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Review

Lessons from crystals grown in the Advanced Protein Crystallisation Facility for conventional crystallisation applied to structural biology

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Abstract

The crystallographic quality of protein crystals that were grown in microgravity has been compared to that of crystals that were grown in parallel on earth gravity under otherwise identical conditions. A goal of this comparison was to assess if a more accurate 3D-structure can be derived from crystallographic analysis of the former crystals. Therefore, the properties of crystals prepared with the Advanced Protein Crystallisation Facility (APCF) on earth and in orbit during the last decade were evaluated. A statistical analysis reveals that about half of the crystals produced under microgravity had a superior X-ray diffraction limit with respect of terrestrial controls. Eleven protein structures could be determined at previously unachieved resolutions using crystals obtained in the APCF. Microgravity induced features of the most relevant structures are reported. A second goal of this study was to identify the cause of the crystal quality enhancement useful for structure determination. No correlations between the effect of microgravity and other system-dependent parameters, such as isoelectric point or crystal solvent content, were found except the reduced convection during the crystallisation process. Thus, crystal growth under diffusive regime appears to be the key parameter explaining the beneficial effect of microgravity on crystal quality. The mimicry of these effects on earth in gels or in capillary tubes is discussed and the practical consequences for structural biology highlighted.

Keywords: Protein structure; Crystal quality; Microgravity; Diffusion; Advanced Protein Crystallisation Facility

Contents

Abbreviations: μg, microgravity; ADH, alcohol dehydrogenase; APCF, Advanced Protein Crystallisation Facility; AspRS-1, aspartyl-tRNA synthetase (form 1 of eubacterial type); B, atomic displacement parameter; CcdB, poison of DNA-topoisomerase II complexes; *d*, resolution limit; ESA, European Space Agency; FWHM, Full Width at Half Maximum; g, ground; Hyp, hydroxyproline; I/σ, intensity to standard deviation ratio; ISS, International Space Station; M_r, molecular weight; DCAM, Diffusion Controlled Crystallisation Apparatus for Microgravity; PCDF, Protein Crystallisation Diagnostic Facility; PDB, Protein Data Bank; $(PPG)_{10}$, synthetic polypeptide $(Pro–Pro–Gly)_{10}$; VDA, Vapour Diffusion Apparatus.

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1. Background

Crystal production is frequently a bottleneck in protein crystallography [1–3]. Nowadays, this is especially true for membrane proteins [4] and high-throughput approaches applied to drug discovery [5] and proteomics [6,7]. In the latter context, it is usually observed that only 1 out of 10 target proteins yields crystals that are suitable for structure determination [6,8]. A reason is the plastic architecture of proteins that decreases their propensity to crystallise. Therefore, in all current protein crystallisation methods, homogeneous solutions are driven smoothly towards supersaturation where nucleation and growth occur. On the other hand, the crystallisation process itself is influenced by a diversity of variables including molecular purity, ionic strength, pH, composition, temperature, pressure and gravity, making the whole process complex. Given these difficulties it is understandable that structural biologists eagerly await any effort aimed to develop strategies to increase the success rate of protein crystallisation [9–11].

Early studies on inorganic and small organic molecules had shown that any difference between the crystal and solution densities triggers buoyancy-driven convection and crystal sedimentation or floating. These phenomena are detrimental to crystal quality because they perturb mass transport during crystal nucleation and growth. This gravity-dependent effect is weaker under weightlessness [12]. As a consequence, microgravity was envisioned to have a favourable influence on protein crystal growth. In addition, the small size of many earth-grown crystals and/or the poor diffraction properties of others were as many reasons for undertaking crystallisation under microgravity. In 1984 the feasibility of such experiments was demonstrated by the growth of voluminous lysozyme crystals aboard an orbiter [13]. Despite the potential promises, such studies were immediately criticised (e.g. [14]), among other reasons were the excessive cost of assays and limited flight opportunities. After two decades of experimentation in space, the criticism that stays is the marginal contribution of microgravity research to the development of structural biology [15], compared to that of the advances in X-ray and computing facilities and in high-throughput technologies applied to crystallisation. Despite this apparently negative appraisal, it is generally accepted that microgravity research contributed to the better understanding of the crystallisation process and the parameters governing crystal quality (reviewed in [16]). Further, a number of data obtained recently with other facilities support the view that space-grown crystals can be useful for structural biology [17–23]. However, until now this potential has not been enough exploited because it was not exhaustively analysed and well explained.

It is the aim of this review to bring clarification in the microgravity/structural biology dispute and to highlight the positive trends. Conclusions are based on results collected over more than one decade in the Advanced Protein Crystallisation Facility (APCF) with emphasis to those allowing better determination of protein 3D-structures. In order to encourage structural biologists to benefit from the knowledge gained from microgravity research, it describes also how conventional crystallisation assays can be modified to create an environment mimicking the one encountered under microgravity.

2. Experimentation in the APCF

Aboard space shuttles or space stations, the gravity level is 10^3- to 10^6-fold lower than at the earth's surface. Several sophisticated instruments have been built over the last two decades to investigate protein crystallisation in such environments (e.g. [13,24–27]). A research program of the European Space Agency (ESA) that involved a number of research laboratories was directed at developing the APCF [28,29] that was built by Astrium GmbH (Germany). This instrument accommodates 48 reactors (with protein volumes ranging from 4 to 470 μl) and operates according to either of three crystallisation techniques (vapour diffusion, dialysis or free interface diffusion) (Fig. 1). The crystallisation process can be monitored with a video camera and an interferometer [30].

The APCF was aboard 7 space missions from which 6 yielded results (Table 1). From June 1993 to December 2002, 474 individual crystallisation assays in all were conducted on a total of 46 different biological particles, including mutants and various crystal forms. These numbers can be compared with those of ~50 missions that carried into space ~10,000 assays in various