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Evaporation Rate in Protein Crystallization Via Vapor Diffusion can be Controlled through a Simple Multistep-concentration Setting in Capillaries

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Abstract

A simple multistep-concentration setting in capillaries was used to control the water-evaporation rate in vapor-diffusion protein crystallization. In the method used, a variety of evaporation rate curves were obtained by using the secondary precipitant solution referred to as "regulatory solution", which is not directly exposed to the protein solution. The curves were applied to the crystallization of lysozyme as a model protein. The results clearly showed that crystal growth is dependent on the evaporation rate. Especially, the decoupling curves in which precipitant concentration in protein solutions increases to a certain point and then decreases to the equilibrium concentration gave the best crystals.

요 약

증기-확산 단백질 결정화의 물-증발 속도를 조절하기 위해서, 간결한 모세관 내의 다중-단계 농도 장착법 (multistep-concentration setting in capillaries)이 이용되었다. "조절 용액 (regulatory solution)"이라고 일컬어지는 2차 침전 용액이 이용되어, 이 방법으로 다양한 증발 속도 곡선들이 얻어졌다. 이 때, 조절 용액은 단백질 용액에 직접적으로 노출되지 않는다. 모델 단백질인 lysozyme의 결정화에 이 그래프들이 적용되었다. 결정 성장은 증발 속도에 달려있다는 것을 실험 결과들이 명백하게 보여주었다. 특히나, 단백질 용액의 침전 농도가 어떤 점까지 증가하다가 평형 농도로 줄어드는 decoupling 곡선이 가장 좋은 결정들을 만들어냈다.

1. Introduction

Vapor diffusion is the most common method to grow protein crystals suitable for X-ray diffraction analysis. In the method, water evaporation between a protein solution and a reservoir solution can be achieved by several techniques such as hanging-drop/sitting-drop and capillary methods. Of those vapor diffusion in capillaries is not widely used, probably because of its lack of advantages over hanging-drop/sitting-drop method. Nevertheless, the capillary method has been successfully used for a few protein crystallization experiments, including the crystallizations of a high molecular weight urokinase[1] and a human Bence-Jones protein in microgravity.[2]

Kinetic aspects of the water-evaporation rate are important to successful crystal growth.[3] The profile of the rate frequently affects nucleation and growth pattern. Unfortunately, it is very difficult to obtain the desired evaporation rate by traditional vapor diffusion methods, because diffusion during the crystallization experiment is dynamic process.[4] Several methods to control the evaporation rate have been reported, including decoupling nucleation and growth in vapor diffusion,[5] temperature-control strategies,[6] dynamic control with a device with a weight-sensitive facility,[7] and diffusion-controlled crystallization with a special apparatus for microgravity.[8] However, most of those require special devices and sometimes sophisticated apparatus that are available only for few researchers.

We report here a very simple approach to control

the evaporation rate in vapor-diffusion experiments using a multistep-concentration setting in capillaries.

2. Experiments

2-1. Sample preparation

Chicken egg white lysozyme purchased from Sigma Co. was used as a model protein without further purification. The enzyme was dissolved in a 0.1 M sodium acetate buffer (pH 4.5) to the concentration of 25 mg/m*l*. All protein solutions were filtered by disposable 0.2 μm sterile syringe filters to avoid heterogeneous nucleation. For the precipitant solutions, NaCl was used as a precipitant and dissolved in pure water.

2-2. Multistep-concentration setting in capillaries

A simple multistep-concentration setting in capillaries was designed to control the water-evaporation rate in vapor diffusion protein crystallization. In the method used, the protein solution and precipitant solutions were placed in the silanized glass capillary tubes, 80 mm in length and 1.0 mm in internal diameter, as shown in Fig. 1. In the setting, the protein solution and primary precipitant solutions (precipitation solutions #1 and #2) were allowed to be equilibrated against the secondary precipitant solution (precipitation solution #3) referred to as "regulatory solution", which is not directly exposed to the protein solution. Solutions were loaded with a 100 μ*l* Hamilton syringe as depicted in Fig. 2.

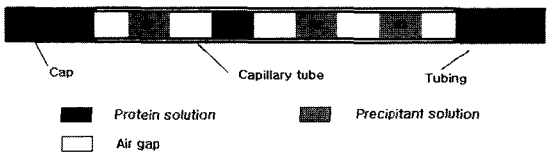

Fig. 1. Multistep-concentration setting in capillaries as described in the text.

Evaporation rate in the capillary setting can be affected by several factors, including the dilution factor that is a precipitant concentration ratio between the two neighboring solutions, the number of precipitant solutions, the chemical nature of a precipitant, the volume of solutions, the length of air gaps, capillary diameter, and temperature. All the factors except the dilution factor were standardized: three precipitation solutions, NaCl as a precipitant, 4 μ*l* of solution volume, 5 mm of air gap, 1.0 mm of capillary tube diameter, and 20°C temperature. The approximate degree of dehydration of protein

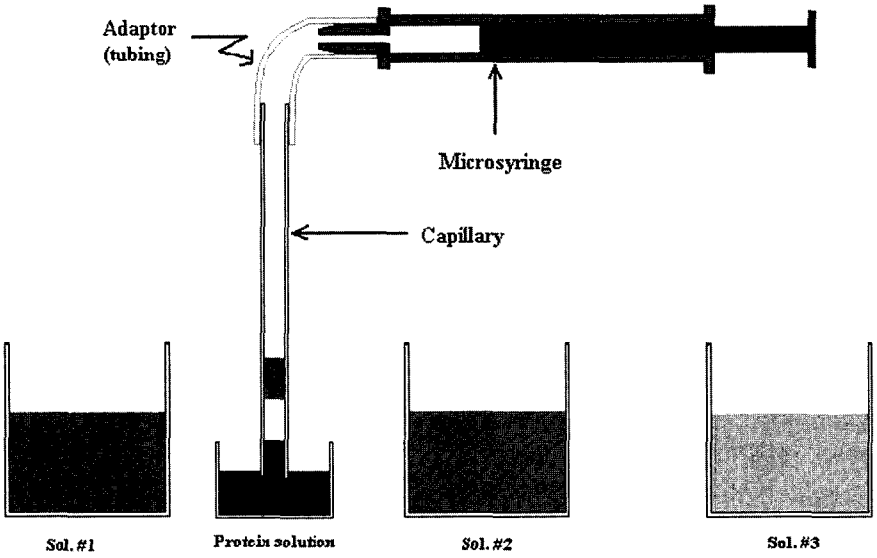

Fig. 2. Sample loading procedure. Solutions were sequentially placed in the capillaries by suction with a 100 μ*l* Hamilton syringe.

Table 1. Relative precipitation concentrations for the capillary settings. For all settings, initial (0.5 M) and final (1.0 M) concentrations of NaCl in the protein solutions are identical

No.	Initial protein solution	Precipitant solution #1	Precipitant solution #2	Precipitant solution #3	Protein solution at equilibrium state
1	1	1	1	5	2
2	1	2	2	3	2
3	1	3	2.5	1.5	2
4	1	3.5	3.5	0	2

solution was set to be 50%. For a set of experiments, the capillary settings with various order and concentration of the precipitant solutions (#1, #2, and #3) were conducted to be identical initial and final conditions in the protein solutions, resulting in maintaining identical environments except the kinetics of equilibration (Table 1).

2-3. Measurement of volume change of solutions in capillary and data conversion

The volume change of protein solutions was evaluated by measuring the change in length of the solution through a microscope (×40 magnification). Each measured data set was normalized to the initial length (volume) of the protein solution and then converted to the degree of dehydration.

3. Results and Discussion

During the course of the experiments, it was found that the high dilution factors between the protein solution and neighboring precipitant solutions were required to overcome the slow equilibration rate, which is a major drawback of the use of capillaries. At the conditions listed in Table 1, the most rapid equilibration rate was comparable to that in conventional hanging-drop settings (data not shown).

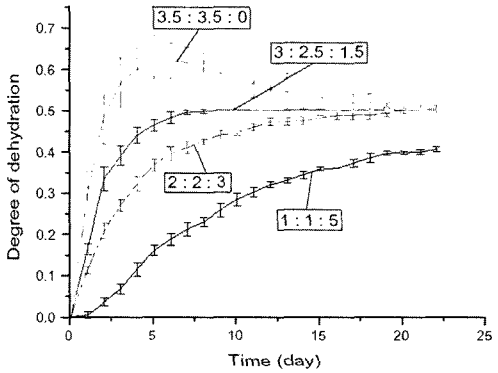

Fig. 3. Water-evaporation rate curves obtained from the experiments described in Table 1. Results for each curve were obtained from the average of five observations.

The silanization of capillaries also was necessary to minimize surface effects.

Evaporation rate in the capillary settings can be efficiently controlled by adopting regulatory solutions and by varying dilution factors. Although it is not possible to predict the exact rate curve for the capillary setting, typical rate curves can be selected, by trial-and-error strategy, for a specific precipitant solution. Four typical curves for NaCl as a precipitant were obtained (Fig. 3) and applied to the crystallization of lysozyme as a model protein. The

Table 2. Crystallization of lysozyme using the multistep-concentration setting in capillaries, based on the curves in Fig. 3. Results for each curve were obtained from the average of five observations

Curve no.	Relative precipitant concentrations (sol.#1 : #2 : #3)	Size and number of crystals in a setting	Type of process	Crystallization methods
1	1 : 1 : 5	Medium 2-4	Conventional process	Capillary setting
2	2 : 2 : 3	Small 4-7	Conventional process	Capillary setting
3	3 : 2.5 : 1.5	Microcrystals 11-15	Conventional process	Capillary setting
4	3.5 : 3.5 : 0	Large 1-3	Decoupling process	Capillary setting
·	Reservoir solution: 2	Micrcrystals Many	Conventional process	Hanging drop

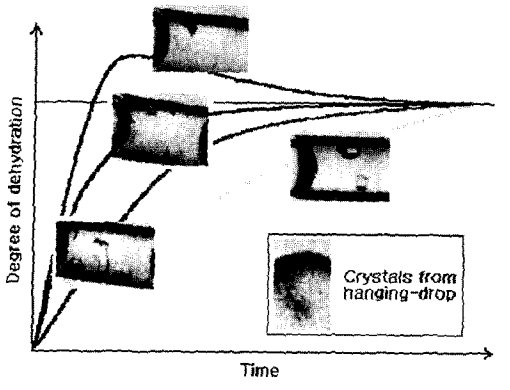

Fig. 4. Lysozyme crystals grown at various evaporation rate profiles as summarized in Table 2.

results clearly showed that crystal growth is dependent on the evaporation rate (Table 2 and Fig. 4). Especially, the decoupling curves in which the precipitant concentration in protein solutions increases to a certain point and then decreases to the equilibrium concentration gave the best crystals. This means that the size of protein crystals was maximized by maintaining the growth conditions in the metastable zone in the solubility diagram.[4] Saridakis and Chayen[5] dramatically improved the crystal quality of human serum albumin using decoupling nucleation techniques, "backing off" from nucleation conditions to the lower concentrations of the precipitant by transferring hanging drops. It is believed that the decoupling condition corresponds to decoupling curves in our experiments.

The use of capillaries in protein crystallization has several advantages, such as compactness and mechanical stability.[2] Aside from the advantages, our results show that the multistep-concentration setting can be efficiently used for protein crystallization in which kinetic factor is important and for height-throughput screening of protein crystallization in the future.

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