Zero Gravity Produces Weighty Improvements

In the stillness of space, proteins form crystals that are much larger and better ordered than on Earth

PROTEIN CRYSTALS RAISED IN OUTER space may be inherently superior to their Earth-bred cousins. That is one implication of an experiment, reported on page 651, in which astronauts on the space shuttle grew protein crystals that were much larger and provided much better structural data than those grown on Earth under identical conditions.

In the past, some scientists had suggested that any improvements in quality gained by growing protein crystals in space would not be worth the added expense of getting them there. The new results, however, provide solid proof that the naysayers are wrong, says Larry DeLucas of the University of Alabama at Birmingham. The improvements we have seen, he says, "are enough to get any crystallographer excited." DeLucas was one of a large group of co-investigators headed by Charles Bugg, also at Alabama, who ran the space shuttle experiment in September 1988.

Improved crystal quality is vital for determining precise protein structures by x-ray crystallography. "The real problem is getting high-quality crystals," DeLucas says, since the resolution of the x-ray data depends acutely on how neatly the proteins line up in the crystals.

The space-grown crystals are already proving their worth. Since their paper was submitted to *Science*, several of the researchers have obtained new information about the structures of three of the proteins carried on the space shuttle.

At Merck, Sharp & Dohme Research Laboratories in Rahway, New Jersey, Manuel Navia has been studying space-grown crystals of porcine pancreatic elastase. This protein has a structure very similar to that of human leukocyte elastase, an enzyme that can damage the lungs and cause emphysema. Because the porcine enzyme is easier to work with than its human counterpart, it is often used in laboratory studies.

Since the early 1970s, several groups have used x-ray crystallography data from porcine elastase crystals to get an approximate structure for the protein. The ultimate goal is to understand how other molecules bind to the protein, information that may aid in designing good elastase inhibitors. Such compounds could lead to more effective emphysema treatments.

But the quality of Earth-grown crystals of the enzyme was relatively poor, and x-ray studies of them gave only a fuzzy picture of the structure of the protein. The crystals grown on the shuttle gave x-ray diffraction data with a resolution of 1.3 angstroms, as compared to 1.65 angstroms for the best Earth-grown crystals of porcine elastase. The space crystals also provided nearly twice as many data points. As a result, Navia says he has been able to determine the protein structure "to a much greater order."

The improvements we have seen are "enough to get any crystallographer excited," DeLucas says.

The crystals of a second protein, isocitrate lyase, were so much better than those available before that they made the difference between go and no-go for a project at Du Pont, says Patricia Weber, a protein crystallographer there. Isocitrate lyase is an enzyme found in nematodes, worms that sometimes live as parasites in plants or animals.

Knowing the structure of the enzyme should help in designing a nematocide, Weber notes. Du Pont had been trying to use Earth-grown crystals of the enzyme to determine its structure, but with little luck. The better crystals transform the problem from "one that is marginal to one that is solvable," Weber says.

And crystallographers at the Center for Macromolecular Crystallography in Birmingham, Alabama, working with scientists from Schering-Plough Corporation in Bloomfield, New Jersey, have been able to solve the structure of γ-interferon for the first time by using x-ray diffraction data from crystals grown during the mission, DeLucas says. Drug companies are interested in using γ-interferon as a possible drug for cancer therapy.

DeLucas and his colleagues originally decided to move their crystal-growing operations to space because many of the limitations on crystal growth on Earth arise from the presence of gravity. No matter how carefully a worker controls the solution in which a crystal grows, gravity will always stir things up, DeLucas explains. Since proteins are in general slightly more dense than water, gravity causes a convective flow in the solution. As proteins are precipitated out of the solution onto the growing crystal, the remaining fluid becomes less dense and flows upward away from the crystal face.

This is not exactly a gentle process, either, DeLucas says—experiments with Earth-bound crystal growth have produced "plumes" that stream off the top of the growing crystal at speeds of almost 1 centimeter per second. The result of this convective flow is that some parts of the crystal will grow faster than others, creating defects in its structure.

Convection creates a second problem, too, DeLucas notes. Once the solution is supersaturated with protein molecules, any disturbance will cause many crystals to start forming throughout the liquid. Without convection, fewer crystals will form, so that each of them can grow larger without interfering with the others.

The obvious cure is to grow the crystals in the near-zero gravity of outer space, as the current experiments have verified.

Of eleven proteins that were used, three produced crystals that were "far superior" to any produced before, DeLucas says. They were larger and provided better x-ray data. This is particularly promising because the crystal growth was done with conditions that were designed to give optimal growth on Earth but not necessarily in space, he notes. With time, they should be able to modify the environment to do even better.

The remaining eight proteins did not perform as well, for various reasons. Six produced "showers of little tiny crystals," DeLucas says, probably because some vibration caused many crystals to start forming all at once.

The group is scheduled to fly a number of other experiments on the shuttle. "Each time we make changes in the hardware to make it better," DeLucas says, with the goal of finding the best conditions for growing crystals in space. The payoff of going into outer space to grow crystals is much greater than can be achieved simply by fiddling with conditions on Earth, Weber adds. "We can work for a long time on the ground here to get an improvement much less dramatic than what we saw with the [shuttle-grown] isocitrate lyase." ■ ROBERT POOL