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The Space Experiment of Protein Crystallization aboard the Chinese Spacecraft SZ-3

Using new flight hardware, a Chinese mission of space protein crystallization has been performed aboard the Chinese spacecraft SZ-3. Preliminary analyses of the experimental results have shown that a few proteins produced better crystals in space. At least, the crystals of cytochrome b5 mutant could diffract X-ray beyond the highest resolution reported so far for the same kind of crystals. In addition, some rules derived from our numerical studies of the liquid/liquid diffusion protein crystallization were proved by the crystallization of lysozyme as model protein in this space experiment, which also clearly showed the advantages and disadvantages of the gelation of the protein solution used in microgravity growth of protein crystals.

Introduction

Due to the peculiar properties of molecules and their crystals, the crystallization of biological macromolecules is a complicated dynamic process depending on many factors. So the growth of good-quality protein crystals is a difficult task and still is the bottleneck of biomacromolecular crystallography, which is the most powerful tool for understanding and exploiting biological macromolecules in life science and biotechnology. Absence or reduction of gravity-driven convection and sedimentation in microgravity makes the crystallization of biological macromolecules an important space biotechnology. The advances in relevant experiments and studies have shown that microgravity could improve the protein crystal growth. At least in some cases, protein crystals of better-quality have been grown in microgravity [e.g.1-3]. However, only a limited success has been made so far due to the complexity of space protein crystallization. The main problem for this space biotechnology is a low success rate of space experiments, usually in the range 20-30% for the proteins involved in the experiments for the first time. The main reason for this is that in many cases the potential benefits from microgravity environment has not been fully exploited [4]. After the space experiments of protein crystallization, which were carried out aboard the Chinese recoverable satellites [5,6], a new flight hardware has been developed [7,8], and used for the mission of protein crystallization aboard the Chinese spacecraft SZ-3, the third unmanned spaceship which launched on March 25, 2002. The experiment and its results of preliminary analyses are given below.

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Materials and Methods

In total 14 proteins and two mutants of one protein were involved in this mission, and their samples were provided by more than 13 research groups from Chinese and Canadian universities and institutes (see Table 1 and references 7-14). The flight hardware was made by Shanghai Institute of Technical Physics in collaboration with Beijing Institute of Biophysics, Chinese Academy of Sciences [7,8], and is the second generation of Chinese facilities for space protein crystallization. It could contain 50 vapor diffusion chambers with volume of protein solution from 10 to 30ul, 10 liquid/liquid diffusion chambers, and has an activation mechanism to ensure that the crystallization process could start or stop at certain time. The crystallization in 40 vapor diffusion chambers can be performed at temperature 20±1°C, and other 10 vapor diffusion chambers at temperature 4±1°C.

Experimental samples were collected in Beijing in the third week prior to launch, and the sample preparation and loading occurred within 2 days before the launch. Except the two Canadian proteins which each shared 6 vapor diffusion chambers, each of 11 proteins and a mutant of nitrogenase MoFe protein shared on average three vapor diffusion chambers. Among

them, bar-headed goose hemoglobin and neutral phospholipase A2 were crystallized at temperature 4°C, and other proteins at temperature 20°C. Lysozyme and another mutant of nitrogenase MoFe protein were crystallized using the liquid/liquid diffusion chambers. Duration of crystal growth in space was about 7 days from 25/3 to 1/4/2002. The recovery hardware was transported by a helicopter for harvesting and examining the crystals in a laboratory, Institute of Biophysics, Beijing. The crystals grown in this mission were first examined by microscopy, and then frozen for X-ray diffraction. The diffraction limits of the frozen crystals were obtained by a MarResearch IP detector with a low-power fixed X-ray anode.

Protein	*Supplier*	*PI & Reference*
Human dehydroepiandrosterone sulfotransferase	CHUL Research Center and Laval University, Canada	S. X. Lin [9]
Mutant of cytochrome b5	Inst. of Organic Chem., CAS	Z. X. Xia [10]
Neutral PLA2	Inst. of Biophysics, CAS	S. Z. Chen
Hen egg-white Lysozyme	ibid	R. C. Bi
Gastrodia antifugal protein	ibid	D. C. Wang
Phosphoenolpyruvate carboxykinase	University of Saskatchewan, Canada	L.T. Delbaere [11]
Haemorrhagin I from snake venom	Inst. of Life Science, CUST	M.K. Teng [12]
Acutothrombin C	ibid	M. K. Teng
2 mutated nitrogenase MoFe proteins	Inst. of Botany, CAS	J.F. Huang [13]
Ca++ binding protein S100A1	Lab of Struc. Biol., Qinghua University	Z. Rao [14]
DNA binding protein Ssh10b	ibid	Z. Rao
Bar-headed goose hemoglobin	Inst. of Life Science, Peking University	Z. Q. Hua et al. [15]
Antibacterial polypeptide LC1	ibid	G.Y. Lu
Basic phospholipase A2	Inst. of Biophysics, CAS	Z. J. Lin et al [16]
Pig heart F1-ATPase	ibid	S.G. Li [17]

Table 1: Proteins involved in the space experiment

Results and Discussion

The rate of crystal production in the 60 samples is 75%, about the same as the ground control experiment. Among the 16 proteins involved in the mission, several proteins have yielded large crystals, i.e. mutant of cytochrome b5, phosphoenolpyruvate carboxykinase (PCK), human dehydroepiandrosterone sulfotransferase and lysozyme as model protein although the sizes of space-grown crystals are obviously larger than the ground-grown counterparts only in the cases of lysozyme and other two proteins, a mutant of nitrogenase MoFe protein and haemorrhagin I. The diffraction experiments of some larger protein crystals with a low-power X-ray source have shown that at least one kind of protein crystals grown in space could diffract to higher resolution than the known limit reported so far. For example, a large crystal of cytochrome b5 mutant could diffract X-ray beyond 1.7Å, much higher than 2.1 Å reported for the same kind of crystals [8]. In addition, partial diffraction intensities were collected with space- and ground-grown crystals of the cytochrome b5 mutant in similar size, and the graph of the signal-to-noise ratio of diffraction intensities as a resolution function (Fig. 1) has shown that the diffraction quality of the space-grown crystal is much better than the ground-grown counter-

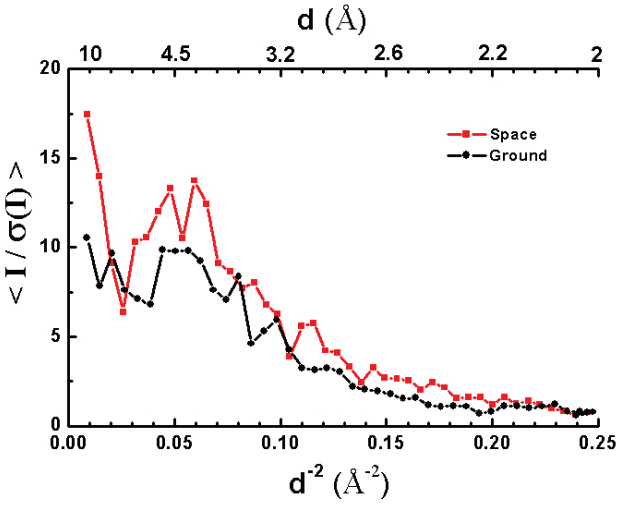

Fig.1.: Graph of signal-to-noise ratio of measured intensities as a function of resolution for the space-grown (▪) and Earth-grown (•) crystals of cytochrome b5 mutant.

Case No.	Lp/Ls mm/mm	Cp mg/ml	Cs w/v%	Cps w/v%	Results		
					Number	Max. size(mm)	Morphology
1	10.5/3.5	25	15	3	50	0.25	B
2*	10.5/3.5	25	24	0	150	0.25	A
3	10.5/3.5	25	24	0	4	0.7	B
4*	10.5/10.5	25	12	0	100	0.35	A
5	10.5/10.5	25	12	0	>200	0.1	C

* with agarose gel in protein solution.

Table 2: Parameters and results of liquid/liquid diffusion crystallization for lysozyme. Where Lp/Ls is the ratio of chamber lengths for the protein and salt solution; Cp, Cps are concentrations of protein, salt in the protein solution respectively, and Cs is the concentration of salt in the salt solution. A, B, C are simplified morphology grades in sequence from good to bad grade.

part. In addition, a space-grown PCK crystal could diffract to 1.8 Å resolution obviously higher than other PCK crystals grown in this mission. To get more and more accurate knowledge, further analyses, in particular the diffraction analysis of the crystals obtained in this experiment will be done subsequently. It should be hopeful to get some intensity data sets with synchrotron radiation source, which could be used for improving the accuracy of structural determination for a few proteins involved in this space experiment.

In this mission, the crystallization of lysozyme as model protein, focusing on method studies, was performed using the liquid/liquid diffusion chambers and different crystallization conditions. Some rules derived from our numerical studies [18] of the liquid/liquid diffusion have been verified by this experiment, i.e. the crystallization with better parameters regarding the ratio of the protein solution length to the salt solution one, the concentrations of protein and salt have produced much better results of lysozyme crystallization (See Table 2). In addition, the crystallizations with the same conditions (i.e., Case 2 and Case 4 in Table 2) have shown the following apparent advantages and disadvantages of gelled protein solution in microgravity-growth of protein crystals: the crystal morphology could be improved, but the number of nuclei increased. The former is consistent with the reported results for other protein crystals [19]. The latter shows that the microgravity could not alter the ground phenomenon that the agarose gel increases the nucleus number of lysozyme crystallization [20]. In addition, it is interesting to find that for the same crystallizing conditions, e.g. in Case 1, the space experiment produced more sea urchin-like crystals. At present, this could not be understood in detail. Moreover, crystals were observed in the precipitant solutions of both ground and space experiments. It is understandable for ground experiments, because the crystals would sediment in gravity environment. But for space experiment, it seems more complicated. G-jitter and other disturbances on the spacecraft may be responsible for this phenomenon. It can be seen that as in other cases of space experiments, the success rate of protein crystallization in this mission is low, and only a few proteins may produce better-quality crystals in space. This may be related to many factors. First, it depends on the crystallization duration and the number of samples shared by one protein. In our case, the time for crystallization in space was only 7 days, and a protein can share on average 4 crystallization chambers. In this case, several proteins, e.g. bar-headed goose hemoglobin and pig heart F1-ATPase, produced very small crystals due to the short crystallization period or the soft crystallization conditions e.g. lower precipitant concetrations in a few chambers. Although the crystallization conditions were optimized before the space experiment, the optimization was done on Earth, in particular under restricted conditions e.g. limiting the growth time to 7 days. This kind of restriction may lead to difficulties to grow better-quality crystals, because the optimized crystallization conditions may not be good conditions for growing the crystals in space. Furthermore, as an important problem in this biotechnology, the selection of experimental samples could not be based on the sensitivity to improve crystal quality by microgravity. Some related studies have shown that the effect of microgravity on protein crystallization may depend on the crystallization system, which may concern the properties of protein sample, crystallzation conditions and crystal properties etc. [e.g.21-23]. In order to understand and exploit the relevant intrinsic rules governing this space biotechnology, more efforts should be paid to mechanism studies of detailed improvement effect of microgravity on protein crystals. The technique improvement and sample selection based on these rules, without doubt, could increase the success rate of this kind of space experiments.

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