Bottled Enzymes Make Complex Chemicals

Genetic engineering, which took barely a decade to pervade every biological discipline from biochemistry to systematics, now promises to revolutionize the organic chemistry lab.

With the help of molecular biology, researchers may one day make complex substances in a bottle in much the same way nature creates them in a living cell — and more efficiently, says A. Ian Scott, an organic chemist at Texas A&M University in College Station.

Scott seeks to produce large quantities of substances that organisms typically make slowly in microgram amounts. But like the organisms, he plans to rely on enzymes to do the work for him. Last week, at an American Chemical Society national meeting in Washington, D.C., Scott described his progress using sets of enzymes to make vitamin B12, penicillin, and anticancer alkaloids derived from the rosy periwinkle (SN: 5/30/92, p.366).

"As far as I know, this is the first application of genetic engineering toward trying to understand the biosynthesis of these complicated molecules," says Ronald Breslow, a bioorganic chemist at Columbia University in New York City. "There's lots of molecules that organic chemists work on that [this approach] could apply to."

For years chemists have counted on commercially available enzymes to speed chemical reactions. But these researchers typically incorporate just one or two enzymes at key points in a synthesis, or they use enzymes to get a particular starting material, says Mark A. Findeis, a bioorganic chemist at Targe-Tech, Inc., in Meriden, Conn.

"We're going for the much more difficult enzymes; many of these had been inaccessible until now," says Scott. "And the novelty is putting a lot of these together, like a cocktail."

Scott has spent decades trying to learn how cells make vitamin B12. To do this he had to extract enzymes from living cells. "Often we couldn't get enough to catalyze the reaction," he recalls.

But after others identified the genes that encode the B12-yielding enzymes, Scott began using genetically altered bacteria to mass-produce the enzymes he needs. Molecular biologists insert these genes into bacteria, which then churn out enough enzyme to allow Scott and his colleagues to piece together the pathway's 15 or so steps leading to basic B12.

To determine the order of enzymes in this chemical cascade, Scott and his colleagues use a powerful nuclear magnetic resonance (NMR) spectroscopic technique. By starting with precursor compounds containing a heavy carbon isotope, the researchers can monitor the intermediate products produced.

Thus they can observe whether a particular enzyme modifies a chemical to form the next intermediate along the cascade, Scott explains. Enzymes are quite fussy about the chemicals they work on, typically modifying one intermediate and not others along the cascade. So for each step, the scientists just try each enzyme and monitor with NMR for any chemical changes in that intermediate.

"We've gotten to the point where we can go to the seventh step [in the B12 synthesis]," he adds.

At first Scott's team tried the enzymes one at a time, waiting for each to finish its chemical transformation before adding the next one down the pathway. But one day, when pressed for time, Scott added several enzymes at once to the starting materials. To his surprise, the reaction proceeded smoothly.

"Now we put five or six [enzymes] in at a time and leave the bottle for a few hours," he says. He hopes eventually to put all the enzymes involved in B12 synthesis in a single flask at once.

Already he has demonstrated one-flask synthesis with penicillin. This approach cannot compete with commercial production, but it lets researchers try to improve upon this drug by making mutant enzymes and seeing what they produce, Scott says.

In other experiments, he and his colleagues are identifying the periwinkle's enzymes for making medically useful alkaloids. Lacking the genes for these enzymes, the researchers extract a key enzyme, determine its amino acid sequence, and from that sequence reconstruct a gene.

"This kind of effort is requiring the use of a whole passel of enzymes for specific purposes," notes Findeis. "It's beginning to get away from traditional organic chemistry."

That shift is changing how chemists view other disciplines. "Rather than stand apart from molecular biology, we feel it is so much a part of our life now," says Scott. *— E. Pennisi*

Clues to the brain's knowledge systems

The peculiar inability of a 70-year-old woman to name animals has led scientists to propose that the brain harbors separate knowledge systems, one visual and the other verbal or language-based, for different categories of living and inanimate things, such as animals and household objects. Moreover, testing of the woman suggests that verbal knowledge about the physical attributes of members of a category exists apart from verbal knowledge about their other properties, according to neurologist John Hart Jr. and psychologist Barry Gordon, both of Johns Hopkins University in Baltimore.

The woman, referred to as K.R. by the researchers, suffered brain damage in both temporal lobes as well as patches of damage elsewhere.

K.R. showed no ability to name animals portrayed in pictures. Nor could she name animals based on recordings of easily recognizable sounds they make. Yet pictures and sounds associated with other living or inanimate things posed no problems for her.

K.R. also lacked the ability to identify physical attributes of animals, such as color or number of legs. For instance, when asked about the color of elephants, she claimed they are orange. However, she retained functional knowledge about animals, such as the realization that elephants are not kept as pets.

Further tests indicated that K.R. specifically lacked verbal knowledge about the physical attributes of animals. Her naming of animals did not improve with the aid of physical attributes as clues, such as a picture of an udder following a picture of a cow. But clues involving nonverbal perceptions of an animal, such as the sound "moo," significantly boosted K.R.'s naming accuracy.

She also correctly matched pictures of animal bodies to the appropriate heads and knew when an animal's picture portrayed the wrong color. But when asked, she still could not say which color belonged on, say, a lion pictured as gray.

A verbal system in K.R.'s brain must have mediated her intact knowledge of the functional properties of animals, while a separate visual system allowed her to recognize the physical attributes of animals in pictures, Hart and Gordon contend in the Sept. 3 NATURE.

The verbal system contains "subdomains" of knowledge, they add, since K.R. could not identify visual physical attributes of an animal from its name, but could recall other information about the same animal from its name.

Areas throughout the brain probably coordinate these knowledge systems, the researchers conclude. *— B. Bower*