PROTEIN CRYSTALLOGRAPHY

PCG MICROGRAVITY EXPERIMENTS FLYING HIGH

New York—This January's launch of the Shuttle Columbia (STS-32) carried an array of protein crystal growth (PCG) equipment for experimentation on 24 proteins—including repeat experiments on those flown on STS-26, the September 1988 Space Shuttle mission. The investigators' principal aim remains basic science—determining the parameters that affect PCG—and refining the vapor diffusion apparatus used. But obtaining high-resolution crystals of some of these proteins also would contribute to commercial efforts in rational drug design.

Last fall, the principal investigators for the PCG experiments flown on STS-26 (*Bio/Technology* 6:1263, Nov. '88), published their results (*Science* 246:651, 3 Nov. 1989). The data showed significant improvement in the ability to grow more ordered crystals in microgravity for three of the 11 proteins studied; they go a long way toward convincing skeptics that the potential benefits of PCG in microgravity outweigh the high cost of experimentation in space.

Two crystals of γ interferon flown by investigators at the University of Alabama-Birmingham (UAB) for Schering-Plough (Madison, NJ) were significantly larger than those produced on earth (one was approximately 50 percent larger than the largest crystal previously obtained) and possessed a similar morphology. The space-grown crystal displayed an increase in measurable data throughout the resolution range, as well as an overall lower B value, indicating increased internal order in the crystal. According to head PCG investigator Charlie Bugg (University of Alabama-Birmingham, UAB), earth-grown crystals of γ interferon "only diffract to about 3 angstroms, whereas in space we can get to 2.7 angstroms...which can make the difference in defining side chains" of the molecule.

Results with porcine elastase were similarly impressive. Sponsored in 1988 by Merck (Rahway, NJ) under the supervision of principal investigator Manuel Navia, the space-grown crystal yielded significantly more data at all resolution ranges, with enhancement in the ultimate resolution at which measurable data can be obtained. And the B value of the STS-26-grown isocitrate lyase crystal from DuPont (Wilmington, DE) also was significantly lower. Of the remaining eight proteins, six did not produce crystals large enough for diffraction

analysis, one produced but two crystals (only enough for a partial data set), and the other could not be analyzed because of problems during data collection.

There are so many variables in PCG that temperature is used as a constant—either 22°C, 4°C, or, in some cases, 37°C. The STS-32 crew will have conducted some 120 PCG experiments over the 10-day mission—60 at 22°C and 60 at 4°C. Because the lower temperature slows the vapor diffusion process and, as a result, the kinetics for crystal growth, a molecule settling into the lattice has more of a chance to equilibrate before another one sits on it and "freezes in" defects. Thus, the sample of reverse transcriptase supplied by Burroughs-Wellcome (Research Triangle Park, NC) for the STS-26 mission, for example, did not form well at 22°C. And the three-day duration of that flight did not allow sufficient time for low-temperature experiments.

Bugg notes that there are more ground-based data on reverse transcriptase than on any other protein. "Everything you can conceive of has been tried to grow this crystal...[Researchers] must have run thousands of experiments." But despite producing "beautiful 0.5 mm crystals, they are all disordered." And while Bugg acknowledges that "biochemical heterogeneity may be a problem that microgravity cannot solve, [we] are enhancing the internal order of some crystals." Compounds being flown for the first time on STS-32 include human growth hormone (Eli Lilly, Indianapolis, IN), satellite tobacco mosaic virus (University of California-Riverside, UC-R)—the largest biological molecule to be crystallized in space—and aldose reductase (UAB and affiliated startup Biocryst), which is diffi-

cult to crystallize on earth at all. UC-R also will re-try crystallizing canavalin, but this time at the lower temperature.

Navia left Merck recently to join Vertex Pharmaceuticals (Cambridge, MA), a startup company pursuing structure-based pharmaceutical design, as the head of its crystallography group. Obtaining better crystals for analysis is critical to the rational drug design process. "All structures are put through a refinement process," he explains, which is data-driven. "It's a molecular modeling exercise where the model is constrained by the calculated equivalent of the observed diffraction data." As an example, for the space-derived crystal of porcine elastase, "going from 19,000 reflections to 37,000 demands a more restricted—and accurate—model." And more companies—including Genentech (So. San Francisco, CA)—are joining the Center for Macromolecular Crystallography at UAB, one of the National Aeronautics and Space Administration's 16 Centers for the Commercial Development of Space.

Yet Navia cautions that "a lot of people still dispute the data" on the value of microgravity PCG. Bugg agrees with that assessment. He points out that any experimentation in space carries with it "a lot of hype and enthusiasm. That's a formula for skepticism." Further, the amount of data and the number of experiments run are small. "It's not a tremendous way to do science," he admits. But while he's "still not convinced it's a cure-all for PCG, the finding that we've altered the internal molecular order is significant...Anyone who's looked at the data and the theoretical reasons for running PCG in microgravity ends up not being skeptical about the rationale" for doing the experiments.

—Mark Ratner

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