PROTEIN REFOLDING PROMOTED BY MULTI-DILUTION MICROCHIP

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ABSTRACT

To study the removal of the denaturant from denaturated proteins during protein refolding, we prepared two microfluidic chips. We found that the model proteins prepared by a microfludic chip with a multi-reaction step showed higher secondary structures and enzymatic activities compared with proteins prepared by batch. In addition, this refolding process was achieved within a few minutes.

KEYWORDS: Protein Refolding, Microfluidics Chip, Denaturated Protein, Inclusion Body

INTRODUCTION

The recombinant proteins are useful for studies of biological functions of genes and bioelements. However, expression proteins in *E. coli* often leads to production of the aggregated form in insolubilized inclusion bodies. These inclusion bodies are subjected to solubilization by denaturants and subsequent refolding methods, i.e., dialysis in order to recover the bioactive form. Many successful results were obtained by this procedure however, the method is not universally applicable because of the lack of sufficient data on the kinetics of removal of detergents or denaturant [1].

Microfluidic reaction system is a superior analytical device that enables strict control of solution flow [2]. Mixing of different solutions can be controlled by using efficient microfluidic design. In addition, the system eases kinetic analysis of chemical reactions because the fluidic system is characterized as laminar and therefore, the reaction time can be simply manipulated by the channel length. Studies of protein folding kinetics using microchips have been reported [3, 4], however, there is no study yet on the recovery of bioactive proteins from denaturated protein. In the present study, we examined the removal process of denaturant from denaturated proteins. Urea-denaturated citrate synthase (CitSyn), which is recognized to be difficult to refold [5] and urea-denaturated ZAP70 kinase domain, which is a tyrosine kinase recovered from inclusion bodies, were used as model proteins.

THEORY

Denaturated proteins in concentrated denaturant solution generally are dialyzed against a buffer, and hence, exposed to descending concentration of the denaturant. Using the one-step dialysis, denaturant concentration decreases with increasing time. As the concentration of denaturant is decreased, the rate of folding into the intermediate and native structures increases. However, the rate of misfolding or aggregation will also increase. To solve this problem, the step-wise dialysis has been widely utilized. Denaturated proteins are first brought to equilibrium with high denaturant con-

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centration, then with middle concentration, and with low concentration. However, this method is not universally applicable and usually small molecules are added as co-solutes. Moreover, it takes a few days. In contrast to dialysis procedure, laminar flow in the microchips helps in the continuous removal of the denaturant from denaturated proteins. To study the effect of laminar flow on protein refolding, we designed two microfluidic chips, MR1 and MR2 (Figure 1). The denaturated proteins directly reacts with a buffer in MR1 (one-step reaction), while MR2 involves a multi-reaction step.

EXPERIMENTAL

Microchips were fabricated on polydimethylsiloxane as previously reported [6]. Porcine heart CitSyn was purchased as a suspension in 2.2 M ammonium sulphate. The folded CitSyn was prepared by dialysis, and the secondary structure is comparable to that of the reported wild-type protein [5]. The denaturated CitSyn was prepared in 2.5 M urea. The activity of CitSyn was measured as described previously [5]. The *N*-terminal His_6-tag ZAP70 kinase domain (mouse 337-597) was expressed in *E. coli* (JM 109). The Zap70 protein in inclusion body was dissolved in 8M urea and was purified by nickel-chelating column. The denaturated proteins (250 μg/mL) were diluted 10-fold by 20 mM Tri-HCl (pH 7.5) or 50 mM PBS (pH 7.0) by microfludic chips. Far-UV circular dichroism (CD) spectra were measured on a JASCO J-820 spectropolarimeter at room temperatur

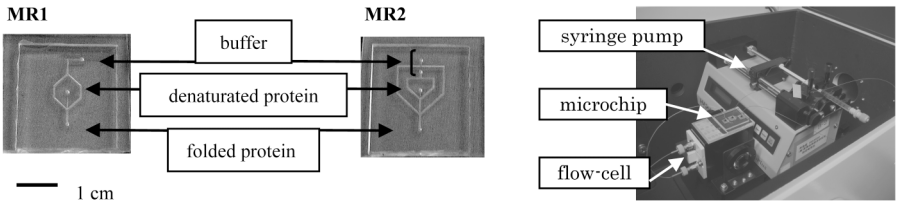

Figure 1. Microfluidic chips (left). The microchip has a dimension of 500 μm depth and 500 μm width. Assembled microreaction system (right).

RESULTS AND DISCUSSION

The refolded CitSyn by MR1 showed the same secondary structure and enzymatic activity as the batch sample. In contrast, the CitSyn prepared by MR2 with a multi-reaction (2-fold to 5-fold) showed higher helical structure (Figure 2a) and activity compared with those of the batch protein (Figure 2b). This protein refolding process was achieved within a few minutes. In addition, this method does not need any additive, suggesting that the continuous removal of urea in laminar flow may produce the intermediate and native structures, not misfolding or aggregation. Moreover, the mild reaction with buffer (2-fold to 5-fold) is a better refolding process than rapid reaction (5-fold to 2-fold or MR1). Next, we studied the refolding of ZAP70. The urea-denaturated ZAP70 formed the helical conformation in a 10-fold diluted solution. The CD spectrum of refolded ZAP70 by MR1 showed the same spectrum as batch sample. Like CitSyn, the secondary structure of ZAP70 prepared

by MR2 with a multi-reaction step also showed higher helical structure compared with that of the batch protein (spectra not shown).

CONCLUSIONS

In this study, we showed that the microfluidic chip with the multi-reaction step is useful for protein refolding without the addition of any small molecules. In addition, this protein refolding was achieved within a few minutes. Results from this study can be useful for quick recovery of bioactive proteins from inclusion bodies.

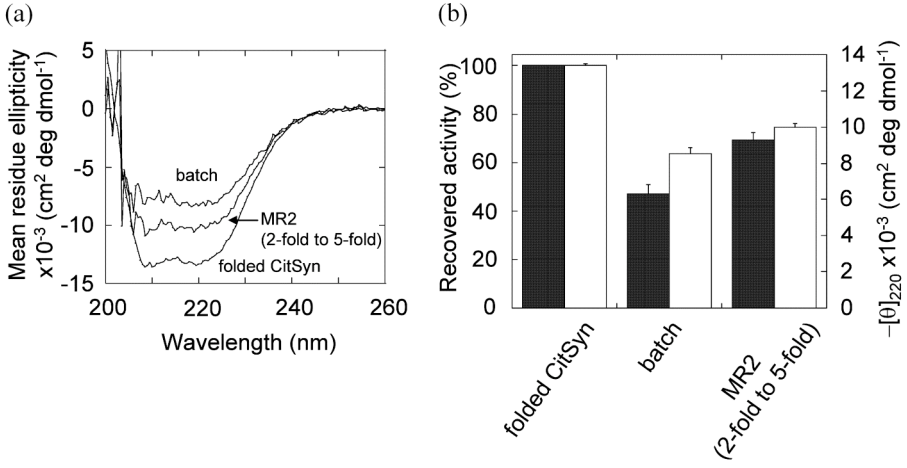

Figure 2. (a) CD spectra of the refolded CitSyn (25 μg/mL). (b) The enzymatic activity (closed bar) and ellipticity at 220 nm (opened bar) of refolded CitSyn.

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