Protein crystal growth in space, past and future

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Abstract

The Center for Biophysical Sciences and Engineering (CBSE) at the University of Alabama at Birmingham has performed protein crystal growth experiments on more than 39 US space shuttle missions. Results from these experiments have clearly demonstrated that the microgravity environment is beneficial in that a number of proteins crystallized were larger and of higher quality than their Earth-grown counterparts. Improvement in crystal quality is judged by analysis of ultimate diffraction resolution, individual peak mosaicity, and electron density maps. There are now a number of protein crystals that exhibited resolution improvements of 0.5–1.5Å. Mosaicity studies revealed dramatic decreases in peak widths for the microgravity-grown crystals. These microgravity results plus data from a variety of other investigators have stimulated various space agencies to support fundamental studies in macromolecular crystal growth processes. The CBSE has devoted substantial effort toward the development of dynamically controlled crystal growth systems which allow scientists to optimize crystallization parameters on Earth or in space. These systems enable monitoring and control of the approach to nucleation and post-nucleation growth phases, thereby dramatically improving the crystal size and X-ray diffraction characteristics. The CBSE is currently designing a complete crystallographic laboratory for the International Space Station including: a crystal growth rack, which will support a variety of crystallization hardware systems; an X-ray diffraction rack for crystal characterization or a complete X-ray data set collection; and robotically controlled crystal harvesting/cryopreservation systems that can be operated with minimal crew time via telerobotic and/or robotic procedures. Key elements of the X-ray system include unique X-ray focusing technology combined with a lightweight, low-power source. The X-ray detection system is based on commercial CCD-based technology. This paper will describe the X-ray facility envisioned for the International Space Station. © 2002 Elsevier Science B.V. All rights reserved.


1. Introduction

For more than a decade, the National Aeronautics and Space Administration (NASA) has supported a relatively small research program in biotechnology. One component of this research involves the study of the physical and chemical factors influencing macromolecular crystal growth. The crystal growth research was stimulated by results from an experiment designed by a German physicist, Dr. Walter Littke, and performed in a microgravity environment on the US Space Shuttle. Two proteins, beta-galactosidase and lysozyme, were crystallized using a unique
Comparison of the space-grown crystals with crystals grown in control experiments on Earth, demonstrated a dramatic increase in crystal size and visible quality for the space-grown crystals. These preliminary results suggested that the space shuttle’s microgravity environment could be beneficial for macromolecular crystallization.

Based on these initial results, NASA developed a ground-based, peer-reviewed research program for the study of fundamental factors affecting macromolecular crystallization processes. In addition, NASA supports a small flight investigator program designed to evaluate the singular effect of microgravity on crystallization. Microgravity results have revealed measurable improvements in X-ray diffraction resolution for ~20% of the proteins crystallized [2–7]. Similar results have been obtained from flight programs sponsored by the European Space Agency (ESA), the Japanese Space Agency (JSA), the Russian Space Agency (RSA), the Chinese Space Agency and the Canadian Space Agency (CSA) [2,7–11].

The field of crystallography has experienced a number of significant advances such as improvements in X-ray detectors and X-ray sources, high-speed computers and enhancements in structure determination methods. The combination of crystal cryo-protection and new crystallographic techniques allows the determination of a complete protein structure from one crystal in less than a day. High-intensity synchrotron sources combined with crystal cryo-preservation methods have reduced the need for multiple crystals of large size. Today, most protein structures can be determined using crystals <200 μm in each dimension. In spite of these improvements, the production of crystals of sufficient size and quality continues to be the major impediment in determining protein structures.

The advent of genomics has generated vast amounts of genetic information from humans, bacteria, parasites, viruses and other species and has led to an exponential expansion of proteomic research. The biological function of each protein is inextricably linked to its three-dimensional structure. Thus, in the next decade, there will be a major increase in the demand for protein structural information. While the majority of X-ray structural solutions will continue to result from crystallization in Earth-based laboratories and data collection at synchrotrons, it is also certain that there will be a significant number of proteins that are either not amenable to crystallization or produce crystals of insufficient quality for X-ray crystallographic solution. The crystallographic community must continue to explore any and all approaches in an effort to increase the yield of crystals suitable for X-ray diffraction analysis. Support from space agencies for basic research aimed at improving our understanding of the factors affecting crystal size and quality will continue to expand experimental success on Earth and space.

Although NASA’s first microgravity experiment occurred more than a decade ago, the total number of microgravity protein crystallization experiments performed remains extremely small. This is due to the infrequency of US space shuttle flights, the small experiment volume available for macromolecular crystallization and the limited sample capacity in most of the experimental hardware systems. These constraints are compounded by detrimental factors such as the unreliability of space shuttle flights, the short duration of space shuttle flights, potentially harmful gravitational forces experienced on re-entry, instability of temperature control during sample transfer operations, and other logistical difficulties. In spite of these difficulties, the space shuttle has proven to be a valuable experimental laboratory that has provided a glimpse of microgravity’s potential to affect biological and physical processes. The International Space Station (ISS) will provide a sophisticated laboratory where long duration, iterative biological experiments can be performed. In addition, scientists will be able to analyze crystals via X-ray diffraction so that crystallization experiments can be optimized or cryo-preserved on orbit. High-quality crystals will be robotically cryo-preserved for subsequent analysis in Earth-based laboratories.

The Center for Biophysical Sciences and Engineering (CBSE) at the University of Alabama at Birmingham (UAB) is a NASA Commercial Space Center. The Center has flown payloads on 38
space shuttle flights and two International Space Station increments in support of its microgravity protein crystallization program. This program involves an international co-investigator team located in more than 40 universities and pharmaceutical companies. The key contributions of the co-investigator team are to provide valuable protein samples for microgravity crystallization and to perform the comparative X-ray diffraction analyses of protein crystals using conventional laboratory equipment or synchrotron radiation.

2. Space experiment hardware

The CBSE has designed and built several protein crystal growth hardware systems. Currently, the Center’s high-density protein crystal growth system (HDPCG) is aboard ISS increment 6A. This payload launched aboard STS-100 on April 19, 2001, and will return to Earth aboard STS-105 in August 2001. The HDPCG is a compact and efficient new vapor diffusion experiment assembly, which contains 1008 individual experiments. The vapor diffusion experiments are conducted in growth cells which are arranged in blocks of six experiments and stored in a tray assembly housed in a Modified Commercial Refrigerator/Incubator Module as shown in Fig. 1.

Prior to launch, the crystal growth experiments are loaded into the growth cells. The protein droplets are premixed and placed in the protein inserts, which can accommodate 5–40 µl, and the precipitant solutions are placed in the precipitant reservoirs. The loaded HDPCG assembly is installed into a CRIM-M and stored in a shuttle middeck locker for transport to the ISS and then transferred to an Express Rack.

The CBSE has also developed a dynamically controlled protein crystallization system (DCPCG) that incorporates a controlled flow of dry nitrogen (N₂) gas instead of a reservoir solution to extract water from the growth solution [12]. A second crystallization system dynamically controls solution temperature via miniature thermo-electric devices. Using information from non-invasive diagnostics (laser light scattering and microscopic video), active control of these parameters can, in real-time, affect the supersaturation condition of the protein solution. This allows precise control over the rate of increase of protein supersaturation in real-time, thereby optimizing crystal growth. The space-flight system screens up to 38 different evaporation profiles simultaneously, using chambers in which the protein solution is deployed as a containerized solution (Fig. 2). The dynamically controlled vapor-diffusion system will fly aboard ISS 7A.1, launching on STS-105 in August 2001.

Fig. 1. High density protein crystal growth assembly installed in CRIM-M.

Fig. 2. Dynamically controlled vapor diffusion system.
3. Conclusions

A comprehensive review of NASA’s microgravity protein crystallization result program is provided in a report by C. Kundrot and coworkers [2]. The following results are highlighted in this report:

- There was a positive correlation between success rate and the number of times a protein was flown.
- There are a number of examples where the improvements seen in the details of the protein structure were greater than those predicted based on the X-ray resolution improvements alone.
- Both poor- and well-diffracting crystals have benefited from growth in microgravity.
- There are a number of cases where the total amount of X-ray diffraction data was more than doubled for the microgravity-grown crystals.

Based on this review, it is clear that crystal growth in microgravity is a valuable tool, which will become a more useful access to microgravity if expanded in the International Space Station. Therefore, the technology of microgravity crystal growth is advancing rapidly. Plans for the International Space Station include a complete crystallographic laboratory. This facility will support a variety of crystallization hardware systems, an X-ray diffraction rack for crystal characterization or complete X-ray data set collection, and a robotically controlled crystal mounting system with automated cryo-preservation capabilities [13,14]. Designated the X-ray crystallography facility (XCF), the entire facility can be integrated into one international standard payload rack and operated either by the crew or tele-robotically from the ground. The prototype facility has been designed and built by the CBSE and several suppliers and integrated into an International Standard Payload Rack (ISPR) (Fig. 3).

The CBSE has also developed a video command and monitoring system (VCMS), which will allow the crystals growing in the high-density protein crystal growth assembly and other crystal growth hardware to be monitored and evaluated while on orbit. The VCMS will be installed on ISS during ULF-1, and is currently scheduled to launch in November 2002.

Marshall Space Flight Center is developing an interactive biological crystallization system (IBC) to allow crystallization experiments to be prepared on orbit to optimize crystal growth in the microgravity environment and to conduct crystallization experiments continually on ISS. This facility will interface with the XCF.

The new flight hardware recently launched and that under development will greatly expand the scope, capacity and success of macromolecular crystal growth experiments in microgravity. With the completion of the IBC and the XCF a complete crystallization laboratory will be available on board ISS and macromolecular crystallographers will have, for the first time, the opportunity to fully use the microgravity environment.
environment to meet the challenges of structural genomics.

Acknowledgements

This work was sponsored by the NASA Cooperative Agreement (NCC8-126) and NASA Grant (NAS8-40189).

References