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Crystallization of the collagen-like polypeptide $(PPG)_{10}$ aboard the International Space Station. 3. Analysis of residual acceleration-induced motion

Dario Castagnolo,[a] Chiara Piccolo,[a] Luigi Carotenuto,[a] Alessandro Vergara[b] and Adriana Zagari[c,d]*

[a]MARS Center, via E. Gianturco 31, 80146 Napoli, Italy, [b]Dipartimento di Chimica, Università degli Studi di Napoli, Monte S. Angelo, 80126 Napoli, Italy, [c]Dipartimento di Chimica Biologica, Università degli Studi di Napoli, via Mezzocannone 6, 80134, Napoli, Italy, and [d]Istituto di Biostrutture e Bioimmagini, via Mezzocannone 6, 80134, Napoli, Italy

Correspondence e-mail: zagari@chemistry.unina.it

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$(PPG)_{10}$ crystallization experiments onboard the ISS using the Advanced Protein Crystallization Facility have shown parallel and coherent crystal motions. The residual acceleration profiles and the history of the ISS Increment 3 mission allow a quantitative interpretation of these motions. Two events determine the observed crystal motions: the undocking of the Space Shuttle and a change in the ISS attitude required for power generation. No correlation between these motions and the crystal quality is apparent.

1. Introduction

Reduced-gravity environments (μ*g*) have several benefits for protein crystallization (Kundrot *et al.*, 2001; DeLucas, 2001; Lorber, 2002; Vergara *et al.*, 2003). Since the early 1980s, protein crystallization experiments have been carried out in μ*g* (Littke, 1984), first aboard Space Shuttles and more recently aboard the International Space Station (ISS).

Despite the optimistic scenario envisaged for μ*g*, it is not the quiescent environment it is supposed to be. Several kinds of motion have been observed by optical microscopy onboard the Space Shuttle and the International Space Station (ISS) (Boggon *et al.*, 1998; García-Ruiz & Otalorá, 1997; Lorber *et al.*, 2000; Kundrot *et al.*, 2001; Vergara *et al.*, 2003). Very often only a few crystals moved, with speeds ranging from 1 to 200 $\mu m\ h^{-1}$, even in the presence of spurts and lulls. The effect of these motions on the crystal quality has been discussed in experimental (Snell *et al.*, 1997; Esposito *et al.*, 1998; Otalorá *et al.*, 1999) and theoretical (Vekilov & Alexander, 2000) works and has been the subject of several review articles (Boggon *et al.*, 1998; Kundrot *et al.*, 2001; Vergara *et al.*, 2003).

The only attempt to find a correlation between acceleration data and observed motions was performed by Snell *et al.* (1997). Although the acceleration measurement system SAMS used during the IML-2 mission did not provide residual gravity acceleration measurements (Canopus Systems Inc., 1996, 1997; Jules *et al.*, 2001), a qualitative agreement between crystal motions and accelerations was found. On the same issue, this paper intends to investigate the correlation between residual accelerations measured by the Microgravity Acceleration Measurement System (MAMS) recorded onboard ISS and crystal motions, and eventually their effect on crystal quality.

Several theories have been proposed regarding the effect of μ*g* on crystal quality and these have recently been reviewed by Vergara *et al.* (2003). In particular, Vekilov's theory (Vekilov & Alexander, 2000) refers to the effect of acceleration on the crystal quality, one of the issues treated here. This theory gives a system-dependent interpretation of the μ*g* effect: it is beneficial for crystals with a diffusion-controlled growth rate and detrimental for crystals with a kinetically controlled growth rate. Conversely, forced flow on earth or in hyper-gravity environments is supposedly beneficial for crystals with kinetically controlled growth.

In the framework of our current research on protein crystallization under μ*g*, we carried out a crystallization experiment on a collagen-like polypeptide $(PPG)_{10}$ onboard the ISS. A complete set of crystallization environment comparisons could be performed: in solution on earth, in solution under μ*g*, in gel on earth and in gel under μ*g*. The results of the space mission ISS-3 are presented in three parts. $(PPG)_{10}$ crystallization conditions and the video imaging are reported in Part 1 (Vergara *et al.*, 2002); X-ray diffraction analysis of $(PPG)_{10}$ crystals is reported in Part 2 (Berisio *et al.*, 2002).

The two major issues in the present analysis are (i) the comparison of the acceleration profile and crystal motions and (ii) the influence of motions on crystal quality.

In order to prevent drift of the crystals, agarose gel has extensively been used as crystallization medium both on earth and under μ*g* (Biertümpfel *et al.*, 2002). Here, the outcome of crystallization in gel during the ISS Increment 3 (ISS-3) mission is used as a reference for results in a quiescent environment.

2. Methods and materials

Comparisons of acceleration profile and crystal motions require definition of reference frames for both the ISS and the Advanced Protein Crystallization Facility (APCF), in which crystals were grown. Details of the APCF have been reported elsewhere (Bosch *et al.*, 1992; Vergara *et al.*, 2003).

The Principal Investigator Microgravity Services (PIMS) report acceleration data to the microgravity scientific community using the ISS analysis coordinate system (X, Y, Z), which is shown in Fig. 1. The ISS analysis system is derived using the local vertical/local horizontal (LVLH) flight orientation. The origin is located at the geometric centre of the Integrated Truss Segment (Jules *et al.*, 2001). The X axis is parallel to the longitudinal axis of the module cluster. The positive X axis is in the forward (flight) direction. The nominal α joint rotational axis is parallel with Y. The positive Y axis is in the starboard direction. The positive Z axis is in the direction of the nadir and completes the right-handed Cartesian system.

During the ISS-3 mission (August–December 2001), the APCF was located in position 4 of rack 1, which was in position O (overhead) 2 (Jules *et al.*, 2001). Since the ISS was mainly in the XVV (X body axis toward the velocity vector) Z-nadir TEA (torque equilibrium attitude) flight attitude, the APCF coordinate system (x, y, z) results as being oriented such that $x = -Z$, $z = X$, $y = Y$.

The signal reported by the Microgravity Acceleration Measurement System (MAMS) Orbital Sensor Subsystem (OSS) onboard the ISS provides the principal investigator with a measure of the residual gravity acting on the experiment. MAMS–OSS data are collected at ten samples per second, bandpass filtered with a cutoff frequency of 1 Hz and sent to Ground Support Equipment (GSE) for further processing and storage. There are numerous gaps in the data owing to data transfer and other problems that arose from being a payload during early ISS operations. PIMS is currently storing the OSS data as raw acceleration files and also as trimmean-filtered data that are compensated for bias. The adaptive trimmean filter provides an estimate of the quasi-steady acceleration signal by rejecting higher magnitude transients such as thruster firings, crew activity *etc*. The trimmean-filter algorithm used by the MAMS GSE operates on a sliding window of 480 samples every 16 s. The filtering procedure sorts the data by magnitude, calculates the deviation from a normal distribution and trims an adaptively determined amount from the tails of the data. The quasi-steady acceleration level is computed as being the arithmetic mean of the trimmed set. Further information concerning the trimmean filter can be found in Hogg (1974) and in Canopus Systems Inc. (1996, 1997).

Methods and materials referring to $(PPG)_{10}$ crystallization experiments on the ISS are discussed in detail in Part 1 of this series (Vergara *et al.*, 2002). In particular, motions were monitored for five selected crystals during the entire period of experimentation in μ*g* and the following analysis refers to these crystal motions.

3. Residual acceleration and crystal motions

Prior to searching for a correlation between accelerations and crystal motions we considered: (i) the source of the acceleration during ISS-3 and (ii) the motions observed aboard the two μ*g* platforms (Space Shuttle and ISS).

(i) The quasi-steady regime is comprised of accelerations with frequency content below 0.01 Hz and magnitudes expected to be of the order of 2 μ*g* or less. These low-frequency accelerations are associated with phenomena related to the orbital rate, primarily aerodynamic drag. However, gravity gradient and rotational effects may dominate in this regime, depending on various conditions and on the location of the experiment relative to the vehicle's centre of mass. A final source of acceleration to consider in this regime is venting of air or water from the spacecraft. This action results in a nearly constant low-level propulsive force. The different quasi-steady environment characteristics seen on the ISS-3 are primarily related to the altitude and attitude of the station. Variation in atmospheric density with time and altitude contribute to the differences in the aerodynamic drag component. Different attitudes will affect the drag component owing to the variation of the frontal cross-sectional area of the station with respect to the velocity vector.

(ii) Comparing ISS (Vergara *et al.*, 2002) and Space Shuttle missions (Carotenuto *et al.*, 2001), comparable average velocity and crystal growth rate of $(PPG)_{10}$ have been observed, but completely different kinds of motion: onboard the Space Shuttle a simultaneous motion towards casual directions occurs, whereas aboard the ISS simultaneous and parallel motions were observed. This is related to peculiarities of the ISS. A complete list of crystal motions observed in the APCF in various missions over the years is reported elsewhere (Vergara *et al.*, 2003).

Since the MAMS sensor is located in the same rack in which the APCF lies (Jules *et al.*, 2001), both gravity-gradient and rotational drag accelerations can be considered to be negligible in the MAMS coordinate system. A comparison with Snell *et al.* (1997) is difficult because SAMS, in contrast to MAMS, measures accelerations caused by vehicle, crew and experiment disturbances (vibratory/transient accelerations, which occur in the frequency range 0.01–400 Hz).

In order to analyze the complete time spectrum of the experiment, the acceleration data in the ISS system g_X, g_Z have been

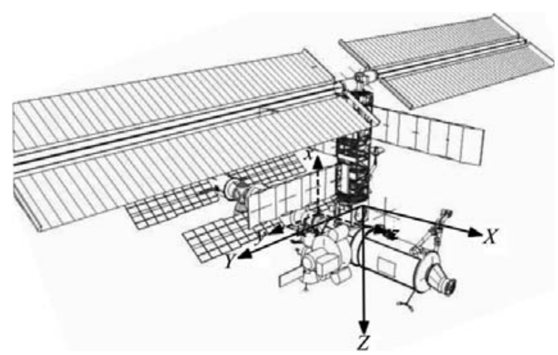

Figure 1
ISS coordinate system (X, Y, Z) in the ISS configuration during Increment 3. (x, y, z) is the coordinate system of the APCF facility. The xyz origin represents where the MAMS sensor and APCF were allocated.

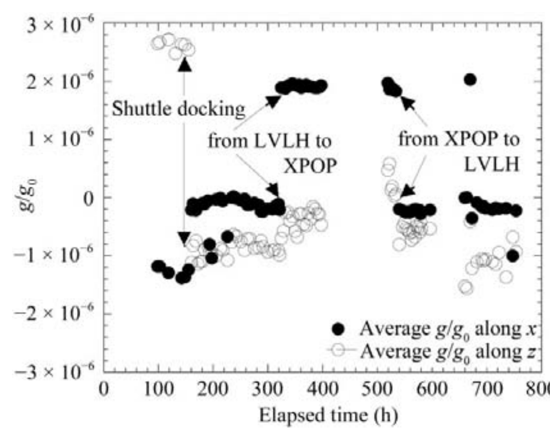

Figure 2
Daily average of MAMS gravity-level data (g/g_0, with 1 corresponding to gravity on earth) from 18/8/2001 to 20/9/2001. Two events caused steep variation: the Space Shuttle undocking from the ISS and the changes in attitude (from LVLH to XPOP and *vice versa*). LVLH refers to the standard ISS attitude, whereas XPOP refers to the sun-tracking attitude adopted during power generation.

averaged for each sample data subsystem each day and reported in Fig. 2. The overall paths of $(PPG)_{10}$ crystals along x during the 960 h of experimentation inside the APCF have been shown in Fig. 2 of Part 1 (Vergara *et al.*, 2002), whereas along the y and z axis they are much smaller. Data along both x and z show all the crystals to have almost coherent and parallel displacements.

The component g_Z is the driver for the displacement along x, while there is a marginal correspondence between g_X and displacements along z. $(PPG)_{10}$ crystal paths along the x axis indicated a movement that changes direction 160 h after reactor activation. On the other hand, the data of crystal movements along the z axis did not show similar variations. The residual gravity accelerations measured during the same period show a change of g_Z occurring late on 20 August, which corresponds to this change of crystal drift in the x direction.

This change of g_Z sign coincides with shuttle STS-105 undocking. Therefore, it would be recommendable to start experimentation after shuttle undocking for future crystal-growth experiments.

Furthermore, the change of sign for g_X reported in Fig. 2 after 300 h corresponds to a change of ISS attitude from LVLH to XPOP (X principal axis perpendicular to the orbit plane) that lasted about 9 d. XPOP is a sun-tracking quasi-inertial flight attitude used for power generation. Nevertheless, this event has a slight impact on crystal drift since only a moderate slowing down occurred for all the crystals both along x and z.

4. Crystal quality

The crystal quality of $(PPG)_{10}$ showed a significant improvement in the diffraction resolution limit achieved from µ*g* experimentation during the STS-95 mission (Berisio *et al.*, 2000), whereas during the ISS-3 mission the maximum resolution was the same as that for earth-grown crystals (Berisio *et al.*, 2002). In both cases an APCF dialysis reactor was used (Carotenuto *et al.*, 2001; Vergara *et al.*, 2002), but in the first case asynchronous and incoherent motions were observed and in the second case synchronous and parallel motions were observed. Furthermore, crystals grown in gel on earth and under µ*g* (both motionless) showed an even lower maximum resolution (Berisio *et al.*, 2002). Therefore, in this case there is no correlation between crystal motions and $(PPG)_{10}$ crystal quality. This evidence seems to be out of line with theories invoking this correlation.

It is worth comparing the quality rank mentioned above with previous investigations of various protein systems crystallized in the APCF, where motions have either been observed or supposed to occur. With regard to the comparable crystal quality between the two sub-environments earth and µ*g*, we recall a recent statistical analysis restricted to APCF experiments (Vergara *et al.*, 2003): the result was that 52% of experiments corresponded to improved quality in µ*g*, 39% to unchanged quality (as in the present case) and 9% to worse quality. With regard to the comparable crystal quality between gel on ground and gel in µ*g*, the only suitable comparison is with Lorber *et al.* (1999), in which the same resolution was observed (as in our case), but a lower mosaicity was measured from µ*g*-grown crystals. Unfortunately, no topography or rocking-curve analysis is available for $(PPG)_{10}$ crystals. As regards the comparison between solution and gel performed in other studies (Miller *et al.*, 1992; Otalorá *et al.*, 1999; Zhu *et al.*, 2001), this is the first case reporting a decrease of crystal quality on going from solution to gel. It is possible that some specific effects of the $(PPG)_{10}$ –gel pair invert the usual trends, as discussed in Part 2 (Berisio *et al.*, 2002).

Therefore, considering the effect of µ*g* on $(PPG)_{10}$ crystals grown during ISS-3 missions in the four investigated environments, no apparent correlation between crystal quality and crystal motion can be assessed, which is consistent with the high number of interplaying parameters affecting the crystallization.

5. Conclusions

A comparative analysis of the effects of motion on $(PPG)_{10}$ crystallogenesis and crystal quality onboard two different µ*g* platforms (Space Shuttle and ISS) and in four different crystallization environments (solution on earth, solution under µ*g*, gel on earth and gel under µ*g*) has been reported. Equal average velocity of crystals but different kinds of motion were observed onboard the ISS and Space Shuttle: acceleration onboard the ISS produces synchronous and parallel crystal motions. The interpretation is enclosed in the peculiar residual acceleration profile onboard the ISS.

MAMS and crystal size experimental data quantitatively justify the observed crystal motions. Therefore, for the first time, a direct correlation between the crystal motions and acceleration has been proved. A change in the ISS attitude required for power generation does not significantly influence the crystal motion. Conversely, an inversion in the direction of the crystal motion arose from the undocking of the Space Shuttle. The useful lesson learnt for future experimentation on the ISS is to recommend starting protein crystallization in solution after this event, whenever possible.

Finally, no correlation between motions and $(PPG)_{10}$ crystal quality has been observed.

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