteins exist if Z-DNA didn't serve some purpose?

So Rich had charged Zhang with the task of searching for a yeast protein that binds to Z-DNA. With such a protein in hand, Rich theorized, they could put their knowledge of yeast genetics to work by studying where Z-DNA forms during the recombination of chromosomes. In the October 1992 EMBO JOURNAL, Zhang and his colleagues reported the discovery of such a protein, which Zhang named zuotin ("zuo" means left in Chinese). When they later deleted the genetic code for zuotin from yeast cells, they found that the cells barely survived and grew very slowly, says Rich.

Zhang *et al.*/PNAS

Molecular building blocks self-assemble into a membrane that stains red.

After Zhang and Rich determined the sequence of amino acids in zuotin, they noticed an odd section located 305 amino acids into the 433-amino-acid protein. "It was the regularity [of the sequence] that seemed so unusual," Rich recalls. In that 16-amino-acid stretch, every other amino acid was an alanine. Sandwiched between each pair of alanines was an amino acid with either positive or negative charge. Alanine molecules, which are not charged, tend to turn away from water, while the charged amino acids are attracted to water.

A computer program designed to predict the structure of proteins based on their amino acid sequence concluded that this stretch should twist like a tele-

phone cord into what's called an alpha helix. But in June 1990, when Zhang made the peptide and then analyzed its twist with an instrument called a spectropolarimeter, he realized that it looked nothing like a telephone cord. "I was very disappointed," he recalls.

Frustrated, Zhang started to pencil in all the atoms sticking out of the amino acids in this peptide. As he did this, he began to realize that rather than forming an alpha helix, peptides with this amino acid sequence can arrange into a different protein structure known as a beta sheet. In beta sheets, peptides line up in parallel zigzags called beta strands. Typically, weak interactions between hydrogen atoms of adjacent strands stabilize the strands into a pleated sheet. In this peptide, the zigzags place alanine only at the tops of a zig and all the charged amino acids at the bottom of a zag.

He also realized that the peptide is self-complementary: The order of amino acids is such that a second copy readily layered over the first because side chains made up of positive amino acids pair off with negative side chains.

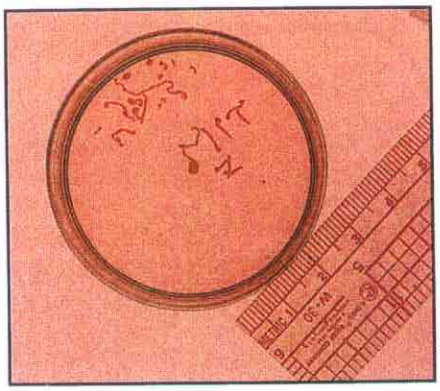
At first Zhang thought he had uncovered a way to investigate protein replication. If one part of the peptide could act as a template to form a second copy, then couldn't this stretch have generated some of life's first proteins? Despite many attempts, he was unable to get the peptide to make copies faster with the template than without.

Then, while playing with his son one evening, Zhang was struck by the similarity between his peptide and his son's Legos. If the self-complementarity of Legos — with bumps that fit snugly into matching indentations — makes stable structures possible, then the same should hold for multiple copies of the peptide, he thought. Could he create molecular building blocks of macroscopic dimensions by stacking his Lego-like peptides in a staggered arrangement?

Around the same time, MIT graduate student Todd C. Holmes made a surprising observation. As part of his study of Alzheimer's disease, he was adding peptides — such as those that form the plaques characteristic of this disease — to cells growing in laboratory dishes. But when he introduced a solution of Zhang's peptides to these cell cultures, he saw something Zhang had never witnessed: The peptides seemed to coagulate into a thin piece of plastic wrap. "That's unusual," notes Rich. "It's such a small molecule, yet it self-assembles to make a large structure."

Holmes could tell this membrane existed because under his microscope it deflected a little light and made visible fringe patterns. Zhang had used a different type of microscope that did not show these patterns.

Scientists "write" letters by squirting dissolved peptide into a salt solution.



Zhang/MIT

As a result of these insights, Zhang began to see the peptide in a much different light — less as part of a Z-DNA-binding protein with particular biological properties and more as a new kind of material. He started to look more closely at the formation and properties of the peptide membrane.

Under the scanning electron microscope, the membrane resembles high-density felt, he says. Something about Holmes' cell culture had caused the peptides to precipitate out of the solution and to form 10- to 20-nanometer-wide fibers that then twisted together. Zhang and his colleagues narrowed down the cause of this precipitation to a sodium phosphate salt used in the cell culture. They also discovered they could make the membrane visible with a stain called Congo red.

They then evaluated many kinds of salts and observed that the peptide membrane formed most easily in lithium salt solutions, with sodium and potassium providing less conducive environments. But divalent salts — those with magnesium or calcium, which readily give up two electrons when they become charged in solutions — wreaked havoc, causing the peptides to clump into visible globs.

The membrane has proved quite tough, Zhang, Rich, and their colleagues report in the April 15 PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES. It does not disintegrate when the researchers add enzymes that normally chop up proteins. Nor does it fall apart when exposed to heat or to an acidic or alkaline liquid.

When Zhang and his co-workers take the membrane out of the salt solution, it shrivels like dried fruit while remaining structurally intact. The salt seems to prompt the formation of strong ionic bonds between the oppositely charged amino acids in adjacent peptides. Such charge-charge interaction is something materials scientists have not yet really investigated in their quest to make self-assembling peptides, says Tirrell.

Like Legos, Zhang's peptides seem to link up into long strands. But unlike Legos, which are stiff, these flexible peptide chains tend to twist and make other kinds of links with adjacent strands, so strands eventually interweave to form a membrane, Zhang explains.

He does not know whether the salts become part of the membrane or just encourage complementary sections of adjacent peptides to bond. And Tirrell suggests that no one can really know how the peptides bond to one another until X-ray diffraction studies reveal the true crystal structure of the linked peptides.

Zhang also experimented with variations on the peptide. Its 16 amino acids actually consist of a sequence of eight that repeats; he made peptides containing different multiples of that sequence. A peptide only eight amino acids long proved too short to form stable macroscopic structures, says Zhang. Peptides with 12 amino acids worked better, but not as well as those with 16. And peptides with 40 or 50 amino acids simply bent back upon themselves, forming molecular hairpins. "The length of the peptide is very important," Zhang concludes.

Completely taken by this peptide, called EAK16, Zhang decided to see just what he could do with it. He made a solution of peptides, added the red dye, and then carefully squirted it into salt water using a pipette with a 100-micron-wide opening. As the solution hit the salt, the peptides formed a thin red thread. With a little practice, Zhang found he could "write" with the peptide, and he spelled out "MIT" with it. "The hard one is the M because it has several bends," he says.

He has begun searching through protein databases for other peptide building blocks. "There are many, many repeats that have the potential to do the same thing [as EAK16]," he says.

Likewise, Rich believes EAK16 may be a model for other self-assembling sequences. These sequences need not include the same amino acids, just ones with similar charges and water-loving properties. "[EAK16] is only one member of what can become a very large class of compounds," says Rich. Several amino acids exist that could fill each position, and protein engineers have already begun making synthetic amino acids not found in nature. "There are many variants we could make," he adds.

For Rich, EAK16's self-assembling properties represent one of nature's many surprises and a novel diversion from his quest to understand Z-DNA's role in cells. But for Zhang, the experience may mean much more.

"I'm a molecular biologist, so this whole thing is completely out of character for me," says Zhang. "I am now turning more and more to materials science." □