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PROTEIN CRYSTAL GROWTH ABOARD THE U.S. SPACE SHUTTLE FLIGHTS STS-31 AND STS-32

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Several advances in the technology involved in determining protein crystal structures have facilitated several new and existing applications for protein crystallography /1/. Crystallographic studies of biological molecules such as proteins and nucleic acids /2, 3/ have played a key role in establishing the structural foundations of molecular biology and biochemistry. Such studies have been important in revealing structure function relationships that are of major importance in understanding how macromolecules operate in biological systems. Recently crystallographic studies of proteins have become of interest to the pharmaceutical, biotechnology, and chemical industries, as promising tools in protein engineering, drug design and other applications to biological systems. Since relatively large, high quality single crystals must be obtained before a structural study can be pursued using this method, protein crystal growth has become a topic of considerable importance. One promising new development in protein crystal growth involves studies of crystal growth processes in the microgravity environment obtainable in space /4, 5, 6/. Beneficial effects of the microgravity environment on protein crystal growth include the minimization of density driven convective flows /7, 8/ that accompany crystal growth, the elimination of sedimentation of growing crystals, and the ability to perform containerless processing of protein crystals.

The first microgravity protein crystal growth experiments were performed on Spacelab I by Littke and John /4/. These experiments indicated that the space grown crystals, which were obtained using a liquid-liquid diffusion system, were larger than crystals obtained by the same experimental system on earth. Subsequent experiments were performed by other investigators on a series of space shuttle missions from 1985 through 1990 /9/. The results from two of these shuttle flights (STS-26 and STS-29) have been described previously /6, 9/. The results from these missions indicated that the microgravity grown crystals for a number of different proteins were larger, displayed more uniform morphologies, and yielded diffraction data to significantly higher resolutions than the best crystals of these proteins grown on earth. This paper presents the results obtained from shuttle flight STS-32 (flown in January, 1990) and preliminary results from the most recent shuttle flight, STS-31 (flown in April, 1990).

HARDWARE DESCRIPTION

The space shuttle experiments involve crystal growth by a vapor diffusion technique which is closely related to the widely used hanging drop method of protein crystal growth on earth /10/. A detailed description of the hardware developed for space shuttle flights has been described previously /5/. However, figure 1 shows the basic principle behind the design of the apparatus developed for protein crystal growth by vapor diffusion techniques. Each experiment takes place within a sealed chamber that has a volume of approximately 5.3 cubic centimeters with clear plastic windows for visual and photographic monitoring of crystal growth. Prior to activation of the experiment, the protein solutions are contained within double-barrel syringes which are stopped during launch and landing (Figure 1a). The two barrels of the syringes are filled with protein and precipitant solutions respectively. The experiment is activated by withdrawing the stopper and extruding the protein and precipitant solutions simultaneously onto the syringe tip (Figure 1b).

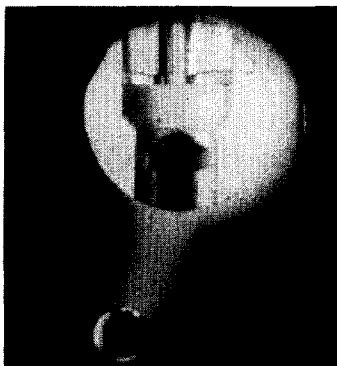


Fig. 1a. - Vapor-diffusion - Stopped configuration. The protein and precipitant solutions are contained within each barrel of the syringe which is sealed by an opposing plunger. An absorbent material that holds the reservoir solutions surrounds the protein droplet, thereby providing exposure to a large surface area so that equilibration times are adequate for short duration shuttle missions.

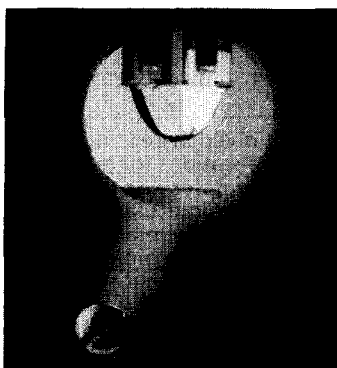


Fig. 1b. - Vapor-diffusion - Unstopped configuration. The protein and precipitant solutions have been extruded onto the syringe tip and are equilibrating with the surrounding reservoir solution contained by the wicking material.

The combined protein/precipitant droplet equilibrates with a wicking material saturated with an equilibration solution. One entire vapor diffusion tray with dimensions of 35.8 cm. x 1.66 cm. x 8.6 cm. contains twenty crystal growth chambers (Figure 2). Three vapor diffusion trays are contained in a refrigerator/incubator module (R/IM) which occupies one middeck locker on the shuttle.

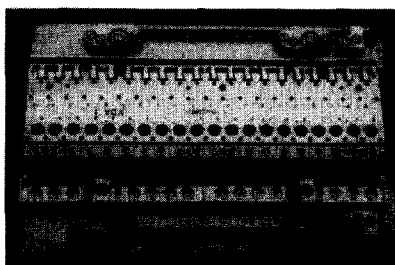


Fig. 2. Vapor-diffusion crystal growth hardware. One complete vapor diffusion tray

consists of twenty syringes and opposing plungers that are operated simultaneously using a mechanical ganging device. The clear circular windows allow the astronaut to photodocument (via 35 mm camera or camcorder) crystals growing within the droplets.

CRYSTAL ANALYSIS

Detailed x-ray diffraction studies were performed with area detector systems /11/ using rotating anode x-ray generators with copper targets, at various laboratories associated with the co-investigator responsible for a particular protein experiment. For those proteins that produced space-grown crystals that were large enough, three-dimensional x-ray diffraction data sets were collected. The techniques and crystal growth conditions used for the space experiments closely parallel the vapor diffusion experiments that have been used in numerous studies on earth with the various proteins. Consequently, three-dimensional x-ray diffraction data sets obtained from space-grown crystals were compared with the best data sets that had been obtained from earth-grown crystals of these proteins using area detector systems and experimental protocols similar to those followed for obtaining data from the space-grown crystals.

RESULTS AND DISCUSSION

There are a variety of methods that can be used to assess the quality of a macromolecular crystal. Standard x-ray diffraction still photographs can be used for qualitative evaluation of diffraction resolutions. However, because evaluation of diffraction resolutions from still photographs is highly subjective, and is often dependent on crystal orientation, the primary evaluation used for these analyses depended on three-dimensional intensity data sets for comparison of space and earth-grown crystals. These intensity data sets can be used to analyze crystal quality in a variety of different ways. Intensities are evaluated as functions of Bragg angles, including analysis of the percentage of data above background levels throughout the data collection range. The intensity data are used to make plots of average $I / \sigma(I)$ values, where I is intensity, versus diffraction resolution, and plots of percentages of data above various σ levels as a function of resolution. In addition, data sets for earth grown and space grown crystals can be compared using relative Wilson plots, also known as difference Wilson plots /2/, which are useful for assessing changes in the internal order of protein crystals. In addition to this detailed quantitative analysis, the principal investigators associated with each protein flown on the shuttle, are asked to make a qualitative assessment of the microgravity crystals based on microscopic analysis with photo documentation.

In general, analyses from the last four U. S. shuttle flights (STS-26, STS-29, STS-32, and preliminary data from STS-31) indicate that protein crystals grown in microgravity may display more uniform morphologies and yield diffraction data of higher quality than the best crystals of these proteins grown by any method on earth. Several proteins flown on STS-26 in September, 1988, produced diffraction data that extended significantly to higher resolutions than the best crystals for these particular proteins grown on earth /6/.

We will report here results from proteins flown on STS-32 and preliminary results from STS-31.

The STS-32 experiments included Canavalin, a protein extracted from Jack Bean. Canavalin crystals are much denser than the solution from which they are grown and, consequently typically sediment to the bottom of the hanging drop or container. As a result, they often grow as fused aggregates with several deformed faces. In microgravity, canavalin crystals grew dispersed through the droplets as shown in figure 3a, which resulted in uniform morphologies for nearly all of the canavalin crystals (Figure 3b).

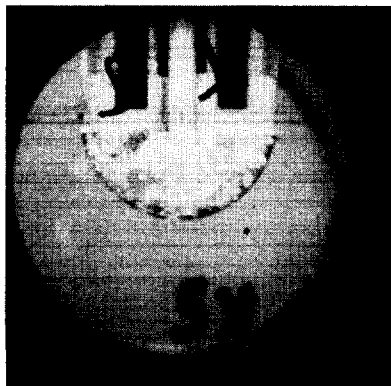


Fig. 3a. Canavalin crystals in vapor diffusion apparatus grown in microgravity

environment. The crystals appear white due to polarized photography; it is evident that the crystals are evenly dispersed throughout the droplet. They do not suffer sedimentation effects as do earth-grown crystals.

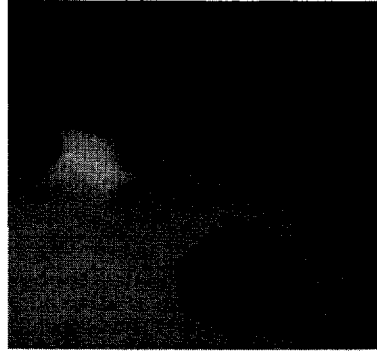


Fig. 3b. Canavalin crystals grown in microgravity. The crystals display uniform morphologies and more isotropic growth characteristics than do typical earth-grown crystals.

Full data sets were collected on two space-grown crystals although one other crystal was simply not as good and may have suffered some degradation before the analysis was performed. The earth-grown crystal used for comparison was not only perfect by microscopy, but about four times larger in volume than the space-grown crystal. When the data is analyzed using a plot of $I/\sigma(I) > 5$ versus resolution, it can be seen that the data for the earth and space grown crystals extend to almost exactly the same resolution but the space-grown crystal yields considerably more and better data over the entire range from infinity to the maximum resolution (Figure 4).

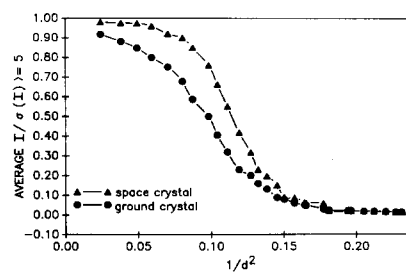


Fig. 4. Comparison of diffraction intensity data for space-grown and earth-grown crystals of Canavalin.

A further analysis of the difference in quality as a function of direction and reciprocal space, shows that the most significant improvement was along the unique three-fold direction, or L-index. This was expected since the diffraction pattern falls off anisotropically and most severely in the L direction.

A second protein, isocitrate lyase, was also flown on STS-32. Ground-based crystallization experiments have /12/ invariably resulted in the growth of dendritic clusters (figure 5a). An improved habit for isocitrate lyase was observed from shuttle flights STS-26 and STS-32. Although some dendritic growth was found in the space samples, a number of well formed prisms (figure 5b) were obtained.

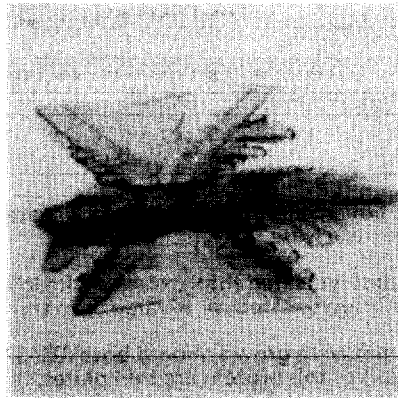


Fig. 5a. Isocitrate lyase crystals. Typical dendritic morphology for crystals grown on earth. The dimensions of this dendritic cluster are 0.74 mm and 0.46 mm, respectively.

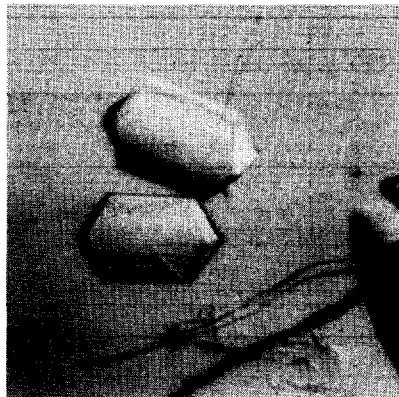


Fig. 5b. Isocitrate lyase. These prisms of isocitrate lyase were grown on STS-26. The crystal dimensions are approximately 0.4 mm x 0.25 mm x 0.4 mm.

These prisms belong to the same space group as the earth-grown dendrites, but they yield better intensity data throughout the intensity range (figure 6).

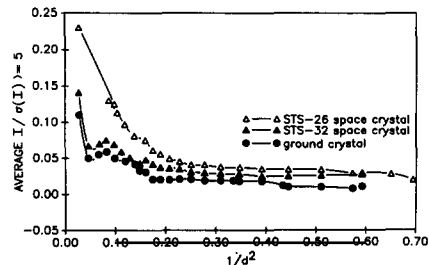


Fig. 6. Comparison of diffraction intensity data for space-grown and earth-grown crystals of isocitrate lyase.

In addition, a relative Wilson plot indicated that, except at the lowest resolution range, the space-grown crystal had a significantly lower effective B-value than the earth-grown crystal.

Crystals of human serum albumin were grown aboard both STS-32 and STS-31 shuttle missions. Although ground-grown crystals of this protein are thin plates, the microgravity-grown crystals were often thicker than their typical earth counterparts. X-ray analysis of the crystals once again shows that the microgravity-grown crystals produce data of higher quality throughout the resolution range (figure 7).

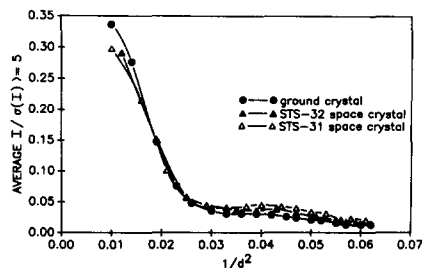


Fig. 7. Comparison of diffraction intensity data for space-grown and earth-grown crystals of human serum albumin.

Crystals of Anti-HPr Fab fragment were also grown on shuttle flight STS-31. Two small crystals were obtained from the microgravity experiment. One of these crystals was among the best in appearance ever seen with perfectly developed faces. Data from one of the space-grown crystals was compared with the best earth-grown crystal (which was ten times larger in volume than the space crystal). The x-ray data analysis does not show a significant difference between the space crystal and the earth-grown crystal. However, one must realize that the volume of the space crystal was only one-tenth that of the earth-grown crystal. The fact that the smaller space crystal produced similar relative intensities to the large earth crystal demonstrates the superb quality of the space-grown crystal. In figure 8, data from the space-grown crystal are compared with data from an equal-sized earth-grown crystal; the space-grown crystal diffracts better than the earth-grown crystal in every resolution range.

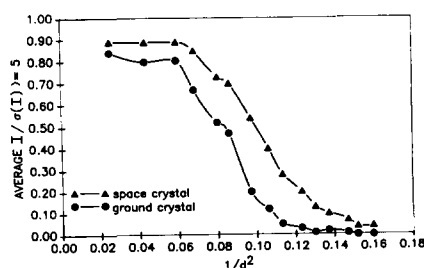


Fig. 8. Comparison of diffraction intensity data for space-grown and earth-grown crystals of Anti-HPr Fab fragment.

Furthermore there is essentially no diffraction beyond 3.0 Å for the equal-sized earth-grown crystal whereas the space crystal is still diffracting at 2.8 Å. The increase in quality is further indicated by analysis of the average mosaic spread of the space crystal (0.65) compared to that of the earth-grown crystal (0.74). A lower mosaicity typically indicates that the crystal is more highly ordered. A relative Wilson plot performed using the space crystal data compared to the best earth-grown crystal also indicated that the space-grown crystal was more highly ordered at the molecular level. The production of smaller crystals from space is believed to be due to the fact that conditions had to be modified to accommodate the short (4½ days) shuttle mission for STS-31. Anti-HPr Fab fragment crystals typically require eight or nine days for optimum crystal growth conditions.

A lysine-49 phospholipase A₂ protein isolated from venom of eastern cottonmouth was used for crystallization experiments aboard STS-32. Three data sets from crystals of the protein produced on the shuttle mission were compared to two data sets from similar crystals produced in ground based experiments. A strong correlation between crystal size and effective resolution dominates the comparisons by both $I/\sigma(I)$ greater than 5 versus resolution and relative Wilson plots, with larger crystals producing higher resolution data sets regardless of the gravity conditions during crystal growth. In one case, however, where data sets are from shuttle-grown and earth-grown crystals of equal size, the data set produced by the shuttle-grown crystal is marginally better in effective resolution. The distribution of crystal sizes seen in shuttle results is not unlike that typical of earth bound experiments, although considerably larger crystals have been obtained on earth. In contrast to the earth bound experiments, however, a much larger fraction of shuttle crystals exhibit the ideal, untruncated square bi-pyramid habit. In earth bound experiments, perfectly shaped bi-pyramid specimens are very rare. In fact it was not realized what the ideal crystal shape was until the results of the shuttle experiments were examined. In a typical experiment performed on earth, crystals are found to nucleate on or gravitate to the borders of the droplet and grow to some truncated permutation of the ideal shape. In contrast, crystals produced on STS-32 typically showed the ideal square bi-pyramid habit and the truncated variance was the rare exception.

The results from the last four shuttle missions can be summarized as follows: Microgravity experiments can yield crystals that are larger, display more uniform morphologies and yield diffraction data to significantly higher resolution than the best crystals of these proteins produced on earth. For each shuttle mission, approximately twenty percent of the proteins flown are found to exhibit better morphologies or better quality data than their earth-grown counterparts. Approximately forty percent do not yield crystals at all and the remaining forty percent yield crystals that are either too small for x-ray analysis or produce data of poorer quality than the best earth-grown crystals. It should be noted that the results of a single space experiment (typically each protein is allotted five crystallization chambers) is being compared with the best crystals ever produced for that particular protein by any method on earth. With the present hardware, investigators have no ability to optimize the crystal growth conditions in microgravity and it is believed that this often accounts for results where either no crystals are obtained from microgravity or crystals that are far too small for data analysis.

There are a number of explanations for the decreased resolution observed when crystallized macromolecules are subjected to x-ray radiation. These include protein heterogeneity, impurities in the crystallizing solution, the rate of crystal growth, inherent thermal motion of the macromolecules, and gravity-induced solutal convection. It is clear that microgravity cannot minimize or eliminate some of these unwanted characteristics. One hundred percent success rates are not expected. The U. S. space shuttle simply provides a unique environment for examining the effects that reduced gravity levels have on crystal growth. Under microgravity conditions, convective flow patterns that accompany crystal growth are minimized, thus generating a more controlled environment at crystal interfaces. Because protein crystals are relatively weakly bonded, with water bridges playing predominant roles, molecular packing patterns may be more regular in the absence of convective turbulence.

REFERENCES

1. L. J. DeLucas and C. E. Bugg, "New Directions in Protein Crystal Growth", *Trends Biotechnol.*, 76, Vol. 5, 188 - 193 (1987).
2. Blundell, T. L. and Johnson, L. M., 1976, *Protein Crystallography*, Academic Press, New York.
3. Kossiakoff, A. A., "The Application of Neutron Crystallography to the Study of Dynamic and Hydration Properties of Protein", *Ann. Rev. Biochem.*, 54, Pg. 1195 - 1227 (1985).
4. W. Littke and C. John, "Protein Single Crystal Growth under Microgravity", *Science*, 225, 203 (1984).
5. DeLucas, L. J., Suddath, F. L., Snyder, R., Naumann, R., Broom, N. B., Pusey, M., Yost, V., Herren, B., Carter, D., Nelson, B., Meehan, E. J., McPherson, A. and Bugg, C. E., "Preliminary Investigations of Protein Crystal Growth Using the Space Shuttle", *J. Crystal Growth*, 76, 681 - 693 (1986)
6. L. J. DeLucas, C. D. Smith, H. W. Smith, S. Vijay-Kumar, S. E. Senadhi, S. E. Ealick, D. C. Carter, R. S. Snyder, P. C. Weber, F. R. Salemme, D. H. Ohlendorf, H. M. Einspahr, L. L. Clancy, M. A. Navia, B. M. McKeever, T. L. Nagabhushan, G. Nelson, A. McPherson, S. Koszelak, G. Taylor, D. Stammers, K. Powell, G. Darby, and C. E. Bugg, "Protein Crystal Growth in Microgravity", *Science*, Vol. 246, pp. 651-654 (1989)
7. M. Pusey, W. Witherow, R. Naumann, "Preliminary Investigations into Solutal Flow about Growing Tetragonal Lysozyme Crystals", *J. Crystal Growth*, 90, 105 (1988)
8. R. L. Kroes and D. Reiss, "Properties of TGS Aqueous Solution for Crystal Growth", *J. Crystal Growth*, 69, 414 (1984).
9. L. J. DeLucas, C. D. Smith, H. W. Smith, S. Vijay-Kumar, S. E. Senadhi, S. E. Ealick, D. C. Carter, R. S. Snyder, P. C. Weber, F. R. Salemme, D. H. Ohlendorf, H. M. Einspahr, L. L. Clancy, M. A. Navia, B. M. McKeever, T. L. Nagabhushan, G. Nelson, A. McPherson, S. Koszelak, G. Taylor, D. Stammers, K. Powell, G. Darby, C. E. Bugg, "Protein Crystal Growth Results for Shuttle Flights STS-26 and STS-29", *J. of Crystal Growth*, in press, (1989).
10. A. McPherson, *Methods Enzymol.* 114, 112 (1985).
11. A. J. Howard, et al., "The Use of an Imaging Proportional Counter in Macromolecular Crystallography", *J. Appl. Crystallogr.*, Vol. 20, Part V, 383 - 387 (1987).
12. P. C. Weber and J. V. Schloss, unpublished data, 1990.