ON-CHIP SINGLE MOLECULE ASSAY DEVICE INTEGRATING CELL LYSIS AND PROTEIN EXTRACTION-PURIFICATION-ASSAY COMPONENTS FOR GENETICALLY ENGINEERED PROTEINS

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ABSTRACT

We have developed a single molecule assay device applicable to the evaluation of genetically engineered proteins. Cell lysis, protein extraction, purification, and assay were sequentially performed on a chip. The cell lysis part was evaluated by absorptiometry and proved to retrieve enough proteins for the assay. Target proteins were purified and assayed on a functionalized channel surface. The single molecule assay on the fabricated chip provides compatible results to a bulk-scale assay.

Keywords: Cell lysis, On-chip purification, Single molecule assay, Protein assay

1. INTRODUCTION

Many fractionated components for protein engineering have been developed such as devices for electroporation, cell culture, cell lysis, and protein assay. They, however, are not well integrated because a processed sample from each device is not directly applicable to the next due to differences of sample amount or concentration. Only a report realized processes from cell lysis to fluorescence assay [1], but protein activity was not evaluated quantitatively. Therefore, we propose a novel assay device along with cell lysis and protein purification, which is applicable to the E. coli-based gene expression system. The device necessitates only a countable number of proteins thanks to the on-chip single molecule assay.

(a) Cr/Au were patterned on a coverslip. Electrode gap and height are 50 µm and 150nm, respectively.

(b) Assay part device consisted of Y-shape microchannel (H: 100 µm, W: assay channel 540 µm, cell inlet channel 60 µm, buffer inlet channel 440 µm) and Ni-NTA coated coverslip.

Figure 1. Devices for cell lysis (a) and extraction-purification-assay (b).
2. EXPERIMENTAL

We first fabricated two devices for cell lysis and extraction-purification-assay (Fig. 1) for verification of each component, which were finally integrated for the sequential procedure (Fig. 2). A target protein was F$_1$-ATPase expressed in *E. coli* known as a rotary molecular motor [2]. Cells were lysed by square pulse voltages applied by triangular electrodes. The amount of extracted protein was measured by absorptiometry.

Crude sample from cell lysis device (applying voltage; 250 V, pulse width; 50 μsec, duty ratio; 0.01%, number of pulses; 1800) without any pretreatment was injected to extraction-purification-assay device for its evaluation. F$_1$-ATPase molecules are trapped on the channel surface by affinity between Ni-NTA coated on a glass and His-tag expressed in F$_1$-ATPase. Sequentially, streptavidin-coated beads was injected and immobilized on biontynlated moiety of the protein. The bead rotation was monitored by a microscope after injecting ATP. The total processes from cell lysis to assay were performed in the integrated device by just injecting 4 μl solution containing cells.

3. RESULTS AND DISCUSSION

For cell lysis evaluation, OD$_{280}$ corresponding to protein concentrations are plotted depending on applied voltage and pulse width (Fig. 3). Higher electric field and wider pulse provide efficient cell lysis and protein extraction. Extracted protein concentration is lower than one obtained by bulk-scale ultrasonic lysis, however, it is high enough for the single molecule assay. Extraction-purification-assay part is evaluated by the number of rotating beads density depending on the channel position (Fig. 4). When cells are lysed on a chip, rotating beads density has peak across the channel. Diffusion in a Y-shape microchannel makes sample concentration gradient, and automatically optimizes sample concentration for the assay. This is an advantage over the bulk scale optimization which is usually realized by preparing several samples with discrete concentrations in tubes. The total revolutions of three beads assayed in the integrated device are compared with results obtained by the conventional purification and assay (Fig. 5). It proves the integrated device has possibility to replace the bulk experiments, since the protein activity is comparable each other.

4. CONCLUSIONS

On-chip electrical cell lysis and protein extraction were optimized for *E. coli*-based gene expression system. The extracted target protein was purified and assayed in the chemically modified microchannel. We integrated two devices to one chip and demonstrated the
Figure 3. Results of cell lysis: (a) Relationship between absorbance and applied voltages. (b) Relationship between absorbance and applied pulse width.

Figure 4. Relationship rotating beads density and all immobilized beads density on coverslip to channel across position: PDMS walls of cell inlet side and buffer inlet side in assay channel were defined 0 μm and 540 μm as channel position, respectively.

Figure 5. Analysis of rotating beads in an integrated device as shown in (Fig. 2): (a) Observation of a bead rotated by single F1-ATPase molecule in the device (frame rate: 15 Hz). (b) Analysis of rotating beads using F1-ATPase by conventional purification and the integrated device.

sequential single molecule assay from cell lysis to rotation analysis. Results prove the total process time decreased from a few days to one hour without losing protein activities. The presented device can be expanded by integrating other components such as electroporation or cell culture for sequential evaluation of genetically engineered proteins.

REFERENCES