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An investigation on the effect of surface roughness of crystallization plate on protein crystallization

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ABSTRACT

Surface treatment by oxidizing the crystallization plates can significantly promote protein crystallization and requires no change to routine screening protocols; therefore, it is potentially useful for practical protein crystallization screening. However, experiments have shown that the amount of oxidants and the treatment process need to be optimized to achieve effective results. Searching for the suitable amount of oxidants, temperature and processing time for surface treatment of the plate will increase the workload and decrease the efficiency of the screening process. To solve this problem, a series of trials to determine suitable surface treatment conditions were conducted. Based on these experiments, not only was the most suitable processing condition for the optimal protein crystallization screening hits discovered but also the relationship between the treatment process and the protein crystallization screening hits was explored. With these results, the surface treatment of protein crystallization plates became easier and more effective, allowing the oxidizing surface treatment method to be applied on plates for use in routine protein crystallization screening.

1. Introduction

The vapor diffusion crystallization technique, in which the solvent in the crystallization droplet evaporates into the reservoir solution and the droplet is concentrated in the nucleation zone [1], is the most widely crystallization technique used in protein crystallization screening [2,3]. Moreover, the vapor diffusion method is a typical utilization of heterogeneous nucleants. Because a solid surface can provide a heterogeneous nucleation site, the crystallization of proteins usually occurs through heterogeneous nucleation. However, the solid surface of the sitting pits in sitting-drop vapor-diffusion crystallization plates has never been optimized for heterogeneous nucleation, and this untreated roughness in the solid surface may not be a suitable condition for successful crystallization. To solve this problem, other techniques have been proposed and tested that would act as a heterogeneous nucleant in crystallization screening. Methods such as mineral substrates [4,5], porous materials [6,7], polystyrene nanospheres [8] and hair [9–12] have proven useful in inducing nucleation and increasing the number of crystallization hits. However, these methods require the addition of nucleants in each crystallization droplet and a considerable amount of labor.

Recently, we developed a surface treatment method of oxidizing the crystallization plates. This method can enhance the success rate of protein crystallization and avoid the extra step of adding nucleants [13]. In this method, the crystallization plates were modified using oxidation so that the solid surface of the plates can facilitate crystallization. The oxidized crystallization plates (OCPs) were found to increase the number of protein crystallization screening hits that contain crystals and improve the reproducibility. Nevertheless, further experiments verified that there is an obstacle to the wide application of this method because the effects of the surface treatment have a great influence on the consequences of protein crystallization screening. Optimizing the amount of the oxidants and the proportion of the treatment reagents should aid in achieving positive effects for crystallization on the plate surfaces. Furthermore, the reaction temperature and processing time also need to be explored to determine the optimal conditions. This work is conducted repeatedly before every protein crystallization screening, which is tedious and time consuming. Moreover, it is also very necessary to adjust the treatment conditions to reduce nucleation number so as to meet the requirement when

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excessive nucleation becomes a nuisance.

The above problems reduce the applicability of the OCP method. To solve these problems, we use commercially available crystallization plates for oxidation tests by orthogonal experimental design. All the oxidants, reagents, temperature, and processing time are included as factors in the orthogonal experimental design. Moreover, appropriate levels for each factor were chosen to facilitate crystallization screening results. After the treatments, both crystallization screening experiments and reproducibility experiments were used to verify the effects of the oxidation treatment on the final crystallization results. Based on the experimental results, we found a relationship between the crystallization screening hits and the surface roughness of the OCPs. Additionally, this relationship demonstrated universality for all tested proteins. In this report, we describe the relationship between the treatment process and protein crystallization screening hits, and we give our recommendation as to the optimal treatment process.

2. Materials and methods

2.1. Materials

In this study, hen egg white lysozyme (HEWL; lysozyme; lys; catalogue No. 100940, Seikagaku Kogyo), proteinase K (pK; catalogue No. P6556, Sigma-Aldrich), concanavalin A VI (con; catalogue No. L7647, Sigma-Aldrich) and catalase (cata; catalogue No. C40, Sigma-Aldrich) were used without further purification.

The Index™ crystallization screening kit (catalogue No. HR2-144) was obtained from Hampton Research. HEPES sodium was purchased from Beijing Chemical Factory. The oxidant potassium dichromate was purchased from Tianjin Chemical Reagent Factory, and concentrated sulfuric acid (98%) purchased from Xi'an Sanpu Chemical Reagent Limited Company (China).

2.2. Methods

To test the relationship between the efficiency of crystallization and the surface of the oxidized plates, crystallization plates were oxidized by potassium dichromate and concentrated sulfuric acid in using various ratios at a proposed temperature and processing time. Afterward, atomic force microscopy (AFM) was utilized to scan the surface morphology and roughness of the crystallization plates. Subsequently, these OCPs were used for a screening study and a reproducibility study in which 96 droplets were dispensed into the pits in the crystallization plates. The plates were then sealed and incubated in a temperature controller.

2.2.1. General surface treatment method of crystallization plates by oxidation

Commercially available crystallization plates (Intelli-Plate, refer to Supplementary material Fig. S3) were used for surface treatment by oxidation, and the orthogonal experimental design was used to produce various surface effects on the OCPs. The oxidants, reagents, temperature, and processing time are included as factors in orthogonal experimental design (the Supplementary material Table S1 shows details).

2.2.2. Surface morphology and roughness detection of the plates

Atomic force microscopy (AFM) was utilized to characterize the surface properties of the crystallization plates before and after surface oxidation. The atomic force microscope (BioScope, Bruker, USA) scanning conditions were as follows: scanning mode: tapping mode, scanning area: 10×10 μm, and probe: NSC11/AIBS from NT-MDT (Russia). After oxidation treatment, a 10 μm^2 area was chosen on each crystallization plate to test the average surface roughness by AFM, which was calculated by WSxm 5.0 Develop 7.0 from the scanning data. The average roughness represents the height in the vertical direction of the structural characteristics of the nucleation surface.

2.2.3. Crystallization trials

Two types of crystallization experiments (crystallization screening study and reproducibility study) were conducted to test the crystallization efficiency of the OCPs.

2.2.3.1. Crystallization screening study. Four proteins, lysozyme, proteinase K, concanavalin A VI and catalase, were dissolved in 25 mM HEPES sodium buffer pH 7.0. The proteins were prepared at an initial concentration of 20 mg ml^{-1} and were then mixed with the Index™ screening kit at a ratio of 1 μl: 1 μl by crystallization robot (Screenmaker; Innovadyne Technologies Inc., USA). The crystallization plate was initially prepared by filling each well with 80 μl reservoir solution, sealed with Crystal Clear Tape (Hampton Research, catalogue No. HR4-506) and then placed into a temperature controller at 293 K. Crystallization screening hits (here a "hit" is defined as a crystallization condition that yielded protein crystals observable by the automated image reader) were identified using the captured images.

2.2.3.2. Crystallization reproducibility study. The reproducibility of crystallization was tested by dispensing 96 droplets from the same crystallization solution using the automated crystallization robot. The crystallization droplet containing the protein and the reservoir solution in a 1:1 ratio was equilibrated against the reservoir solution. The initial concentrations in the droplets included 30, 40, 50, or 60 mg ml^{-1} lysozyme; 0.2 M sodium acetate buffer, pH 4.6; and 30 mg ml^{-1} NaCl. All other procedures were the same as described for the screening study. The crystallization success rate, which is defined as the ratio of the number of crystallized droplets to the total number of droplets, is used to represent the reproducibility of all the OCPs.

3. Results and discussion

3.1. Surface characterization of the oxidized crystallization plates

The effect of the oxidation treatment on the crystallization plates is closely related to the changes in the surfaces of the plates. Therefore, morphology and roughness analyses were used to detect the surface characteristics of the OCPs.

We scanned all the oxidized crystallization plates in the orthogonal experimental design using AFM, and we found that the surface roughness either increased or decreased after the surface treatments (shown in Supplementary Fig. S1). The surface roughness data for the crystallization plates are shown in Table 1. The data show that the surface roughness changes with changes in the combination of potassium dichromate, concentrated sulfuric acid, reaction temperature and processing time. The average roughness ranges from 3.98 to 32.03 nm for the OCP group, compared with 21.74 nm for the control. These data indicate that the surface of the treated crystallization plates is closely related to the oxidation treatment procedures. Our previous experiment showed that the surface of treated plates was smoother than that of untreated ones [13]. The current results showed that, under wider treatment conditions, the surfaces of treated plates can be either smoother or rougher than the surfaces of untreated plates.

3.2. Crystallization results

3.2.1. Crystallization screening test

In the crystallization screening tests, four proteins were utilized for testing the applicability of the OCP method. The number of crystallization screening hits for each protein varied obviously after different oxidation treatment procedures (shown in Supplementary Table S2).