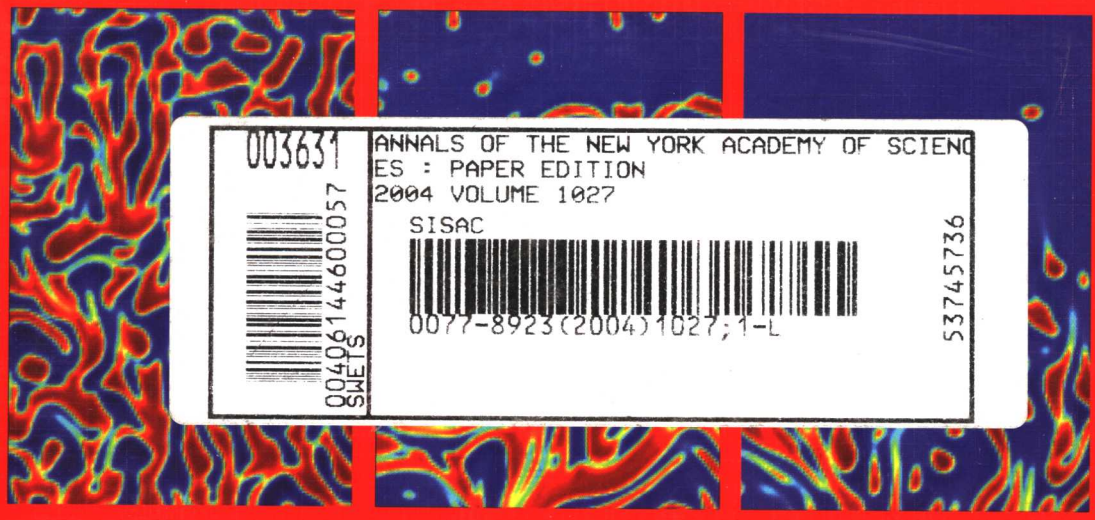

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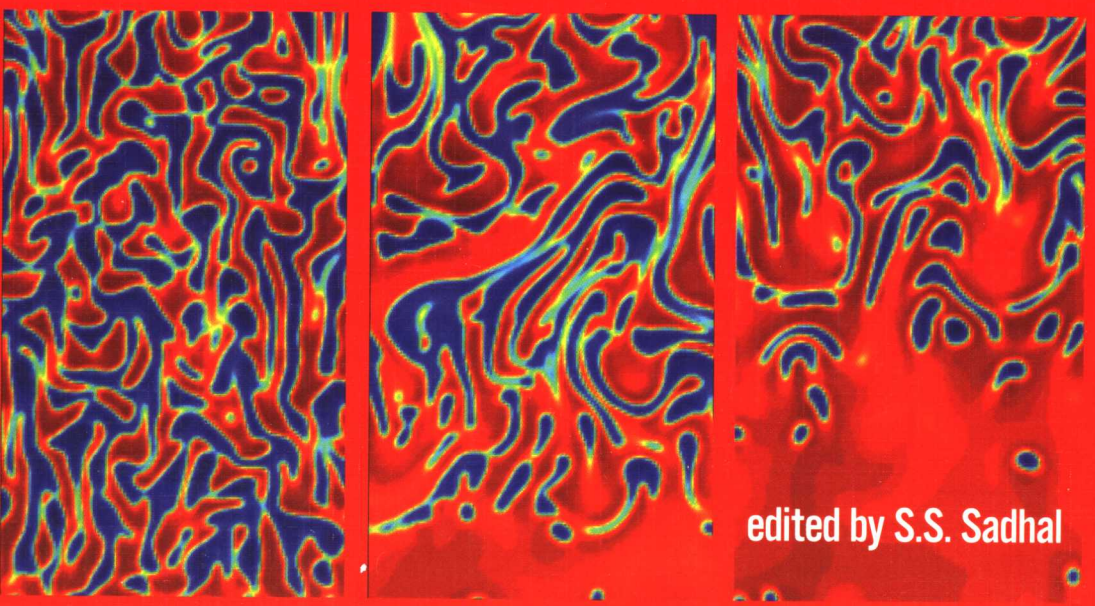

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TRANSPORT PHENOMENA IN MICROGRAVITY

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Effects of Buoyancy-Driven Convection on Nucleation and Growth of Protein Crystals

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ABSTRACT: **Protein crystallization has been studied in presence or absence of buoyancy-driven convection. Gravity-driven flow was created, or suppressed, in protein solutions by means of vertically directed density gradients that were caused by generating suitable temperature gradients. The presence of enhanced mixing was demonstrated directly by experiments with crustacyanin, a blue-colored protein, and other materials. Combined with the vertical tube position the enhanced convection has two main effects. First, it reduces the number of nucleated hen-egg-white lysozyme (HEWL) crystals, as compared with those in a horizontal capillary. By enabling better nutrition from the protein in the solution, convection results in growth of fewer larger HEWL crystals. Second, we observe that due to convection, trypsin crystals grow faster. Suppression of convection, achieved by decreasing solution density upward in the capillary, can to some extent mimic conditions of growth in microgravity. Thus, impurity supply, which may have a detrimental effect on crystal quality, was avoided.**

KEYWORDS: **protein crystals; hen-egg-white lysozyme (HEWL); porcine pancreatic trypsin; crustacyanin; nucleation; growth; buoyancy-driven convection; mimicking microgravity**

INTRODUCTION

X-ray structure determination is the most powerful tool for revealing the fundamental relation between molecular structure and biological function of proteins in biosolutions. However, obtaining high-quality crystals suitable for X-ray diffraction remains the major-bottleneck to structure determination. A profound knowledge of the details of the crystallization process is indispensable in order to achieve high-quality crystals.

Several different approaches can be used to grow relatively large and high-quality protein crystals. As well as control over crystal nucleation,[1,2] in the work reported in this paper, we use (in a batch method) buoyancy-driven convection in an attempt to replenish the exhausted solution in the crystal vicinity, thus ensuring faster growth and larger protein crystals. Conversely, by suppressing the buoyancy-driven convection, microgravity conditions were to some extent mimicked in ground experiments.

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No matter how complex a crystal growth process is two main consecutive stages occur, namely mass transport to the crystal surface and incorporation of the elementary building blocks on it. (Simultaneously, during the second stage, latent heat is dissipated to the ambient solution.)

Nutrition is usually the rate-determining step under pure molecular diffusion. Forced agitation, which enhances solute delivery, is used in the practical growth of large and relatively homogeneous inorganic (e.g., optic and segnetoelectric) crystals. Convection replenishment was used long ago in the so-called hydrothermal synthesis of quartz crystals.[3,4] It is more effective than diffusion, since the material is transported from (almost) the entire solution volume. Slow growth under pure diffusion control leads to increased exposure times of (broader) interstep terraces, and hence, to enhanced impurity incorporation.[5] The need of fine-tuning the agitation velocity is evident, since due to the agitation impurities are carried as well. The excessive mass brought by intensive flow cannot be utilized if the increased impurities result in worse crystals. Experimental skill is, therefore, required to adjust the flow rate in order to obtain an acceptable ratio of impurities versus crystal building blocks that impinge on the crystal surface. Usually a gentle flow, created by means of slow solution stirring, has proved to be optimal. Under microgravity conditions buoyancy driven convection is avoided but Marangoni convection is always present, leading to solution agitation.

Buoyancy driven convection was evoked or suppressed in our work by putting a glass capillary tube with the protein solution in a temperature gradient, which creates a density gradient, directing it in a desired position with respect to the gravity vector.

To enhance buoyancy driven convection the heaviest solution, at 4°C, is placed at the top of a capillary tube that holds the protein solution, and the lightest solution at, 20°C, is placed at the bottom of the capillary. The heavier liquid flows down in this case and slowly mixes the protein solution. A reversed temperature gradient was used in an attempt to suppress buoyancy driven convection.

Temperature gradients have been used for growing protein crystals and other crystals.[6–8] Luft *et al.*[8] describe a gradient established by running hot and cold water through channels machined into opposite ends of an aluminum plate. Micropipettes containing the protein solution equilibrate thermally with the plate by surface contact. In this way, the authors[8] performed a blind search for optimal crystallization conditions of proteins. Recently, Mao *et al.*[9] used a high-tech experimental approach to create a temperature gradient.

Temperature gradients were used in the present work for two purposes. First, to create (or suppress) buoyancy driven convection, and second, to create a supersaturated gradient with proteins whose solubility is temperature dependent. The generated supersaturated gradient enabled us to find optimal crystallization conditions for these proteins.

EXPERIMENTAL

Experimental Procedure

The principle of creating a temperature gradient is very simple—heat is extracted from the one end of a special tube that contains the glass capillary. The temperature gradient was created with the chosen orientation, vertical or horizontal.

Glass capillary tubes were filled with the desired solutions. The capillary tubes were closed tightly with Teflon caps, inserted in the temperature gradient, and fixed there.

Nucleation of protein crystals was investigated by means of the so-called double-thermal-pulse technique.[10] Quenching created the higher supersaturation that was necessary for nucleation. By cooling (the entire glass capillary containing the solution) we enforced crystal nucleation only in that part of the capillary tube where the supersaturation exceeded that corresponding to the metastable zone. After a lapse equal to the nucleation time, the glass capillary tube was removed from the temperature gradient and replaced at ambient temperature, corresponding to the metastable zone, in order to grow visible sized protein crystals. These crystals were counted and their number related to the corresponding nucleation time.[10]

Investigation Strategy

The essential idea in this project is as follows.

1. To evoke motion in the solution contained in the capillary tube, we used buoyancy driven convection. Hence, the coldest (4°C) tube-end was put on top, the bottom being maintained at 20°C. In this way we created conditions favoring buoyancy driven convection; we created an unstable configuration of the density gradient. (As is well known, water density reaches its maximum at 4°C.) Due to gravity, the difference in the solution density induces gravity driven flow. It is initiated by horizontally directed density inhomogeneity due to cooling from the outside. Another factor that favors buoyancy is water viscosity. This is almost twice as low on the capillary bottom, at 20°C, compared with that at 4°C.

However, water at 4°C is only about 0.2% heavier than at 20°C, protein solutions being heavier than pure water. Thus, the convection driving force is rather small. Therefore, an extremely simple test was performed in order to prove the action of buoyancy driven convection in the case under consideration. We compared the replacement of a colored boundary in two (identical) glass capillary tubes, each 10-cm long. Both glass capillary tubes were filled with pure water. On the top of each we put small (1 μl) colored droplets containing crustacyanin, a blue-colored protein (or in other experiments coomassie blue, or blue ink). Contact was then made between the colored droplet and the water. Putting one tube in the temperature gradient with 4°C on top versus 20°C on the bottom, and the second tube acting as a control at 20°C, we measured the propagation distances of the colored solutions. Indeed, they depend on the diameter of the glass tube. For comparison purposes glass capillary tubes with different inside diameters, 0.55 mm and 1.8 mm, were used in the present investigation.

2. In an attempt to eliminate the cause of the buoyancy driven convection (thus mimicking microgravity conditions on the ground) we put the coldest (4°C)

capillary tube end, with the heaviest solution, on the bottom. (It was supported by the air bubble that results from the tight closure, the cap.) Since the upper layer of solution is at a higher temperature, it is lighter. Of course, Marangoni convection remains under both microgravity and Earth conditions. However, the more intensive the flow, the more impurities impinge on the crystal surface.

3. We started our investigations with nucleation of protein crystals. Capillary tubes with a smaller inside diameter (0.55 mm) were used to limit protein consumption. The idea was to use the nucleation as a fine tool in exploring the effects of buoyancy driven convection on protein crystallization, without disturbing the system. Since the nuclei are extremely small[1,2,10] they do not appreciably decrease the solute concentration, and do not create any appreciable local density change. Thus, they could not create any buoyancy driven convection (or so-called natural convection). In contrast (due to the appreciable solute consumption), the macroscopic size growing crystals often evoke (buoyancy created) convection plumbs. Such phenomena have been repeatedly observed by interferometry.[11,12]

During the present investigation the gradient tube was put in both vertical positions, namely with the coldest tube end at the top and at the bottom. Using the double (thermal)-pulse technique,[10] thereby separating nucleation and growth processes, and comparing with the results for those for the horizontal tube position, we tested the effect of the buoyancy on protein crystal nucleation. Provided that protein solubility versus temperature dependence is known, quantitative data on nucleus number versus supersaturation can be obtained (compare also with Refs. 1, 2, and 10).

Protein Solutions

Hen-egg-white lysozyme (HEWL) and porcine pancreatic trypsin were chosen for the crystallization investigations. Reliable solubility data for HEWL are available in the literature for temperatures higher than 5°C.[13–15] (Moreover, a systematic study of the nucleation rate was performed recently under exactly the same conditions as in this paper but in horizontal tube position.[10]) SEIKAGAKU, 6× crystallized lysozyme was used at 30 mg/ml protein in 50 mM sodium acetate, pH = 4.5. The precipitant solution contained 3(w/v)% NaCl. Porcine pancreatic trypsin, the solubility of which is also temperature dependent[16] was obtained from Sigma and used at 15 mg/ml in 33% $(NH_4)_2SO_4$, tris-HCl-buffer, pH = 8.2. Since pouring $(NH_4)_2SO_4$ cause the solution to become turbid it was added in small portions.

RESULTS AND DISCUSSION

Microhydrodynamics

We observed that the (blue) color spread differently in the two (measuring and control) capillary tubes during the same time span. Longer distances were measured in the temperature gradient, 4°C on top (for both capillary diameters), as compared with the distances at room temperature, 20°C, despite the lower diffusivity at the lower temperatures. The difference increased as more time passed. The results for capillary tubes with an inside diameter of 0.55 mm are given in TABLES 1 and 2 for crustacyanin and TABLE 3 for coomassie blue. Since we observed that after 10 h

TABLE 1. Data for α-crustacyanin, 5 mg/ml

Duration (hours)	Vertical Tube 4°C on top (mm)	Reference Sample at room temperature, 20°C (mm)
2	7.1 ± 0.5	4.0 ± 0.5
6	13.0 ± 0.5	6.0 ± 0.5
10	15.5 ± 0.5	7.5 ± 0.5

NOTE: The table shows the distances (in mm) to which the blue color spreads in the temperature gradient and at room temperature, depending on the time span.

TABLE 2. Data for β-crustacyanin, 5 mg/ml

Duration (hours)	Vertical Tube 4°C on top (mm)	Reference Sample at room temperature, 20°C (mm)
2	8.1 ± 0.5	5.0 ± 0.5
6	15.0 ± 0.5	7.0 ± 0.5
10	18.5 ± 0.5	9.5 ± 0.5

NOTE: The table shows the distances (in mm) to which the blue color spreads in the temperature gradient and at room temperature, depending on the time span.

TABLE 3. Data for Coomassie

Duration (hours)	Vertical Tube 4°C on top (mm)	Reference Sample at room temperature, 20°C (mm)
2	4.5 ± 1.0	2.0 ± 1.0
3	6.5 ± 1.5	3.0 ± 1.5
4	8.2 ± 1.5	4.0 ± 1.5
6	9.0 ± 1.5	4.3 ± 1.5

NOTE: The table shows the distances (in mm) to which the blue color spreads in the temperature gradient and at room temperature, depending on the time span.

the colored substance dilutes substantially in the temperature gradient, longer times were not used. For longer times the colored boundary bleaches and its determination becomes uncertain. Similar results were also obtained with blue ink.

The reason that modest differences were measured may be that not only pure molecular diffusion is acting at 20°C; some flow may be initiated because the colored liquid is heavier. Although not large, the differences unambiguously show the presence of an additional transportation driving force under the temperature gradient; that is, buoyancy driven convection is taking place.

Experiments with wider glass capillary tubes (1.8mm inside diameter) were very instructive. They showed that the colored boundary is replaced about five times faster, both for the temperature gradient and at room temperature. Moreover, the shape of the colored boundary is conic.

Cooling the solution from room temperature to 4°C at the beginning of the experiment makes the solution heavier and it starts to flow down. A narrow trickle should be formed along the capillary axis, where the viscous resistance is smallest. The conic form of the blue colored boundary proves this. It was already noted that toward the bottom of the capillary, the stream is facilitated due to the two-fold lower water viscosity there. Simultaneously, an upcoming fluid flow should be created nearer the capillary walls, despite the larger resistance there. This has to be more intensive in the wider capillary tubes. One can imagine that (after a sufficiently long time) the result is slow solution circulation, although we did not observe it due to the limited sensitivity of our eye. The presence of circulation explains why Poiseuille's law for the flow rate, which depends on the fourth power of the capillary radius, is not obeyed in the case under consideration.

Most important for the present work is the fact that the upcoming flow should replenish the crystal nutrition zone, contributing to faster crystal growth. Presumably, protein crystals, which are big enough and suitable for X-ray structure determination, can be grown in this way. This method of additional "feeding" should work both with proteins of normal and reverse temperature dependent solubility.

Results and Discussion on Protein Crystallization

Due to the small capillary cross-section (diameter 0.55mm) and the relatively low driving force, the buoyancy driven convection causes a gentle slow motion of the solution. The visibly measured rates (in a 0.55-mm capillary) are roughly about 0.5μm per sec. Therefore, we applied relatively long nucleation times of 2, 1, and 0.5hours. The result for the nucleation of HEWL crystals in both scenarios of the vertical tube (averaged over three to eight separate experiments) is presented in FIGURE 1.

Nucleation of HEWL crystals, which has already been studied in the intermediate case, in the horizontal tube position,[10] was used as a reference in FIGURE 1. (Since we changed HEWL sample the data presented are averaged from new control experiments, performed in the framework of the present study.)

It is evident that the tube position is important. First, the number of HEWL crystals nucleated in both vertical tube positions is decreased substantially. The explanation is that, before growing to visible sizes, some crystals fall down because they are loosely connected to the glass wall. However, the assumption that the crystals may be "homogeneously" nucleated has to be rejected for several reasons.

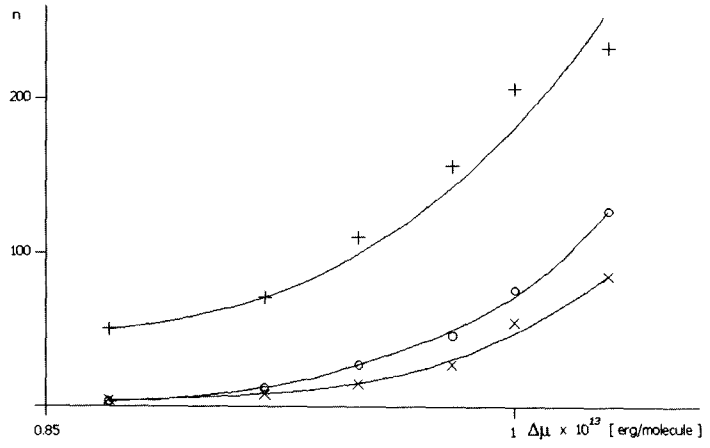

FIGURE 1. Dependence of the number of crystal nuclei n on supersaturation $\Delta\mu$ for two hours: +, in a horizontal tube position; ○, in a vertical tube with suppressed buoyancy; and ×, in a vertical tube with buoyancy convection (due to buoyancy, the $\Delta\mu$ value is changed and we are only able to show the nominal $\Delta\mu$ values).

1. In previous papers[1,2] we have described the effects of templates on crystal nucleation. A monomolecular layer of poly-L-lysine deposited on a glass substrate diminishes the number of nucleated hen-egg-white lysozyme (HEWL) crystals. In contrast, hydrophobization of glass surfaces by means of hexamethyl-disilazane stimulates nucleation. The fact that the templates were already exerting strong effects during the nucleation stage convinced us that heterogeneous nucleation was prevalent.

2. Most HEWL crystals stick strongly to glass or to the template,[1] the adhesion strength showing clear anisotropy.

3. True homogeneous nucleation is hardly possible. According to (classical) nucleation theory heterogeneous (crystal) nucleation is easier. Although "born" in the bulk solution some crystals may be nucleated heterogeneously on larger protein molecules that are present as impurities in the solution.

As is shown in FIGURE 1, when the coldest solution is at the top of the capillary the number of the HEWL crystals is reduced, as compared with their number in the reverse vertical tube position. The effect persists for a nucleation time of one hour, but seems to disappear for shorter times; for example, 30 min. We consider this fact as evidence that slow buoyancy-driven convection is acting. It appears that by trying to equalize supersaturation along the capillary, the convection reduces the highest supersaturation values.

Our study on convection effects exerted on the protein crystal growth was also performed using porcine pancreatic trypsin. The experiments show that trypsin crystals grew to visible sizes, by means of the thermal-double-pulse nucleation technique, for 24 h (in some cases for 21 h) only in the case when the coldest solution (4°C) was at the top of the capillary. No crystals were observed in the control sample,

under exactly the same conditions but in the horizontal capillary tube. We found that under conditions without agitation, due to the buoyancy driven convection, trypsin crystals required substantially (many times) longer times for their growth to visible sizes. Evidently, buoyancy contributes to faster growth; *vice versa*, this fact is another, indirect proof for the presence of buoyancy driven convection.

CONCLUSIONS

Buoyancy effects on the nucleation (and growth) of protein crystals were investigated. This was done by monitoring the results of a series of experiments on protein crystallization in both vertical and in horizontal tube positions. We draw the conclusion that solution replenishment due to buoyancy driven convection can contribute to faster growth of protein crystals.

Bringing fresh solution, the buoyancy acts in two different ways. On one hand, the additional feeding results in fewer larger crystals. However, we suppose that convection brings impurities, which cause detrimental effects on crystal quality. Therefore, in the present work we are attempting to suppress (at least to some extent) buoyancy driven convection in order to grow purer protein crystals of high quality.

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REFERENCES

1. TSEKOVA, D., S. DIMITROVA & C. NANEV. 1999. Heterogeneous nucleation (and adhesion) of lysozyme crystals. J. Cryst. Growth **196:** 226–233.
2. NANEV, C. & D. TSEKOVA. 2000. Heterogeneous nucleation of Hen-egg-white lysozyme—molecular approach. Cryst. Res. Technol. **35:** 189–195.
3. BALLMAN, A.A. & R.A. LAUDISE. 1963. The Art and Science of Growing Crystals. J.J. Gilman, Ed. New York.
4. LAUDISE, R.A. 1970. The Growth of Single Crystals, Prentice-Hall, Inc., Englewood Cliffs.
5. FRANK, F.C. 1958. Growth and Perfection of Crystals. R.H. Doremus, B.W. Roberts & D. Turnbul, Eds.: 411–418. J. Wiley & Sons, Inc. New York.
6. ATAKA, M. & S. TANAKA. 1979. Growth of large crystals. Chem. Abstr. **90:** 46940t.
7. ZEPPEZAUER, M. 1971. Enzyme purification and related techniques. *In* Methods in Enzymology, Vol. XXII. W.B. Jakoby, Ed.: 253–269. Academic Press, New York.
8. LUFT, J.R., D.M. RAK & G.T. DETITTA. 1999. Microbatch macromolecular crystallization on a thermal gradient. J. Cryst. Growth **196:** 447–449.
9. MAO, H., T. YANG & P.S. CREMER. 2002. A microfluidic device with a linear temperature gradient for parallel and combinatorial measurements. J. Am. Chem. Soc. **124:** 4432–4435.
10. PENKOVA, A., N. CHAYEN, E. SARIDAKIS & C. NANEV. 2002. Nucleation of protein crystals in a wide continuous supersaturation gradient. Acta Crystallographica D **D58:** 1606–1610.
11. ONUMA, K., K. TSUKAMOTO & I. SUNAGAWA. 1988. Role of buoyancy driven convection in aqueous solution growth; a case study of $Ba(NO_3)_2$ crystal. J. Cryst. Growth **89:** 177-188.

12. ONUMA, K., K. TSUKAMOTO & I. SUNAGAWA. 1989. Effect of buoyancy driven convection upon the surface microtopographs of $Ba(NO_3)_2$ and CdI_2 crystals. J. Cryst. Growth **98:** 384–390.
13. RIES-KAUTT, M. & A.F. DUCRUIX. 1992. Phase diagrams. *In* Crystallization of Nucleic Acids and Proteins: A Practical Approach. A.F. Ducruix & R. Giegé, Eds.: 195–218. Oxford University Press, Oxford.
14. ROSENBERGER, F. & S.B. HOWARD, J.W. SOWERS & T.A. NYCE. 1993. Temperature dependence of protein solubility—determination and application to crystallization in X-ray capillaries. J. Cryst. Growth **129:** 1–12.
15. FORSYTHE E.L. & M.L. PUSEY. 1996. The effects of the acetate buffer concentration on lysozyme solubility. J. Cryst. Growth. **168:** 112–117. (See also references therein.)
16. CHRISTOFER, G.K., A.G. PHIPPS & R.J. GRAY. 1998. Temperature-dependent solubility of selected proteins. J. Cryst. Growth **191:** 820–826.

Numerical Analysis of the Depletion Zone Formation Around a Growing Protein Crystal

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ABSTRACT: **It is expected that a protein depletion zone and an impurity depletion zone are formed around a crystal during protein crystal growth if the diffusion field around the crystal is not disturbed. The growth rate of the crystal may be decreased and the impurity uptake may be suppressed to result in highly ordered crystals if these zones are not disturbed. It is well known that a microgravity environment can reduce convective fluid motion, and this is thought to disturb the depletion zones. Therefore, we expect that crystals grown in space can attain better quality than those grown on the ground. In this study, we estimate the depletion zone formation numerically and discuss the results of crystallization in space experiments. In case of α-amylase, most of the crystals form a cluster-like morphology on the ground using PEG 8000 as a precipitant. However, in space, we have obtained a single and high-quality crystal grown from the same sample compositions. We have measured the viscosity of the solution, the diffusion coefficient, and the growth rate of protein crystals on the ground. Applying numerical analysis to these values a significant depletion zone was expected to form mainly due to higher values of the viscosity. This might be one of the main reasons for better quality single crystals grown in space, where the depletion zone is thought to remain undisturbed. For protein crystallization experiments, salts are widely used as a precipitant. However, in that case, reduced concentration depletion zone effects can be expected because of a low viscosity. Therefore, if it is possible to increase the viscosity of the protein solution by means of an additive, the depletion zone formation effect would be enhanced to provide a technique that would be especially effective in space.**

KEYWORDS: **protein crystal growth; protein depletion zone; impurity depletion zone; viscosity; space experiment**

INTRODUCTION

When a crystal grows in a supersaturated solution, significant concentration gradients of materials occur that include, not only the protein molecule, but also

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