MICROFLUIDIC REACTOR ARRAY FOR HIGH-THROUGHPUT SCREENINGS OF PROTEIN CRYSTALLIZATION CONDITIONS

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Abstract
We have developed a novel screening system in search for protein crystallization conditions using microfluidic reactor array. In this system, protein solution was accurately and equally dispensed into nanoliter-sized multiple aliquots, and mixed with various kinds of precipitants using pneumatic pressure. This screening system is highly advantageous since the required amount of expensive proteins could be decreased dramatically compared to conventional methods. As model proteins, we performed crystallization of lysozyme and glucose isomerase, and the capability of this system was demonstrated.

Keywords: crystallization, microfluidic reactor array, protein, screening

1. Introduction
In recent years, proteomics and structural genomics are becoming more and more important with the progress of biotechnology. One of the most important analyses is X-ray crystallography of proteins for the discovery of new drugs. However, the primary condition screenings for protein crystallization is sometimes difficult and troublesome. In normal scale, approximately 1 µL of protein solution is required for each screening condition, and more than 100 conditions are usually required for the crystallization of one protein, which is sometimes equivalent to the amount of protein obtained through the cultivation of tons of E. coli. Therefore, a new method, with which we can reduce the required volume of expensive proteins, is required.

In this study, we propose a novel screening system in search for the protein crystallization conditions using microfluidic devices. Microfluidic reactor array [1, 2] is adopted for the ultra-low volume protein crystallization. Using this system, required volume of proteins can be decreased dramatically, less than one hundredth compared to...
the normal scale. Also, by arranging multiple structures in parallel, various conditions can be tested simultaneously, minimizing the dead volume.

2. Experimental

2.1 Microdevice

PDMS microdevices were fabricated through usual soft lithographic techniques and replica molding. Two PDMS plates with different channel depths were bonded after O2 plasma treatment. Schematic diagram of microdevices used in this study is shown in Figure 1. This device consisted of three-dimensional microchannel networks, in which nanoliter-sized liquids could accurately be dispensed and mixed using pneumatic pressure. There were one inlet for the protein solution (Figure 1, A), ten inlets for different precipitant solutions (Figure 1, B), and ten air vent channels (Figure 1, C). Narrow channels (Figure 1, D), whose depths were typically 5 - 15 μm, worked as a kind of valve, for both of hydrophilic and hydrophobic liquid [3]. With this device, single injection of protein solution (approximately 1 μL) was enough to prepare ten aliquots for testing ten crystallization conditions. The size of each mixing chamber was 800 x 500 x 100 μm, and its volume was 40 nL. 10 nL of protein solution and 10 nL of precipitant solution were mixed in this chamber. Each chamber was equipped with an air vent, which was indispensable for the introduction of liquids into the chamber.

![Microdevice](image)

**Figure 1. Microdevice for the testing of ten conditions.**

2.2 Liquid operation

Schematic diagram of liquid dispensing and mixing is shown in Figure 2. Liquid operations were performed by controlling the pneumatic pressure using a pressure controlling apparatus (μ-flow mate, Arbiotech Inc., Japan).

Lysozyme (100 mg/mL in acetate buffer, pH 4.5) and glucose isomerase (21.7 mg/mL in MES-HCl buffer, pH 6.5) were used for model protein solutions, whereas 0.8 M potassium/sodium tartrate, 1.0 M sodium acetate, 2.0 M ammonium acetate, 20% isopropanol, 30% polyethylene glycol 4000, and 30% 2-methyl-2,4-pentanediol were used for precipitants.
After the mixing of protein solution and precipitant, microchannels were sealed with liquid paraffin to minimize the evaporation of solutions. After that, microdevice was preserved in a refrigerator at 4 °C for at least 3 days. Microscope with polarizing filter was used for the observation of crystals since it was suitable for making out optically active molecules such as proteins.

Figure 2. Schematic diagram of liquid dispensing and mixing. (A) Liquid introduction, (B) Liquid (droplet) dispensing, and (C) Mixing.

3. Results and discussion

3.1 Liquid Dispensing

First, we tested whether various types of precipitants could successfully be dispensed. Participants could be classified into three groups; salt solution group, PEG solution group, and alcohol solution group. Introduction pressure was controlled to be 3 kPa. As a result, it was observed that salt solution stopped in front of the narrow channel, whereas PEG solution and alcohol solution stopped at the end of the narrow channel. However, droplets of all these solutions with precise volumes were successfully dispensed and introduced into the mixing chambers.

3.2 Crystallization

We observed protein crystallization in microdevices. Micrographs of protein crystals formed in the microdevices are shown in Figure 3; (A) is lysozyme and (B) is glucose isomerase. A mixture of 0.1 M HEPES and 0.8 M potassium/sodium tartrate (pH 7.5) for lysozyme, and a mixture of 30% PEG 4000, 0.1 M Tris-HCl, and 0.2 M lithium sulphate (pH 8.5) for glucose isomerase, were used as the precipitants, respectively. For the comparison with the normal scale, we performed crystallization in plastic wells using

Figure 3. Crystals of proteins formed in microdevices. (A) is lysozyme, (B) is glucose isomerase.
approximately 1 μL of protein solutions, and same precipitants. Micrographs of formed crystals in the normal scale is shown in Figure 4. It can be seen that the shapes of protein crystals in the microscale were the same as those in the normal scale, demonstrating the suitability of this microdevice. With this microdevice, required volume of protein solution for testing one condition was 10 nL, which was less than one hundredth compared to the normal scale.

4. Conclusions

We have successfully performed protein crystallization with ultra-low volume protein solutions, minimizing the dead volume. This system will soon be automated since liquid operation is very simple and highly reproducible. In addition, such a microscale crystallization technology will become a useful tool in the field of high-throughput screening for proteomics and structural genomics.

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References