Minerals for Growth

Mineral surfaces turn out to be useful platforms for growing protein crystals

By IVARS PETERSON

Proteins and biological processes are intimately intertwined. These enormous, complicated molecules form the basis for structures such as hair, muscle and skin. As enzymes, they regulate biochemical reactions. As hormones such as insulin, they act as chemical messengers in the body.

An understanding of how a particular protein functions requires a detailed knowledge of how it is put together. The best techniques for precisely locating the thousands of atoms that make up a typical protein involve X-ray or neutron diffraction. By observing the way in which a single protein crystal scatters radiation, researchers can work out its atomic structure. With such structural information, molecular biologists can more effectively engineer enzymes and design drugs for specific purposes. Unfortunately, defect-free, single crystals of proteins are often unobtainable.

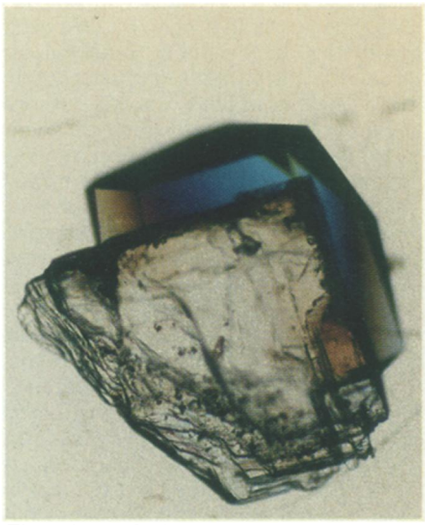
"This is getting to be a serious bottleneck," says biochemist Alexander McPherson of the University of California at Riverside. "A great deal of biotechnology is dependent upon knowing the structures of specific proteins."

In fact, only a small fraction of the thousands of proteins now known have ever been crystallized, he says. As a result, research on proteins is generally restricted to those few proteins whose molecules can be tempted to slip into the regular patterns characteristic of crystals. Even when particular proteins are known to crystallize into a suitable form, conventional techniques for growing such crystals work only one-quarter of the time.

Now, McPherson and Paul J. Shlichta of the Jet Propulsion Laboratory in Pasadena, Calif., have found a crystal-growing technique that may help relieve the bottleneck. Their experimental results show that mineral particles induce the growth of protein crystals, offering the promise of greatly improved control, reliability and reproducibility in protein crystal growth experiments.

That discovery may make it easier to perform crystal-growing experiments in space, and it could increase the supply of high-quality protein crystals for biotechnology research. It's even possible, the researchers say, that this technique may permit proteins that have never before been crystallized to join the crystal elite. Reports of their work appear in the Jan. 22 SCIENCE and in the JOURNAL OF CRYSTAL GROWTH (Vol. 85, p.206).

McPherson started collaborating with Shlichta about two years ago. "The problem was that protein people and crystal people had never really gotten together before," says Shlichta, an expert in crystal growth. "We started by looking very hard at methods used to grow good [small-molecule or inorganic] crystals to see if any of them were applicable to proteins."



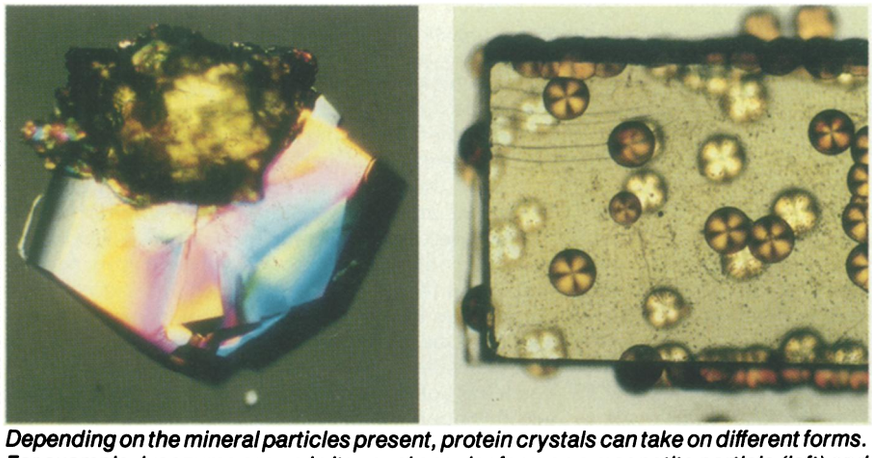
The edges of a lysozyme crystal and its apophyllite substrate appear to be parallel, which suggests the possibility of epitaxial growth.

One of the possibilities was the use of tiny mineral grains as "nucleant" particles, around which protein crystals could be encouraged to grow from solution. Although such a technique has been used for growing crystals of simple molecules, no one had systematically applied the same approach to proteins.

As a first step, McPherson suggested experimenting with proteins that were known to crystallize easily: chicken egg lysozyme, jack bean canavalin and concanavalin B, and beef liver catalase. "The feeling was that if we couldn't do it with these, we might as well shut up shop," says Alexander.

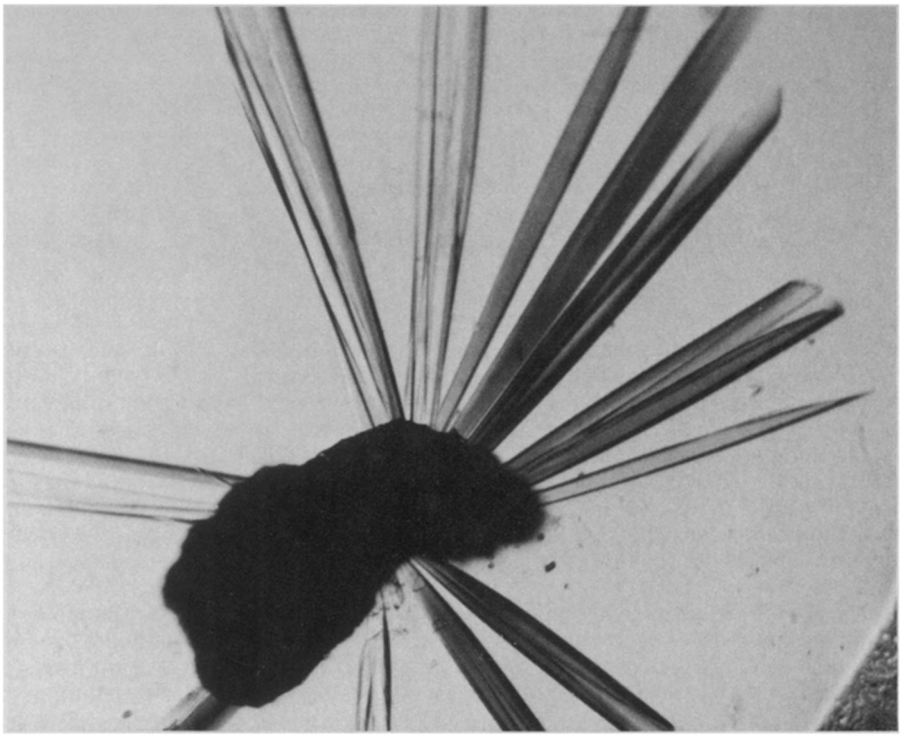
Shlichta sacrificed 50 specimens from his extensive mineral collection, crushing the materials to produce single-crystal samples the size of sand grains. The samples, which covered a wide range of crystal characteristics, included minerals such as magnetite, mica, galena, calcite, talc, topaz and various zeolites.

The proteins were dissolved in water, usually under acidic or alkaline conditions. For example, beef-liver catalase was dissolved in water containing a trace of ammonium hydroxide. As the ammonia evaporated, the solution became supersaturated so that the protein was eventually forced out of solution to form into crystals.



Illustrations: A. McPherson and P.J. Shlichta

Depending on the mineral particles present, protein crystals can take on different forms. For example, lysozyme grows in its usual angular form on a magnetite particle (left) and as spherulites on a magnesium oxide crystal (right).



An array of feathery needles marks the growth of the protein crystal concanavalin B on the surface of the mineral aragonite.

The researchers were particularly interested in how the presence of mineral crystals would influence protein crystallization. "We had a number of surprises," says McPherson. In many of the 452 trials, protein crystals grew much earlier, at lower levels of supersaturation, on the minerals than they did when they crystallized by spontaneous nucleation.

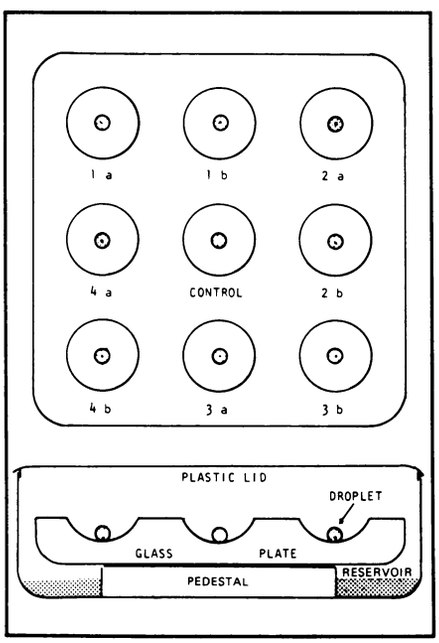
"The minerals that induced early crystallization varied according to protein, although some were common to all," the researchers say. "When nucleation occurred early, it invariably occurred on the surface of the mineral substrate rather than in the surrounding free solution."

Exactly why crystallization occurred in each case has yet to be determined. Factors such as the presence of cracks or imperfections on mineral surfaces and the ability of these surfaces to absorb, hang on to and align molecules all may have had an effect. Many minerals also contain heavy-metal ions such as lead, which may have contributed by attracting certain parts of protein molecules.

"It appears that minerals with absorptive properties – that is, minerals known to bind organic molecules to their surfaces – work best," says McPherson. "These minerals have very interesting surface features."

In several instances, McPherson and Shlichta discovered that the presence of minerals even altered the basic shape of the protein crystals formed. This result suggests that when proteins happen to crystallize spontaneously into needles or very thin plates, which can't be used for X-ray diffraction, the choice of a suitable mineral substrate may alter the protein crystal shape to one that is more amenable to diffraction experiments. "We're always looking for techniques for producing a crystal form that is different from the one that grows spontaneously," says McPherson.

The most striking result was seen in the growth of lysozyme on the mineral apophyllite. In this case, the protein molecules, seeking congenial resting spots on the mineral surface, seem to settle into an orderly arrangement automatically.



Top and side views of the crystallization apparatus used for growing protein crystals.

The protein and mineral crystals not only have parallel edges but also reveal matching crystal lattice characteristics in X-ray diffraction measurements. "This type of epitaxial growth has never been observed before," says McPherson.

The McPherson-Shlichta results may influence the type of experiments done on future spaceflights. Some researchers believe that convection currents interfere with crystal growth by disturbing the way in which protein molecules are stationed or oriented. Such currents are absent under the microgravity conditions of space, leading a few experts to suggest that space may be the ideal place to grow large, defect-free protein crystals. So far, however, spaceflight experiments have been inconclusive, mainly because protein crystallization is hard to initiate.

"What we're looking for is an experiment in which a single crystal will start to grow automatically as soon as you push the button," says Shlichta. That would enable the spaceflight experiment to be completed in the short time available while guaranteeing a crystal product. The lysozyme-apophyllite system, because it appears to produce large, well-formed protein crystals readily and reliably, may fit the bill (see p. 150).

The use of suitable mineral grains may also increase the size of protein crystals produced on earth. It's a matter of finding a mineral whose lattice closely matches that of a particular protein crystal. Once an orderly layer of protein molecules is nudged into place by the mineral substrate, then subsequent protein layers are more likely to fall into place to create a larger, more uniform crystal.

The McPherson-Shlichta results have already generated considerable excitement among researchers at pharmaceutical and biotechnology companies and among scientists working on the structure of proteins. A team of researchers at Du Pont in Wilmington, Del., recently duplicated many of the experiments and confirmed the results. The Du Pont team is now trying new combinations.

Meanwhile, Shlichta and McPherson face so many promising possibilities that it's hard to decide which experiment to do next. "We're just at the beginning of this," says Shlichta. "It's like opening a box at Christmas. We don't even know what we've got yet."

"Protein crystal growth is a very new area," says McPherson. "In the past, it was left to students and technicians. Only in the last few years, because of its importance in molecular biology, has it really gotten any attention from scientists." He adds, "What we're trying to do by our studies is to take it out of the world of art and to put it back into the world of science." □