The Chemistry of Life at the Margins

Researchers are hoping to find exquisitely sensitive industrial catalysts in the chemistry of enzymes from organisms that live in some of Earth's harshest environments

As far as we know, life never took hold on the baking crust of Venus or on the bitter surface of Mars. But creatures with alien sorts of biochemistries are now turning up in the very harshest environments on earth—in the depths of oil wells, in arctic ice, in desiccating salt marshes, and above steaming heat vents under the ocean. "We are now looking for organisms in places where, 15 years ago, we assumed they couldn't possibly survive," says University of California, Berkeley, biochemical engineer Douglas Clark. Today, Clark and other chemists are taking apart these "extremophiles" and examining the molecules that make them run. Their goal: to put these molecules to use in down-to-earth laboratory and industrial processes.

This interdisciplinary group of chemists, biochemists, and biochemical engineers is most interested in the enzymes that build, assemble, tear apart, and otherwise moderate metabolism in these organisms. "Man has yet to devise a catalyst that's as selective as an enzyme," says biochemical engineer Jonathan Dordick of the University of Iowa. But enzymes from ordinary bugs are too delicate to be of use in most industrial processes—they would cook or fall apart in the high heat or toxic solvents required. That's why chemists are looking to super-hardy enzymes, which they have dubbed "extremozymes," that combine exquisite precision with the toughness needed to survive on nature's margins.

Some researchers are finding natural extremozymes with new and useful properties, says John Baross of the University of Washington, who scours deep-sea vents to collect organisms supplying many of the extraordinary enzymes of interest. Other researchers are seeking to reveal the molecular tricks that endow the enzymes with the power to survive extreme heat, cold, pressure, or salinity. Ultimately, they hope to be able to exploit these tricks to outdo nature and make enzymes that can perform in environments, such as industrial solvents, that would be deadly to even the hardiest of creatures. "We would be utilizing nature's ability to have a very high selectivity but turning it into a system that's purely manmade," says Dordick, one of some 160 extremozyme researchers who compared notes at a recent meeting in Washington, D.C., sponsored by the National Science Foundation and the National Institute of Standards and Technology.

If they succeed, the potential payoff could be enormous, as one successful example shows. Taq polymerase, a natural enzyme from the heat-loving bacterium *Thermus aquaticus*, is the workhorse of the polymerase chain reaction (PCR). It has revolutionized entire areas of biochemistry while racking up hundreds of millions of dollars in sales. And taq polymerase isn't even considered a true extremozyme; although it is hardy enough to survive the repeated heating and cooling steps of standard PCR, it breaks down at the "cool" temperature of 80°C. Now researchers are experimenting with an accelerated version of PCR using hardier enzymes from bacteria adapted to deep-sea vents, where pressure allows the water temperature to reach 120°C.

Heat lover. Bacterium *(right)* extracted from tube worms *(above)* living in scalding hot water by a heat vent in the northeast Pacific.

Novel biochemistry

Biochemists are learning about tricks of extremozymes that go beyond PCR into the realm of entirely novel chemical processes. Take recent findings by Francine Perler of New England Biolabs in Beverly, Massachusetts. Perler found that not only could she control the activity of heat-loving enzymes by changing the temperature, but the temperature also influenced the enzyme to change its activity in a process never before seen in biochemistry.

Perler says she stumbled across this new mechanism while attempting to clone the gene for an enzyme from a heat-vent organism in *Escherichia coli*. She ended up getting enzymes she didn't ask for—nucleic acid–eating endonucleases—that killed the hosts. Perler says she noticed that the genetic sequence she thought coded for her enzyme—a polymerase—seemed to code for a much larger protein instead. She suspected that the large protein was a precursor that split into fragments including the endonucleases.

The changes happened too fast for her to see the precursor, she says, so she spliced the genetic code for just two segments—the endonucleases—into the genes for another protein in a cool-water bacterium. When she coaxed the organism to make this protein at 12°C, she found that, indeed, what came out was a large, inactive protein. As she heated the protein gradually to 90°—a temperature closer to that of the organism's heat-vent home—she observed the large protein split apart, spontaneously producing the endonucleases and three other fragments that spliced themselves together to form a polymerase. "The thing people are excited about is the fact that protein splicing occurs," she says. While several groups have recently reported evidence for fragments splicing themselves together to form enzymes, she says, this is the first time anyone has isolated the precursor and controlled the splicing.

When all the splicing is done, how do the enzymes survive in near-boiling water? Several researchers are starting to gather hints. So far, they have found that extremozymes guard their secrets well. "Nature hasn't taken one tactic for stabilizing a protein and used it to a great extent, but rather, has taken a lot of different things that collectively contribute to the stability," says biochemical engineer Robert Kelly of North Carolina State University. The challenge is to tease out which of the many unique properties of extremozymes impart their special stability.

Researchers have found a few structures that they believe could be important. Kelly has found that a number of high-temperature enzymes have fewer of the amino acids most vulnerable to heat. And Michael Adams of the University of Georgia noted in a recent paper that some extremozymes use a "salt bridge"—a zipperlike series of ionic bonds through the interior that makes the structure difficult to unravel.

Other researchers believe there will be some significant overlaps between the mech-

anisms that enable an extremozyme to resist, say, heat and pressure. "I think there are common mechanisms by which these things survive in extreme conditions," says Berkeley's Clark. Last year, Clark started off on a search for a connection between resistance to pressure and resistance to temperature. He found that enzymes from organisms living in deep-sea vents, where boiling heat and crushing pressure combine, become more stable and more active when the pressure is increased. He then took enzymes from organisms adapted to high temperatures but not to high pressure and found that, surprisingly, these, too gained stability as he added pressure.

The pressure link gives a clue to which of several tricks the proteins are using for heat stability. The enzymes have not only the salt bridges noted by Adams but also a densely packed interior held together by water-excluding hydrophobic bonds. Since pressure destabilizes salt bridges and stabilizes the hydrophobic bonds, says Clark, the enzymes that become more stable under pressure are probably relying on the hydrophobic bonds for high temperature stability.

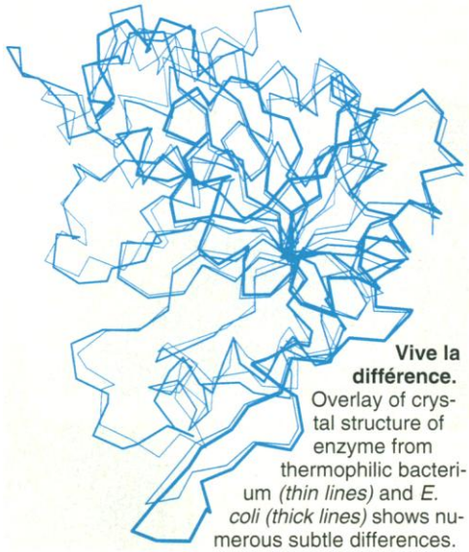
While Clark is looking for clues to thermal stability in the mechanisms organisms use to survive other adverse conditions, biochemist Gregory Petsko of Brandeis University in Waltham, Massachusetts, is trying another route: recreating evolution. He notes that the current genetic evidence indicates heat-loving bacteria appear to be the most primitive organisms known; as life spread to cooler regions, these primitive organisms adapted to temperate climes. But because modern cool-water bacteria differ from their heat-loving ancestors in a hundred subtle ways, Petsko says "I have no idea which of these differences are important and which are there by random genetic drift." To get around the problem, Petsko is trying to make heat-loving organisms that are adapted to cooler temperatures with a speeded-up version of natural selection. "We wanted to do what nature did," he says, but much faster.

He creates thousands of randomly mutated versions of a stretch of the bacterial DNA by amplifying it with an intentionally sloppy version of PCR. He then equips the heat-loving organisms with slightly altered copies of the original DNA, puts them in a cool environment, and sifts for survivors. Most of them, still adapted to heat vents, fail to grow, but a few develop a mutation that helps them adapt to the cool water.

Since the cool-water mutants are only one mutation away from the original, the structural change this imparts should have something to do with temperature stability. So far, Petsko and colleagues have managed to create a cold-water mutant version of an enzyme essential for synthesis of the amino acid leucine in bacteria. Its structure, says Petsko, hints at the nature of the adaptation: Cold water turns the heat-adapted enzyme to an ineffective, rigid brick. The cold-adapted mutant stays flexible.

The ultimate solution

Eventually, Petsko, Clark, and others hope they can learn enough of the extremozymes' tricks to modify enzymes to withstand not only high temperatures but environments even more hostile to life, such as found in the organic solvents used in many industrial processes. Enzymes would be far more precise and efficient than the synthetic catalysts used for most of these processes. But natural enzymes generally don't survive in organic solvents, and the few that do become tens of thousands of times less active.



Vive la différence. Overlay of crystal structure of enzyme from thermophilic bacterium *(thin lines)* and *E. coli (thick lines)* shows numerous subtle differences.

There's also another incentive to make enzymes that can withstand organic solvents: Solvents make some enzymes choosier about the targets they bind to, says Massachusetts Institute of Technology chemist Alexander Klibanov. Last year Klibanov found that about a dozen enzymes capable of choosing between left- and right-handed versions of a target molecule in water become more selective in organic solvents. Selecting between mirror-image molecules is important for preparing many pharmaceuticals, since right- and left-handed versions often have profoundly different biological effects. One reason for the change in selectivity, Klibanov says, is that solvents can make enzymes more or less flexible, and this flexibility in turn can control the way the enzymes select their targets. But there's a cost to this increased selectivity: the enzymes retain only about 0.001% of the activity they had in water. To improve that level of activity, Dordick is looking at extremozymes from another unusual medium—extremely salty water.

The organisms he studies, halophilic bacteria, come from the Dead Sea or from the salt marshes at the south end of the San Francisco Bay, where the water has 30 times the salinity of the sea. These creatures die if taken out of their salt-laden environments, and their enzymes require very salty water to function. Dordick is studying these enzymes with the goal of using their survival tactics to modify other enzymes to work in solvents. "There's no natural environment that mimics an organic solvent," he says. But salt, like solvents, dehydrates enzymes. Salt ions stick to the enzymes, shielding them from water.

Dordick says in the past 6 months he has found that salt can enable both special halophilic and ordinary enzymes to survive in solvents. He learned this, he says, by taking a halophilic enzyme together with some salt water, freeze-drying it, then adding the powdered salt-enzyme combination to an organic solvent. The enzyme came back to life, he says, functioning almost as well as it did in its natural saltwater environment.

As a next step, Dordick says he decided to see whether he could get a similar effect with a freshwater enzyme. He took an enzyme called a protease, which ordinarily splits proteins into peptides, put it in salt water, freeze-dried it to a salty powder, and added it to various industrial solvents. "I found the activity increased dramatically," he says, by a factor of 20,000—making it a fifth as active as it had been in water.

Despite these promising results, California Institute of Technology biochemical engineer Frances Arnold argues that studying natural extremozymes, while interesting, is not the best way to adapt enzymes to industrial processes. "Many properties we are looking for have no counterpart in the natural world," she says. There are no natural pools of industrial solvents, so an enzyme that works in such a solvent is something "nature never had a reason to make."

Arnold is developing a technique to do what nature never intended: a form of guided evolution by natural selection. Like Brandeis' Petsko, she uses the tricks of molecular biology to generate thousands of mutant versions of an organism, the mutations changing the structure of one particular enzyme. By sorting for organisms that survive in some new condition, say a particular solvent, she says, you can create new enzymes to do whatever you wish, wherever you wish. This sort of guided evolution, she says, may yield proteins that go far beyond the extremozymes of the natural world. "Extremozymes are a good place to start for molecular evolution," she says. "But we want to know what is the edge...just how far enzymes can go."

Whatever the edge is, it seems that chemists certainly haven't reached it yet, even though they are now doing enzyme chemistry in regimes that, just a few years ago, would have seemed all but unthinkable.

–Faye Flam

ILLUSTRATION: GREGORY PETSKO