Dissolution Rate of Hen Egg-White Lysozyme Crystal Under Microgravity

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Protein crystallization under micro gravity has been already tried many times in the United States¹⁾ and other countries, and it is reported that about 20 % of proteins were better crystallized under microgravity than on earth. This verified that microgravity is sometimes effective in protein crystallization. However, if these procedures continued to be carried out without clarifying which processes are effective, improved development of protein crystallization cannot be expected. The most effective way to study the process is to carry out protein crystallization experiments, each elementary stage of which is clearly observed. To this end, the dissolution rate of a single crystal of hen egg-white lysozyme has been measured both under microgravity and on earth in order to study the mechanism of protein crystallization.

In May 1997, we had an opportunity to have an experiment on protein crystallization with use of STS-84 space shuttle missions (the time duration of which was 210 hours). The apparatus for protein crystallization by vapor diffusion techniques was available and we have tried to use it for measurement of crystal dissolution rate under microgravity.

The barrels of two syringes (20 ml x 2) were filled with unsaturated protein solutions (hen egg-white lysozyme (HEWL): 0.0wt%, 0.1wt%, 0.2wt%, 0.3wt%; NaCl 3wt% aqueous solution) and a crystal (HEWL: tetragonal form; 1mm $\pm$ 0.2 mm in diameter) was put on the bottom of the one syringe. Dissolution of the crystal was started by extruding the unsaturated solutions onto the syringe tip with the crystal. The crystal dissolved in 40 μ l droplets that are extruded from syringes. The temperature was kept at 20 °C. Just before the Space Shuttle begins returning to the earth, the protein solution was withdrawn back into the syringes by the astronaut, and the melting experiment was finished. In the one syringe the incompletely melted crystal was withdrawn as well. The solution concentration in the other syringe was measured.

In the flight experiment, only the initial and final solution concentrations can be measured. In the control experiment on earth the solution concentrations in the course of dissolution were also measured. 20 undersaturated solutions (40 μ l in volume) were prepared and a crystal (1 mm $\pm$ 0.2 mm in diameter) was put in each solution. The solution concentration was measured at constant time intervals.

When the crystal starts to dissolve, molecules in the crystal surface leave the crystal, the concentration around the crystal becomes high and there occurs a radial concentration gradient centered on the nucleus. Molecules diffuse along the gradient and go farther from the crystal. On earth, the concentration gradient might be disturbed by convection and the high concentration around the crystal might be disturbed more quickly than under microgravity. We might therefore expect that dissolution rate on the ground should be larger than under micro-gravity. However, our experimental results show that the expectation was not correct. What might the reason be? One possible reason is that there might be Marangoni convection effects in the apparatus for protein crystallization by vapor diffusion techniques used for the melting experiment. This effect has also been observed by Chayen et al.²⁾

References

1) L.J. DeLucas and C.E. Bugg, *METHODS*, 1 (1990) 105 - 109

2) N.E. Chayen, E.H. Snell, J.R. Helliwell, P.F. Zagalsky: *J. Cryst. Growth*, 171 (1997) 219 - 225