The capillary convection in protein crystal growth

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Abstract

An optical diagnostic system consisting of the Mach-Zehnder interferometer with the phase shift device and the image processor has been developed for the study of the kinetics of protein crystal growing process. The concentration capillary convection around growing protein crystal was investigated during the process of trichosanthin crystal growth. The observation in real time showed that the concentration capillary convection associated with the surface tension of the crystallizing solution occurs at the vicinity of the surface of the protein mother liquor and directly affects on the outcome of protein crystallization, including the process of growth and the quality of resulting crystal. So far the detailed analysis and the important role of the concentration capillary convection in protein crystallization has been overlooked in both the space- and the ground-based crystal growth experiments. This may be one of the reasons for the majority of the results of space-based investigation shown no improvement.

Keywords: interferometer, phase shift, capillary convection, crystal growth

1. Introduction

Since the peculiar physical-chemical properties such as chemical versatility, conformational flexibility and extreme sensitivity to the subtle variation of external conditions, protein molecules usually require defined solvent conditions for their stability and function. Therefore protein crystals must be grown from chemically complex aqueous solution that is restricted to rather narrow conditions[1]. As a mater of fact, most of these factors are not recognized and meanwhile the thermodynamic and kinetic process are not well understood yet, though considerable process has been made in study of protein crystals and their growth processes.

Among those parameters, the solute convection is known to influence crystal growth and crystal quality significantly. In order to minimize or eliminate the undesirable effects of the convection, many efforts have been made in both space- and ground-based protein crystallization experiment[2-6]. Nevertheless it has long been the subject of a great deal of debate whether the convection and sedimentation, both of which are caused by gravity, are important factors limiting the outcome of the protein crystal growth. If this is really the case, the protein crystal growth under microgravity (μg) conditions should undoubtedly result in marked improvement in both the size and quality of resulting crystals. However, the results of space-based investigations of the protein crystal growth often showed no improvement and even more inferior to those grown on earth, in the majority of cases. Understanding how microgravity (μg) affects the crystal growth process must be preceded by an understanding of the process on ground, and much careful experimental work have to be devoted on this topic[8-12].

In order to understand how solute convection directly affects the outcome of crystal growth, the fluid dynamics approaches

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have been applied to characterize the convection-diffusive supply fields in the crystallizing solutions, and thus to rationalize, e.g., the difference in microgravity and in ground-based crystal growth[7].

During investigations on the process of protein crystal growth by using homemade phase shift Mach-Zehnder interferometer, we observed the concentration capillary convection around the growing crystals and found this convection seriously affects the crystal growth. Carefully studying this fluid behavior within the crystallizing micro-scale drop during the protein crystal growth could provide useful information to optimize crystal growth and hence to improve crystal quality. In this paper, the interesting observations and the detailed analyses would be presented and discussed.

2. Materials

As the model, one proteins, trichosanthin (TCS) was used in this study. The trichosanthin (TCS) is a type I ribosome-inactivating protein (RIP) (247a.a., Mr = 27400Da), extracted from *trichosanches kirilowill maxim* plant root tuber in south china. The dry TCS protein powder, at home purified, was dissolved in double-deionized water aqueous solution containing 5% sodium chloride at 30mg/mL and stirred 2 min, and then centrifuged for 20 min at 10000 rpm. All other chemicals used in the present study were of analytical grade and used without further purification, but all solutions were subsequently filtered with a micro filter of 0.22 μm to reduce noise to ensure the success, before use.

3. Instrumentation

A schematic block diagram of the system arrangement is shown in Fig. 1, the crystallization cell was designed for crystal growth by the vapor diffusion method using either the hanging or sitting drop technique. For the special purpose, it is made of optical glass, and the geometrical shape is shown in Fig. 2. The optical parts of the system with the crystallization cell are mounted on a vibration-isolated table-top platform, and are shield with thermostat box (temperature regulation device) for the dustproof in the light beam and control the temperature.

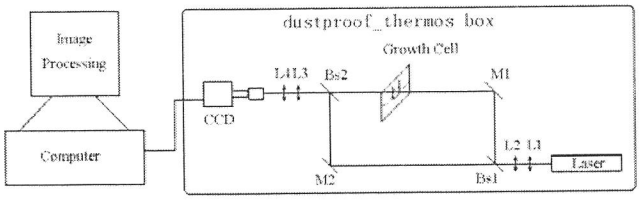


Fig. 1 The optics interferometry system

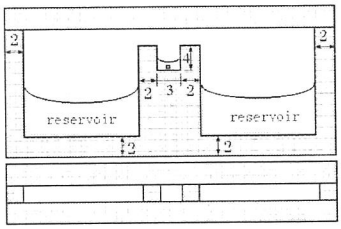


Fig. 2 The modified cell of the vapor diffusion method

4. Experimental procedure

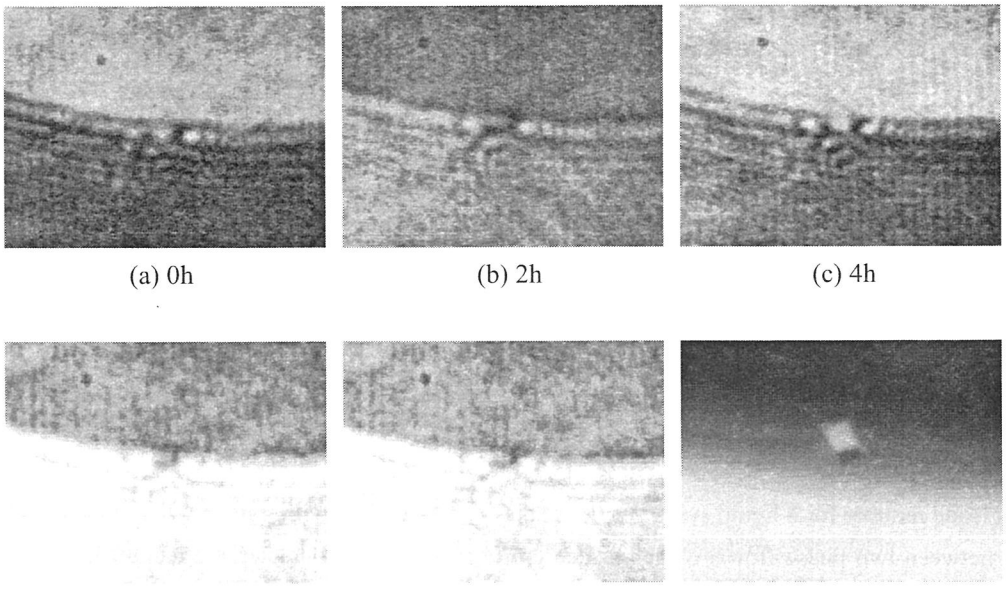
The vapor-diffusion was conducted in the present crystal growth. The crystallization drop was made up as follows: A stock solution of 30 mg/mL TCS in 50 mM sodium citrate buffer at pH 5.3 was mixed in equal volume with a reservoir solution containing 3M KCl and the same buffer at pH 5.3. A droplet (12μL) of this mixture was injected carefully into the growth cell which was purged with the double-deionized water prior, and 400μL of the reservoir was injected into the big chamber of the cell and sealed over it tightly. Initially, the growth cell was allowed to stand about 24h at 295K incubator before start. Then the growth cell was mounted at the sample stage of the micro Mach-Zehnder interferometer and kept at 295K. TCS protein crystallizes in two kinds of space groups under different conditions, but under this present condition, TCS crystallizes in its orthorhombic morphology and empirically demonstrates that this condition gives the best results. During the crystal growth, images of the fringes were captured with an automated image acquisition CCD system.

To measure the protein concentration variation in the crystal growth cell during the experiment, the refractive index of each

sampling drop was determined with a temperature controlled Abbe refractive meter, and established the relationship concentration of protein within the cell versus refractive index.

5. Results and Discussion

The concentration capillary convection around the growing protein crystals were observed and visualized by using Mach-Zehnder interferometer during the course of the trichosanthin crystal growth process. Direct evidence of the presence of the concentration capillary convection in the crystal growth experiment is shown in Fig. 3. The crystal on the liquid surface is shown as in Fig. 3(f) which has been taken by the microscope. The interference fringes of the crystal on the liquid surface are rings on the left and right of this crystal. This phenomenon demonstrates that the crystals correlated with the concentration capillary convection were all along in the vicinity of the liquid surface, and at which the post-nucleation growth rate becomes very small. Thus the consequential resulting crystals suffer not only rather small size but also irregular shape (see Fig. 3f) when the crystal grows on the liquid surface in the sitting droplet.



(a) 0h (b) 2h (c) 4h

(d) 5h (e) 6h (f) 6h

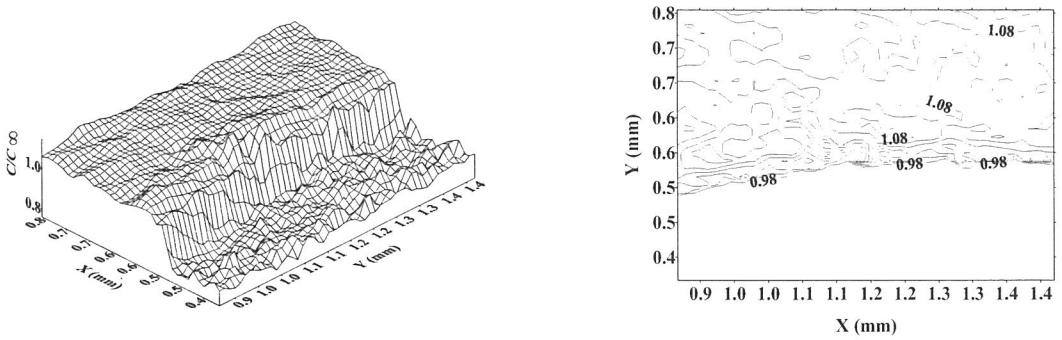
Fig. 3 The fringe of the trichosanthin crystal grown in liquid surface (0.52mm×0.42mm)

In conventional vapor-diffusion methods, the water vaporization is crucial. The equilibration of the vapor-diffusion experiment is driven by the difference between the chemical potential of the water in the droplet and that in the reservoir. In general arrangement, traditionally, the well containing aqueous salt at twice the concentration in the droplet, thus, the water will move from the droplet, where the chemical potential of water is lower, to the well reservoir where the chemical potential of water is higher. This departure of the water shrinks the overall volume of the droplet and diminishes the radius of curvature of the droplet, which serves to decrease its surface area (occur the change of surface tension). On the other hand, the continuous transfer of the water molecules from the interior of the sitting droplet to the surface will condenses and creates a boundary layer rich in water, thus the concentration of the protein and crystallizing agent are decrease, before final equilibrium is achieved. In addition, in conventional crystallizing methods, nucleation and post-nucleation growth proceed randomly in the protein mother liquor. It is therefore difficult to define where is the nucleation position. So, in case of the nucleation occurs in the vicinity of the surface in the sitting droplet method, the post nucleation growth rate should be small and unequal due to the regular growth sampled by the surface tension and unsteady concentration convection, and the lower concentration as well.

Durbin[13] et al have investigated the tetragonal lysozyme crystal growth and the correlation of the morphology with the dependence on concentration, where they observed that the tetragonal lysozyme crystal grown at low concentration are often asymmetric – the equivalent four {101} faces are unequal, whereas crystals grown at high concentration almost always display a symmetrical tetragonal forms[13].

In order to affirm the possibility of the presence of the concentration capillary convection in present experiment, we also evaluated numerically by the value of bond number based on the observed data.

For the trichosanthin crystal growth, the protein concentration gradient distribution obtained in the crystallization solution after two days of crystal growth are depicted in Fig. 4, the concentration changes around the smaller crystal are much less pronounced due to the low growth rate at closely located solution surface. However, the concentration distribution on the line y = 0.5979mm, which across the crystal, are much more pronounced that the concentration capillary convection exists at the sides of the crystal if the crystal grows on the liquid surface, see Fig. 5.



(a) (b)

Fig. 4 The concentration gradient distribution calculated from Fig. 3(a)

The dimensionless Bond number for a liquid system is defined as $Bo = \Delta\rho g d^2/\sigma$ with g the gravitational acceleration, $\Delta\rho$ the density difference between two phase flows, σ the surface tension, and d the characteristic length. In our system, σ = 50 dyn/cm = 5×10^{-2}N/m. The density for crystallizing solutions is 1.36×10^3kg/m^3, the density of the air is considerably as 0.029×10^3 kg/m^3. The characteristic length should be the range in which has the change in concentration, it is estimated as the max value 0.0005m, so the Bond number Bo = 0.132. In fluid dynamic theories, when the Bond number is smaller than 1, the statics action of the fluid will be controlled by the surface tension, the convection introduced by the asymmetry of the surface tension will take an important action in the fluid. Hence, the concentration capillary convection introduced by the asymmetry of the surface tension is the impersonal physical phenomenon in the crystallization of the protein crystal growth in the present experiment.

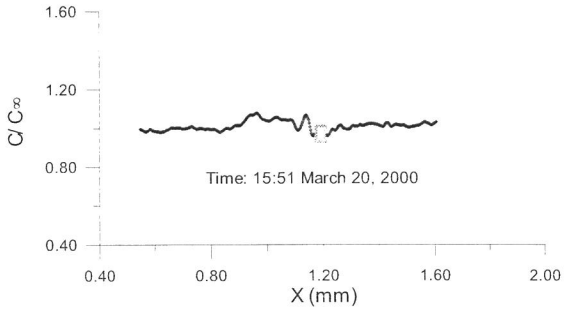


Fig. 5 The concentration distribution on the line y = 0.5979mm

6. Conclusion

The concentration capillary convection has been observed and visualized by Mach-Zehnder interferometer with micro scale crystallizing sample of trichosantrin during their crystal growth under well designed conditions. It seems that the concentration capillary convection arise from the combination of the surface tension with the convective flow in the crystallizing solution. Once occurred the nucleation at the vicinity of the liquid surface of the crystallizing solution, the concentration capillary convection can interfere with the growth rate and the final crystal quality, as are of the undesirable perturbation. To the author's knowledge there has not been any reports about the phenomenon of concentration capillary convection for protein crystal growth and its affection on the outcome of the growing crystals. Although somewhat preliminary, this observation is instructive.

This finding is revealed that it should not be regardless of the adverse effect of the concentration capillary convection in crystallizing solution during the process of protein crystal growth. Unfortunately, however it has been overlooked the important detail of the concentration capillary convection in protein crystallization so far, including space crystal growth experiments. This may be one of the reasons for the majority of the results of space-based investigations show no improvement and even smaller crystal size and more imperfect structures. Understanding of this concentration capillary convection should improve and help our ability to grow good quality protein crystals. So, it should pursue to examine the details of the phenomena described here.

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