Volume 259, number 1, 194-198 FEB 07886 December 1989

Crystallization of proteins under microgravity

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Received 25 August 1989; revised version received 16 October 1989

For the crystallization of proteins under microgravity conditions, a Chinese re-entry system was used, in which 101 experiments of 25 different biological macromolecules were accommodated. From the results obtained we conclude that under microgravity conditions crystal growth can only be expected under those crystallization conditions which also permit crystal growth on earth. A number of space-grown crystals were larger in size and of a better quality in their ability to diffract X-rays than the corresponding ground control crystals grown at the Chinese launch site. However, the space-grown crystals have not reached the X-ray diffraction quality of the crystals obtained under optimal conditions in the home laboratories.

Protein crystallization; Microgravity

1. INTRODUCTION

Growth of protein crystals suitable for X-ray diffraction is a complex process governed by a large number of factors such as pH, concentration of protein and precipitant, purity of the sample, temperature etc., the influence and interplay of which is poorly understood. The influence of gravity on nucleation and growth of protein crystals is also uncertain. Gravity is generally considered unfavourable since it gives rise to convection in the samples and, in many cases, to sedimentation of the crystal nuclei. To assess the role of gravity in protein crystallization, experiments have been carried out in space crafts under microgravity in recent years, but it proved difficult to interpret the results in an unambiguous way. Early reports on protein crystallization experiments on board of Spacelab 1 [1,2] did not quantify the absolute sizes and the X-ray diffraction quality of the crystals obtained, whereas subsequent experiments suffered from various malfunctions [3-5]. In order to clarify the issue, a payload called COSIMA I was launched into space on August 5, 1988, on an unmanned Chinese re-entry system and recovered eight days later. The results of the experiments are reported in this communication.

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2. MATERIALS AND METHODS

A Long March CZ-2C rocket carried the Chinese re-entry capsule FWS-1 in an orbit of 319.5 km apogee and 205 km perigee. The system was launched from the Juiquan space center (Inner Mongolia). The quality of microgravity was stated to be better than $10^{-4} \times g$ by the China Academy of Science and Technology. During the re-entry process the deceleration level increased to $13 \times g$ and ended by opening of a parachute causing a shock of $60 \times g$ for a period of 5-10 ms. The re-entry occurred in less than 10 min.

The crystallization vials for all 101 samples consisted of a flexible transparent tube welded on one end and stuck together with a glass test tube at the other [6] (fig.1). The vials were placed into a $22 \times 21 \times 15$ cm box which was kept at a constant temperature of 20 ± 0.5°C. Ground control experiments were carried out at the launch center, using a second identical set of 101 samples. Here, the sample processing unit was gently turned 180° twice a day because it was thought that otherwise flow of the solutions along the bottom of the vials and mixing might occur.

3. RESULTS AND DISCUSSION

The time schedule of the mission is shown in table 1. The rather long period of 3 weeks between collection of samples and launch was probably not suitable for all proteins. Within 2-3 days after landing, the results were centrally documented by visual inspection and photographing, without opening the vials. All samples were then distributed to the research groups for opening and evaluating sizes, shapes and X-ray qualities of the obtained crystals. Among the 101 samples, 45 did not yield

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00145793/89/$3.50

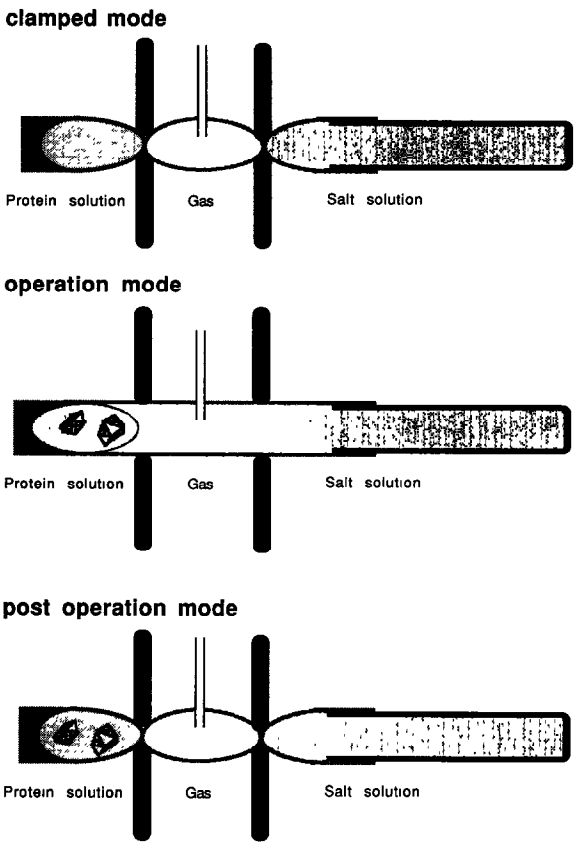

Fig.1. Crystallization vials used for space and ground control experiments. The flexible tube contained the protein solution (5–90 μl) and the glass tube contained the salt solution (600 μl) for equilibration. To prevent the crystallization process from being started before reaching the orbit both solutions were separated by clamping the flexible tubes. After reaching the orbit, the clamps were opened and the equilibration between both solutions was started via a gas compartment of 8 mm length. Before leaving the orbit the compartments were clamped again and left in this state until they returned to the laboratories for analysis. An open capillary was fixed in the wall of the flexible tube to guarantee a pressure compensation during opening and closing of the clamps. The small diameter (0.45 mm) and the length (10 mm) of the capillary prevented diffusion of significant amounts.

any crystals and 27 did not yield crystals sufficiently large for X-ray analysis, neither in the microgravity experiments nor in the ground controls. A total of 29 samples from nine different proteins yielded crystals which were analyzed in the home laboratories; the results obtained with seven of these proteins are summarized in table 2 and fig.2.

An interesting change of morphology has been observed with the space-grown crystals of elongation factor Tu (protein no. 3, table 2): These were complete concave lenses (fig.2-2A), whereas all crystals of this complex grown so far on earth were fragments thereof [7] (fig.2-2B). Presumably owing to their more compact shape these space-grown crystals were mechanically more stable during mounting in X-ray capillaries. For *Streptomyces* lysozyme (Hilgenfeld et al., unpublished; fig.2-4A/B) many of the rod-like crystals grown under microgravity showed fractures perpendicular to the rod axis, and even intact crystals tended to exhibit broad reflection profiles. On the other hand, a transport-sensitive though not rod-like crystal of the ribosomal 50S subunit [8], which was included in the mission, could be recollected without damage.

While other observations made with individual proteins are mentioned in the legend to table 2, several general tendencies can be derived from our experiments: (i) crystal growth only occurred in space under conditions which yielded crystals on earth; (ii) the maximum volume of space-grown crystals were to the most part larger than crystals grown in the corresponding ground experiments (except for protein no. 5, table 2); (iii) the maximum resolution of the diffraction pattern of the space-grown crystals tended to be higher than for the ground control crystals, indicating that the internal order of the space-grown crystals was better than for the ground control crystals; (iv) sizes and X-ray diffraction qualities of crystals obtained in the home laboratories under optimal conditions were in general unequalled by either space-grown or ground control crystals; (v) space groups and unit cell dimensions of the crystals grown in space were identical to those of crystals obtained under terrestrial conditions; (vi) in cases where this was analyzed (proteins nos. 4–6), the internal order of space-grown crystals as expressed by the mosaic spread was inferior to that of the ground control (proteins nos. 4,5) and to that of the home laboratory-grown (proteins nos. 4–6) crystals.

In conclusion, some interesting effects of microgravity have been demonstrated, but in no case there is an improvement over the crystallization outcome in the individual labs noticeable. It should be kept in mind that besides gravity there are a number of other parameters that influence the crystallization of biological macromolecules. They deserve much more systematic consideration than they are given today.

Table 1

Time schedule of the COSIMA mission in 1988

	Date	Sample temperature
Collection of samples at the laboratories	July 11–13	0°C
Transport to the launch site	July 16–22	0°C
Integration in flight hardware	July 23–26	4–7°C
Launch	Aug 5	4–7°C
Crystallization in orbit	Aug 5–13	20°C
Landing	Aug 13	20°C
Photographic documentation in China	Aug 15–16	20°C
Distribution of the samples to the laboratories	Aug 25–26	20°C
X-ray analysis	Aug 30–Sept 24	20°C
Performance of the ground control experiments	Aug 1–9	20°C

The time between opening and closing of the clamps was 187 h 40 min

Table 2

Comparison of crystals grown under microgravity and on earth

	Project 1			Project 2			Project 3			Project 4			Project 5			Project 6			Project 7		
Protein	vanadate peroxidase			TET repressor			EF-Tu × GDP			adenylate kinase (wild type)			adenylate kinase (mutant)			lysozyme			thermolysin		
Source							*Thermus aquaticus*									*Strept. coelicolor*			*B. thermoproteolyt.*		
Crystallization conditions, references	[9]						[7]			[10]			[10]			(unpublished)			modified after [11]		
Origin of crystals*	sp	gr	lab	sp[a]	gr[a]	lab[a]	sp[b]	gr[b]	lab[b]	sp	gr	lab	sp	gr	lab	sp[f]	gr	lab	sp	gr	lab
Number in fig.2	–	–	–	1A	1B	–	2A	2B	–	3A	3B	–	–	–	–	4A	4B	–	5A	5B	
Vol. of protein sol. (μl)	15	15	15	15	15	15	10	5	5	20	20	20	20	20	20	60	60	20	60	60	10
Temperature (°C)	20	20		20	20		20	20	4	20	20		20	20		20	20	20	20	20	20
Crystalliz. time (days)	8	8		8	8		8	8	30	8	8		8	8		8	8	3	8	8	6
Maximum crystal size (μm) l	70	nc	500	250	150	400	300	200	700	1500	2000	1200	500	1000	1000	800	350	800	1100	120	900
w	50	nc	150	150	100	200	300	100	300	650	400	500	250	500	400	120	60	120	120	10	200
h	50	nc	150	100	80	100	100	20	140	70	35[c]	150	15[e]	20[d]	20[d]	120	60	120	120	10[g]	200
Maximum crystal volume (%)	2	nc	100	47	15	100	26	2	100	75	31	100	20	100	80	100	11	100	44	0	100
X-ray source and detector**	A	–	A	A	A	A	B	B	B	C	C	C	C	C	C	D,E	D,E	D,E	D	–	D
Maximum resolution (A)	5	nc	2.4	5	7	2.8	3.2	3.5	2.6	2.5	2.5	1.8	3.3	3.3	3	3.1	3.7	3.0	2.3	–	1.8
Mosaic spread	nd	nc	nd	nd	nd	nd	nd	nd	nd	0.25	0.20	0.18	0.9	0.7	0.65	0.4	–	0.3	nd	nd	nd

Maximum values for crystal sizes are given throughout. nc, no crystals observed; nd, not determined; *sp, crystals grown in space during mission; gr, crystals grown in parallel ground control experiments during mission; lab, crystals grown under optimal conditions in home laboratories; **A, Elliott GX-20 rotating anode generator, X-ray film; B, DESY synchrotron (positron side), λ = 1.473 Å, X-ray film; C, Elliott GX-6 rotating anode, X-ray film, Nicolet P2$_1$ diffractometer; D, Elliott GX-21 rotating anode, FAST area detector; E, Rigaku RU-200 rotating anode, Nicolet area detector

[a] Many of the space crystals looked like a cross, with two prisms intergrown (fig.2-1A). Ground control and laboratory crystals were single prisms

[b] Space crystals are perfect concave lenses (fig.2-2A). Crystals grown under terrestrial conditions are fragments thereof (fig.2-2B)

[c] The general thickness of gr crystals is 60 μm, only the gr crystal with the largest volume is 35 μm thick

[d] Crystals are badly twinned

[e] Crystals are even worsely twinned than in d

[f] Space-grown crystals tend to have cracks perpendicular to the rod axis (fig.2-4A)

[g] The ground control experiments were surprisingly unsuccessful, yielding thousands of thin needles (fig.2-5B)

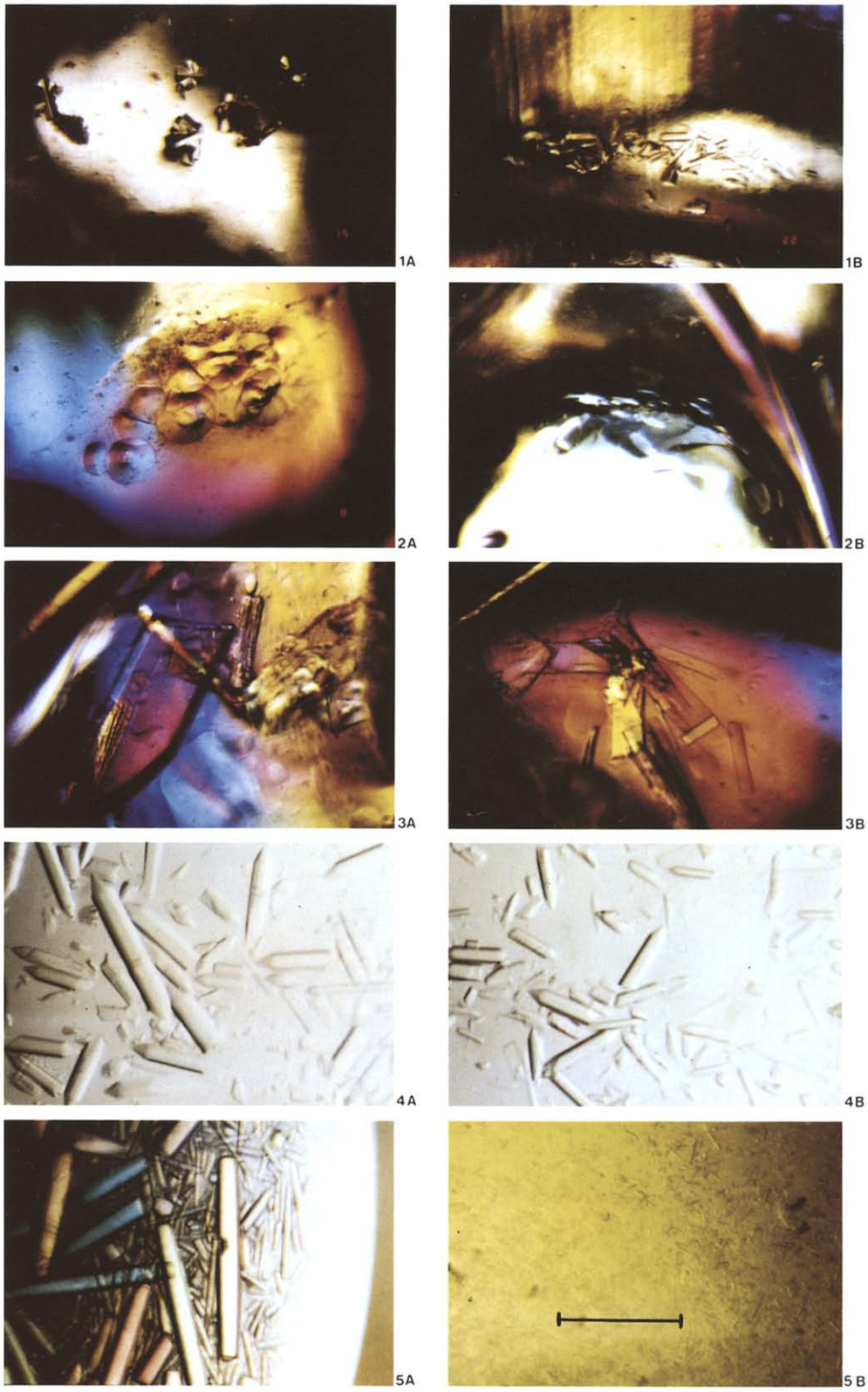

Fig.2. Crystals grown in space (A) and in ground control experiments (B). (1) TET repressor; (2) Elongation factor Tu from *Thermus aquaticus* GDP complex; (3) Adenylate kinase (wild type protein); (4) Lysozyme from *Streptomyces coelicolor*; (5) Thermolysin. Bar = 0.6 mm.

Acknowledgements: E. coli strains producing wild type and mutant PL9 adenylate kinase were kindly provided by Dr Wittinghofer, Heidelberg, FRG. Thanks are due to Dr Hecht for making his Nicolet area detector available. We are grateful for financial support from Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 9; Leibniz-Programm; Bundesminister für Forschung und Technologie; and Fonds der Chemischen Industrie to W.S. and V.A.E.; Deutsche Forschungs- und Versuchsanstalt für Luft- und Raumfahrt; and EMBO to V.A.E.; and Graduiertenkolleg Polymerwissenschaften, Freiburg, to R.H.

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