Impurity effects on protein crystal quality

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Introduction

There have been many attempts of protein crystal growth experiments in microgravity, and thousands of samples have been analysed [1]. It has been reported that microgravity had a positive effect on the crystal quality in many of the samples [2,3]. The effect of microgravity is generally understood as follows: (1) Because of the absence of convection, the depleted zone, i.e. a zone where the protein concentration is lower than the bulk solution because of protein consumption by the growing crystal, around the growing crystal will be maintained and growth will proceed slowly [4,5], (2) Because of the absence of convection, impurities which are preferentially incorporated into the crystal surface will initially be incorporated into the crystal, but later an impurity depletion zone will form which results in less impurity incorporation into the crystal [5,6], (3) Because of the absence of sedimentation, microcrystals formed in the bulk solution do not deposit on the growing crystal [4,7].

We have already proved that the crystal quality actually increases when the supersaturation is lower by using PF BL6A [8]. This supports the above-mentioned first possibility of microgravity effect. In this study, we focus on the above-mentioned second possibility that the impurity deteriorates the crystal quality. If impurity incorporation decreases the crystal quality, microgravity may work as a positive impurity filter.

Experiment

Materials and methods

Hen Egg White Lysozyme tetragonal crystals for X-ray data collection were crystallized by the conventional batch method at a temperature of 20 ºC. Lysozyme dimer was used as a model impurity. Covalently bound lysozyme dimer was purified from commercial lysozyme sample by re-crystallization, gel filtration and HPLC. 93% pure dimer sample was added to 99.99% pure lysozyme monomer sample for crystallization in various rates; 0%, 0.5%, 2% and 5%.

The crystal size was carefully controlled to be the same to compare the crystal quality without compensation. We collected all data at room temperature at the BL-6A of the Photon Factory (PF), Tsukuba, Japan. Nearly complete diffraction data sets were collected using an ADSC Quantum 4R CCD detector by the oscillation method with a wavelength of 0.978 Å. Fifteen samples were analyzed in total. The X-ray diffraction data were auto-indexed and integrated using the program *DPS/MOSFLM/CCP4* [9] and then merged and scaled with *SCALA/CCP4* [10].

Results

The crystal quality was evaluated by four indexes; maximum resolution limit, $<I>/<\underline{\sigma} I>$, R_{merge}, overall B factor. In every index, there was a tendency that the crystal quality decreased as the impurity improved. The results will be reported in detail elsewhere.

NASDA is planning a microgravity experiment on a space shuttle on 2002. The impurity incorporation in space and the crystal quality of space grown crystals will be studied.

References

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