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Expanding pH screening space using multiple droplets with secondary buffers for protein crystallization

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ABSTRACT

We have proposed a rational strategy for selecting a suitable pH of protein solution based on protein biochemical properties. However, it is difficult to use this strategy for biochemical properties unknown proteins. In this paper, a simpler and faster pH buffer strategy was proposed. An additional pH-controlling buffer was added to crystallization droplet mixed with protein solution and commercial crystallization reagents to adjust its pH. The results revealed that protein crystallization success rates were enhanced by this strategy due to expansion of the pH screening space, which was closely related with protein solubility. Thus, the possibility of reaching supersaturation was increased by using this strategy.

1. Introduction

Determining the structure of protein molecules is important for understanding their biological functions [1–3]. X-ray diffraction analysis is used to obtain structural information of proteins. In the Protein Data Bank (PDB), more than 89% of the deposited protein structures were determined using this technique [4–6]. Typically, there are three steps that need to be overcome, including protein purification, protein crystallization screening and crystallization optimization [7–12]. Of these steps, crystallization screening is an important limiting factor in the protein structure studies.

pH affects the surface charge of protein molecules [13], which in turn can impact the packing [14] and stability of the protein molecule during crystallization [15]. Both packing and stability are critically important for protein crystallization. It has also been reported that a change of 0.1 pH unit can result in significant differences in protein solubility [16,17] that ultimately affect the crystallization of a target protein. Kantardjieff and Rupp [18] revealed that a correlation between a protein's isoelectric point (PI) and its crystallizability by using data deposited in the PDB database. Furthermore, Kirkwood et al. [19] used both buffer pH and chemicals in the crystallization solution to estimate the true pH and then analyzed the relationship between the pI of protein and true pH at which it crystallizes. It has also been reported that protein solution pH is critical for crystal nucleation and growth [20–24]. Additionally, crystal size, morphology and qualities are also related to pH [25–28]. All these findings indicate that pH is an important factor in protein crystallization.

The pH values of crystallization droplets are determined by both crystallization reagent pH and the pH of protein solution. During routine crystallization screenings, the pH of protein solution is very important for determining the pH of crystallization droplets. In literature, there are a few studies on the selection of a proper pH of protein solution [29–32]. We have proposed a rational strategy for selecting the pH of protein solution to enhance crystallization. According to this strategy, we can select the protein solution pH before setting up the crystallization droplets [33]. However, this strategy still requires improvement. For example, to use this strategy, protein biochemical properties must be known (e.g., pI value and stable pH range). For new proteins, it is difficult to know their biochemical properties, particularly the stable pH range. Even when selecting a suitable protein solution pH, the protein purification buffer needs to be replaced with a crystallization buffer with a desirable pH. Based on these considerations, a high-throughput and easy-to-operate method needs to be proposed.

Abbreviations: M, microbatch; CDM, cross-diffusion microbatch.

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In this paper, we propose an easier and faster pH strategy to enhance protein crystallization. We directly mixed the pH-controlling buffer with protein solution and the crystallization reagent in one crystallization droplet, after which its effect on protein crystallization and relevant mechanisms were analyzed.

2. Materials and methods

2.1. Materials

Eleven commercial proteins (Table S1) were utilized without further treatment. The proteins were selected as model proteins according to their pI distribution. The pI values of the chosen proteins were distributed over a broad range (from 3.0 to 11.3). Sodium chloride and sodium citrate were purchased from the Chemical Reagent Co. Ltd., (Beijing, People's Republic of China). Succinic acid, sodium dihydrogen phosphate, hydrochloric acid and sodium hydroxide were purchased from Beijing Chemical Factory (Beijing, People's Republic of China). Sodium HEPES and glycine were obtained from Amresco (Solon, USA). The Index™ screening kit was acquired from Hampton Research (Aliso Viejo, USA).

2.2. Preparation of the protein and pH-controlling buffers

All proteins were weighed and then directly dissolved in their respective buffers, which are listed in Table S1. All of the initial protein concentrations were 20 mg/ml, except for glucose isomerase (7 mg/ml) and papain (14 mg/ml). After the preparation, the samples were centrifuged at 10,000 rpm for 10 min to remove impurities. A multiple-component buffer system [34] was used as the pH-controlling buffer. By using this buffer system, a wide pH range can be achieved without changing the chemical components of the buffer. Three chemical reagents were used in this buffer system, including succinic acid, sodium dihydrogen phosphate monohydrate and glycine in a ratio of 2:7:7, respectively, to obtain a 1 M stock solution. This stock solution was diluted with deionized water (R = 18.3 MΩ) to a final concentration of 25 mM and adjusted to the desired pH via the addition of 1 M HCl or 1 M NaOH (PB-10.23991772 pH meter, Sartorius, Germany). The pH-controlling buffer was filtered with a 0.22 μm membrane (Millipore, California, USA).

2.3. Crystallization experiments

To screen four sets of pH buffer in one crystallization plate, we used two types of high throughput crystallization plate, including microbatch plate (abbreviated as M plate) in Fig. S1a and cross-diffusion microbatch plate (abbreviated as CDM plate) in Fig. S1b. Conventional 96-well sitting-drop Intelli Plates (Hampton Research, USA), which were termed SDVD plates in this study, served as the control group (Fig. S1c).

The geometric shapes of the CDM and M plates are shown in Fig. S1. The design of the plates is the same as that in Refs. [35,36]. Detailed structure of these two plates can be found in our previously published paper [35]. The plates are made from a water permeable material. The plates and the wells are arranged according to the Society for Biomolecular Sciences Standard (SBS), which is compatible for high-throughput crystallization using automated system. Both plates contain 96 units with 4 wells in each unit. Therefore, there are 384 wells in total for each plate. The 3D profile sizes of the CDM and M plates are the same as those of the SDVD plate. In the case of the M plate, the 96 units are separated and the 4 wells in each unit are in a sealed space. Thus, any volatile matter can diffuse freely in the same unit but cannot diffuse to other units. In the case of the CDM plate, the 96 units (384 wells in total) shared one common space and the volatile matter can diffuse from one droplet to any other droplets in the crystallization plate.

The sitting-drop method was utilized for the experiment. For both the M plate and CDM plates, pH-controlling buffers with four pH values (i.e., 2, 5, 8, and 11) were directly mixed with the protein solutions, and crystallization reagents from the Index™ screening kit (Hampton Research, USA) at a volume ratio of 1 μl:1 μl:1 μl without reservoir solution were added. For the SDVD plate, crystallization droplets were set up by mixing the protein solution with the crystallization reagents and deionized water at a volume ratio of 1 μl:1 μl:1 μl. The volume of the reservoir solution was 80 μl. An automatic protein crystallization robot (Gryphon LCP, Art Robbins Instruments, Sunnyvale, Canada) was utilized to set up crystallization screening trials.

We then tried to verify that the increase in crystallization success rate was induced by our new pH strategy rather than the CDM plate and M plate. We conducted the experiment by using the CDM plate. For the experimental group, protein solution, pH-controlling buffer and crystallization reagents were mixed with a volume ratio of 1 μl:1 μl: 1 μl. For the control group, deionized water was added as the third component instead of pH-controlling buffer.

After the crystallization trials were prepared, the crystallization plates were placed in a sealed chamber (inner dimensions 28 cm * 23 cm * 11 cm) that was connected to a programmable refrigerated circulator (Polyscience 9712 refrigerated circulator, Polyscience Inc., USA) to control the temperature inside the chamber within ±0.1 K. The temperature was 293 K and the incubation time was 48 h. After incubation, images of the crystallization droplets were captured using an automatic crystal image reader (XtalFinder, XtalQuest Inc., People's Republic of China) equipped with a UV light source (Crystalight 100 UV source, Molecular Dimensions, USA) [37]. Salt and the protein crystal were distinguished by using this device with Izit Crystal Dye (Hampton Research, Aliso Viejo, USA). The crystallization hits were obtained from the images ("hits" are defined by the number of crystallization conditions that yield observable protein crystals under a microscope).

2.4. Determination of the actual pH value

Due to the diffusion between crystallization droplets in one plate, the pH value could have potentially changed during the process of crystallization. The actual pH value was measured. For the CDM and M plates, 1 μl of pH-controlling buffer (at pH 2, 5, 8, and 11), 1 μl of 25 mM HEPES-Na buffer solution (pH 7) and 1 μl crystallization reagent from the Index™ screening kit were mixed in the drop well. For the SDVD plate, 1 μl deionized water, instead of pH-controlling buffer solution, was mixed with the protein and crystallization reagent in the drop well, and 80 μl of crystallization reagent was added to the reservoir well. The actual pH values of the droplets were measured immediately after setting up the crystallization droplet with pH precision indicator strips (Shanghai SSS Agent Co., Shanghai, China) with an accuracy range of ±0.1 pH value.

These crystallization plates were sealed and placed in an incubator at 293 K. After incubation for 48 h, the final actual pH values of the droplets were measured again with pH precision indicator strips. A humidifier was used to prevent evaporation during measurement.

3. Results

3.1. Initial crystallization screening results using pH-controlling buffer

It has been reported that protein crystallization is facilitated over a broad pH range [33]. Therefore, four sets of pH-controlling