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Experiments in Microgravity: A Comparison of Crystals of a Carbohydrate-Binding Fab Grown on the Ground, on Space Shuttle *Discovery*, and on Space Station *Mir*

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Abstract

The Fab fragment of the hybridoma antibody (YsT9.1) specific to *Brucella abortus* has been crystallized on earth using both Linbro plates and ground-based models of the flight hardware, as well as in microgravity on board the space shuttle *Discovery* and the space station *Mir*. Large-scale experiments using Linbro plates gave two different crystal morphologies, pyramidal and rhomboid, depending on conditions. The pyramidal crystals proved to scatter X-rays to higher resolution, and conditions within the ground-based flight hardware for both *Discovery* and *Mir* were adjusted to produce crystals with this morphology. The experiment on *Discovery* produced large crystals in each of ten chambers. The experiment on *Mir* produced crystals in only one of the five assigned chambers, despite the fact that the simultaneous ground-based experiment produced large crystals in every corresponding chamber. Data collection was attempted for crystals from both space and ground-based experiments. Higher resolution data was obtained from crystals grown on *Discovery* than from either *Mir* or ground-based crystals, even though the crystals obtained from *Discovery* were smaller and forced to grow over a much shorter period of time because of the shorter length of the shuttle mission.

1. Introduction

A hybridoma antibody (YsT9.1) with high specificity for the cell-wall polysaccharide of the Gram-negative bacterium *Brucella abortus* was produced by Bundle *et al.* (Bundle *et al.*, 1989; Bundle, Gidney, Perry, Duncan & Cherwonogrodzky, 1984). Papain digestion yielded the Fab fragment, which was crystallized in the orthorhombic space group $P2_12_12_1$, by the hanging-drop method using PEG 8000 as the precipitant. The structure obtained from these orthorhombic crystals has been reported (Rose *et al.*, 1993). Later, crystals in the monoclinic space group $P2_1$ were also grown from PEG 8000 solutions. An unexpected difficulty arose in adapting these protocols for microgravity conditions when it proved difficult to use high concentrations ($\simeq$ 30%) of PEG 8000 within the flight hardware. Further, high-quality crystals could only be grown under these conditions when the solutions were seeded. Therefore, a new set of conditions had to be found that did not require high concentrations of PEG or seeding. It was found that ammonium sulfate (AS) could be used as a precipitant to give large crystals, which were subsequently found to be trigonal. In order to ascertain if the quality of these crystals could be improved, we carried out two crystallization experiments in microgravity – one on the US space shuttle *Discovery* and the other on the Russian space station *Mir*. We now report details of these crystallization experiments on the ground and in microgravity, as well as the results obtained from them.

2. Preparative crystallization trials

2.1. *Experimental*

Working from experimental conditions first reported (Przybylska, 1989; Rose *et al.*, 1993), crystals for the orthorhombic form of YsT9.1 were grown from solutions of 25% PEG 8000 at ranges of pH from 5.0 to 8.5. Trials were undertaken using a variety of different salts and buffering agents (listed in Table 1), with a final buffer concentration in each case of 50 to 100 m*M*. It was subsequently found that the monoclinic form could be grown at some pH values by the addition of approximately 3% AS. However, neither protocol was suitable for use in the flight hardware as they required seeding to produce large crystals. Increasing the concentration of PEG 8000 to 30% caused crystals to appear spontaneously in Linbro plates. Unfortunately, it was difficult to load these concentrated solutions of PEG 8000 into the porous reservoir material of the space shuttle flight hardware. Consequently, a new protocol had to be developed which did not use PEG 8000.

Crystals of the trigonal form of the Fab protein were initially grown at 293 K by the hanging-drop vapor-diffusion method in Linbro plates. A solution containing 3–4 mg ml^{-1} protein in either sodium phosphate or potassium phosphate buffers (0.1 *M*) in the pH range

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Table 1. *Crystal morphologies observed during preparative crystallization trials at 293 K*

pH	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5
11% PEG 8000 + 3%AS + Buffer	×	M	×	×	×	×	M	×
11% PEG 8000 + Buffer	O	O	O	O	O	O	O	O
15% AS + Na-PB	T_P	T_P	T_P	T_P	T	T_R	T_R	T_R
15% AS + K-PB								
15% AS + Tris–HCl	—	—	—	—	T	T_R	T_R	T_R
15% AS + Na-AcB								
15% AS + NH_4-AcB	T_P	T_P	T_P	T_P	T	—	—	—
15% AS + cacodylate								

Notes: × = no crystals. — = experiment not performed. M = monoclinic crystals observed. O = orthorhombic crystals observed. T = trigonal crystals with pyramidal or rhombic morphology observed. T_P = trigonal crystals with pyramidal morphology observed. T_R = trigonal crystals with rhombic morphology observed. AS = ammonium sulfate. PB = phosphate buffer. AcB = acetate buffer. Tris = Tris(hydroxymethyl)aminomethane. Buffer = one of Na-PB or K-PB at any pH, or Tris–HCl at or above pH 7.0, or Na-AcB, NH_4-AcB or cacodylate at or below pH 7.0.

5.5–8.5 and 12–16% saturated AS was centrifuged and equilibrated against a 1 ml reservoir of 41–43% AS in 0.2 *M* phosphate buffer. These conditions were appropriate starting points for the experiments on the space shuttle and *Mir*, and were tested in a September 1991 flight of *Discovery*. However, a malfunction of the flight hardware caused a failure of the entire experiment, producing crystals less than 0.2 mm long, and the experiment had to be rescheduled as part of the International Microgravity Laboratory-1 (IML-1). A complication came when we learned that our IML-1 experiment had to be carried out at 277 K, and so the crystallization conditions were adjusted to this temperature by changing the concentration of AS in the reservoir to 38–40% AS.

2.2. *Results*

The results of the trials in Linbro plates, preparative to tests in the flight hardware, are summarized in Table 1. For all conditions where crystallization occurred, the largest and best formed crystals were found from pH 5.5 to 6.5. This is close to the isoelectric point of the Fab being crystallized, which was found to be approximately pH 5.8.

For trials using PEG 8000 the orthorhombic crystals were observed at all pH ranges that were tested; however, the conditions favoring crystallization in the monoclinic space group yielded crystals at only pH 5.5 and pH 8.0.

The experiments using AS as a precipitant revealed that crystals grown at room temperature and at pH 5.5–6.5 were mono or bipyramidal with sharp edges, measuring up to 1.0 mm along the pyramid axis after 1–2 weeks and up to 1.4 mm after longer period of time. At higher pH values the crystals were of equal size, but their shape was rhomboid without sharp edges. The external morphology observed for the trigonal crystals appeared to be determined by the pH of the solutions, and did not appear to be dependent on the salts and buffers that were used. Pyramidal crystals were almost exclusively observed below pH 7.0, rhombic crystals were almost exclusively observed above pH 7.0, while a mixture of the two morphologies was observed at neutral pH. The trials carried out at 277 K yielded crystals in a manner exactly parallel to the results obtained at 293 K, except that pyramidal crystals were obtained in all cases.

Both forms of trigonal crystals exhibited sharp extinctions in polarized light. Precession photographs showed that the rhomboid crystals did not scatter X-rays past 6.0 Å resolution, while the pyramidal crystals diffracted X-rays to approximately 3.5 Å resolution. Consequently, most of the conditions for the experiments on *Discovery* and *Mir* were adjusted to produce crystals with the more highly diffracting pyramidal morphology. However, because pyramidal crystals were obtained at all values of pH at 277 K (the temperature at which the space shuttle experiments would be carried out), a few chambers were reserved for higher pH on both sets of flight hardware.

3. Crystallization experiments using the flight hardware

3.1. *Experimental*

3.1.1. *Space shuttle flight hardware.* Prior to the experiment on the space shuttle we received from the University of Alabama a double-barrelled syringe apparatus (DeLucas *et al.*, 1986) for protein crystallization in microgravity. Conditions which favored the production of pyramidal crystals had one of the barrels filled with 15 µl of 6–8 mg ml^{-1} Fab solution in 0.2 *M* phosphate buffer, pH 5.5 or 8.0, and the other barrel with 28–30% AS. The solutions were mixed by extruding and withdrawing the solutions from the syringe eight times, with the mixed drop expelled to the tip of the syringe equilibrated with a wicking material saturated with 39–43% AS in 0.2 *M* buffer. After 5–7 d at 277 K we obtained crystals of the Fab fragment.

The US space shuttle mission designated International Microgravity Laboratory-1 was launched on January 22 1992, and returned to Earth on January 29 1992. The ten chambers allocated to our experiment contained 6–8 mg ml^{-1} Fab solution in 0.2 *M* phosphate buffer, pH 5.5 or 8.5, and 28–30% AS in the two barrels, respectively. The reservoirs were filled with 38–40% AS in 0.2 *M* buffer. The shuttle experiment (MS = microgravity/shuttle) and a simultaneous ground control experiment (GS = ground/shuttle) were carried out at 277 K.

3.1.2. *Space station Mir flight hardware.* Before the experiment on *Mir* we received from Payload Systems,

Inc., their apparatus (Strong, Stoddard, Arrott & Farber, 1992) for growing crystals in microgravity by the sitting-drop method. The protein cup was filled with 30 µl of 2–3 mg ml^{-1} Fab solution in 0.2 *M* phosphate buffer, pH 5.5 or 8.0, and 12% AS. The surrounding moat was filled with 41–43% AS in 0.2 *M* buffer. Under these conditions crystals that were visible under the light microscope first appeared after 2–3 weeks at 293–295 K and continued to grow for 2–3 months.

A Russian *Progress* space rocket carrying our experiment was launched on January 25, 1992, and docked with the space station *Mir* 2 d later. The experiment was activated on January 28, 1992. Each of five chambers contained 50 µl of Fab protein (2.1–3.0 mg ml^{-1}) in 0.1 *M* phosphate buffer, pH 5.5, and 12% AS in the central cup and 750 µl of 41–43% AS in 0.2 *M* buffer in the surrounding moat. The experiment (MM = microgravity/*Mir*) was deactivated after 55 d on March 23, 1992, and returned to earth 2 d later. During that mission the temperature on *Mir* fluctuated in the range 293–298 K. A simultaneous control experiment (GM = ground/*Mir*) was carried out on earth. In this experiment the crystals grew at a constant temperature of 291 ± 0.2 K.

3.2. *Results*

X-ray diffraction data were measured on a San Diego Multiwire System two-detector setup mounted on a Rigaku RU-200 rotating-anode X-ray generator operated at 40 kV and 150 mA. For ease of comparison of the data sets, all data collections were carried out using the same time exposure per frame.

Several crystals grew under MS conditions in each of the ten chambers flown on IML-1, the largest of which measured 1.0 mm along the pyramid axis, Table 2. There was no obvious dependence on pH, protein concentration or AS concentration. The crystal used for data collection from MS conditions measured 0.7 × 0.35 × 0.35 mm, and was grown over a period of 7 d on IML-1. A total of 97 124 measurements were made of 17 023 unique reflections (of a possible 17 195) to a resolution of 3.07 Å. Of these, there were 10 605 (62.3%) reflections greater than 2σ.

GS experiments carried out at 277 K produced small pyramidal crystals after 5–7 d, the largest measuring less than 0.5 mm in length and less than 0.15 mm in width. The largest crystal grew from solutions containing 4 mg ml^{-1} protein with 39–40% AS as precipitant at both pH values. Although none of these crystals was found to scatter beyond 5 Å, data collections were attempted for several specimens. However, these crystals died after a few hours in the X-ray beam, and so only a partial data set was obtained. Crystals from the other three sets of conditions did not display significant decay over the course of the data collection; however, attempts to collect data on subsequent crystals were abandoned when it became apparent that crystals began to lose their ability to scatter X-rays as little as two weeks after their recovery from the flight hardware. (This behavior was also noted for the small crystals recovered from the failed September 1991 *Discovery* mission when data collection was attempted at the MacCHESS synchrotron facility at Cornell University, Borisova *et al.*, unpublished results.)

Table 2. *Space-shuttle experiment (MS)*

The corresponding GS experiment produced from 20 to 70 small (< 0.5 mm) crystals in each chamber.

Chamber number	[Protein] (mg ml^{-1})	[AS] (%)	Results	Maximum crystal size (mm)
1	4.0	38	8 crystals	0.4 × 0.2
2	4.0	39	5 crystals	0.6 × 0.3
3	3.5	39	6 crystals	0.5 × 0.35
4*	3.5	40	5 crystals	0.7 × 0.4
5	3.0	40	6 crystals	0.4 × 0.25
6	3.0	41	5 crystals	0.65 × 0.35
7	4.0	38	2 crystals	0.5 × 0.25
8	4.0	39	2 crystals	0.65 × 0.3
9	3.5	38	5 crystals	1.0 × 0.45
10	3.5	40	7 crystals	0.7 × 0.4

* Crystal used for data collection.

Table 3. *Mir experiment (MM and GM)*

Chamber number	[Protein] (mg ml^{-1})	[AS] (%)	Results	Maximum crystal size (mm)
4 (MM)	3.0	41	No crystals	
4 (GM)			6 crystals	1.0 × 0.6 × 0.4
5 (MM)	3.0	42	No crystals	
5 (GM)			30 crystals	0.6 × 0.3 × 0.3
6 (MM)	2.6	42	No crystals	
6 (GM)			2 crystals	2.0 × 1.1 × 0.6
11 (MM)	2.6	43	No crystals	
11 (GM)			7 crystals	0.7 × 0.25 × 0.25
12* (MM)	2.1	43	4 crystals	1.4 × 0.6 × 0.6
12* (GM)			6 crystals	1.1 × 0.6 × 0.6

* Crystals used for data collection.

The only chamber flown on *Mir* (MM conditions) that contained crystals was that in which the protein concentration was lowest (2.1 mg ml^{-1}) and the AS concentration was highest (43%). There were four crystals in that chamber, ranging in size from 0.6 to 1.4 mm, Table 3. The crystal used for data collection from MM conditions measured 1.4 × 0.7 × 0.7 mm, and was grown over a period of 55 d under MM conditions. A total of 85 433 measurements were made of 18 053 unique reflections (of a possible 18 421) to a resolution of 3.0 Å. Of these, there were 10 019 (55.5%) reflections greater than 2σ.

In contrast to the experiment in microgravity, all five chambers of the *Mir* ground control (GM) did contain crystals, the largest one measuring 2 mm along the bipyramid axis. Three of these chambers had several large crystals while in the other two there were many medium-sized ones, but this could not be correlated with

either protein or AS concentrations. The GM experiments were designed for slow crystal growth, and the largest crystals (up to 1.3 mm) were obtained from lower protein concentrations (2.0–2.5 mg ml^{-1}) and higher AS concentrations (42–43%). The GM crystal used for data collection measured 1.1 × 0.6 × 0.6 mm, and was grown over a period of 55 d. A total of 85 645 measurements were made of 17 080 unique reflections (of a possible 18 363) to a resolution of 3.0 Å. Of these, there were 8147 (47.7%) reflections greater than 2σ.

Although the crystals used for data collection were not the same size, each was longer than the width of the collimated X-ray beam (approximately 0.5 mm at the crystal), and consequently had approximately equal portions of its length irradiated during data collection. This means that the crystals from the GM and MM experiments, which were approximately the same width, effectively presented the same amount of scattering material to the X-ray beam. The crystal grown on IML-1 (MS) was about half as wide as the GM and MM crystals, and so displayed proportionately about one-quarter of the scattering material to the X-ray beam.

4. Comparison of crystal qualities

Fig. 1 illustrates the differences between data collected from the crystals grown in GS, MS, GM and MM conditions. For the experiment using the *Mir* flight hardware, it is clear that the MM conditions gave a crystal that produced better data over all ranges of resolution than the GM crystal, although that improvement is quite modest. A more marked improvement is seen for crystals grown in the space shuttle flight hardware, where the resolution for crystals grown in microgravity was effectively extended by approximately 1.5 Å resolution over that for crystals grown on the ground.

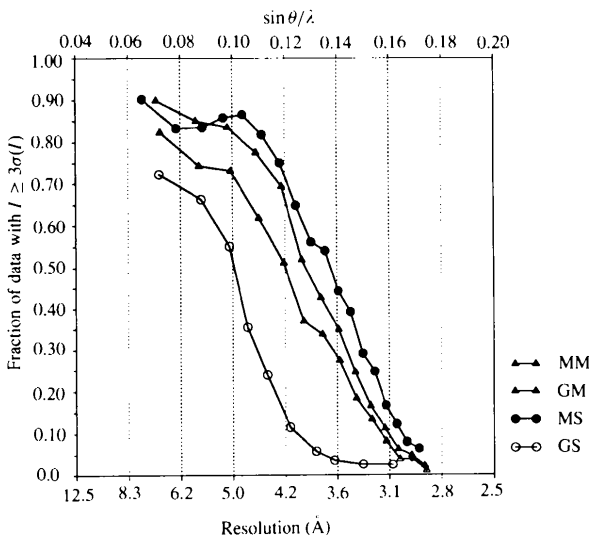

Fig. 1. Comparison of data from crystals grown in microgravity about space shuttle *Discovery* (MS) and space station *Mir* (MM) with data from crystals grown on earth in corresponding flight hardware and over the same span of time (GS and GM). More data were observed from the MS crystal despite the fact that it was about one quarter of the size of either the MM or GM crystal. The crystals grown in GS conditions scattered X-rays poorly.

It is remarkable that the largest crystal grown on the space shuttle (MS) was only somewhat larger than any crystals that grew during the same length of time and in the same equipment on earth, yet displayed much higher scattering ability. Further, in spite of a volume effectively about one-quarter of that for the *Mir* MM and GM crystals, the MS crystals clearly gave much better data. The improvement shown by the MS crystals was 0.5 to 1.0 Å over the best ground (GM), which is consistent with earlier studies (DeLucas *et al.*, 1986). We did not observe the same effect in the *Mir* experiment, possibly because the crystals grown on Earth were already quite large. However, it is interesting to note that both of the crystals grown in microgravity gave better data than the corresponding crystals grown on the ground.

5. Discussion

The crystals grown in space showed an improvement of at least 0.5 Å over the best crystal that was grown on the ground, and these results are consistent with studies that have shown that better quality protein crystals can be grown in microgravity than on earth. However, although caution must be employed comparing a limited number of data sets, the results indicate that the conditions surrounding the microgravity experiment may also be also important. It has usually been found that slower crystallization is advantageous, but we obtained better data from a smaller crystal that grew in space 7 d than from a larger crystal that was in microgravity over 55 d. There was no correlation between the temperature of crystallization and the quality of the resulting crystals; however, we had observed a strong temperature dependence during the crystallization trials, and the observed difference in crystal quality may be attributable to stricter control of the environment on the space shuttle. The temperature was kept at a constant 277 K, while on *Mir* it fluctuated in the range 293–298.4 K. During the first 12 d the average daily temperature decreased by 3.0 K. Moreover, on most days the temperature fluctuated within a range of 1.0–1.5 K. Fluctuations in temperature as small as 0.1 K have been conclusively shown to cause the veiling of lysozyme crystals (Monaco & Rosenberger, 1993).

One other effect detrimental to protein crystallization is vibration. Although records of the levels of vibration are not available, it is known that they are higher on board *Mir* than on the space shuttle (Stone, 1993).

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