EFFECTIVE DILUTION OF PROTEIN FOR SINGLE MOLECULE ASSAY IN AN INTEGRATED ASSAY DEVICE

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ABSTRACT

We present an integrated device, which enables cell lysis, protein extraction, purification, and activity assay for the genetically engineered protein, F₁-ATPase. Evaluations of individual functions for cell lysis and assay proved that the device provides a compatible platform to perform a single molecule assay. In addition to these functions, the effective dilution of the eluted protein was necessary to achieve molecular system for the assay. The comparison of experimental results and numerical study reveals that F₁-ATPase concentration is optimized in a certain range.

KEYWORDS: μTAS, Single Molecule Assay, Molecular Diffusion, Motor Protein

INTRODUCTION

We have previously developed a single molecule assay device applicable to genetically engineered proteins by integrating cell lysis, protein extraction, purification, and assay [1]. Here, we report detail analysis of the device function focusing on cell lysis and dilution phenomena of F₁-ATPase protein.

Many fractionated components have been developed such as devices for electroporation, cell culture, cell lysis, and protein assay. Their integration, however, are still limited to a few cases including ours [1-3]. In our device, the target protein, F₁-ATPase, is diluted automatically by its diffusion at the interface between cell eluate and dilution buffer, in which F₁-ATPase concentration is considered to be optimized for single molecule assay. To understand the phenomena we applied a simple diffusion theory to obtain F₁-ATPase concentration in the Y-shape channel, and compared with assay results.

EXPERIMENTAL AND SIMULATION

Detail functionalities for the integrated device was illustrated in references [1, 2]. Cell lysis function was evaluated by absorptiometry (OD₂₈₀) depending on field strength, cell flow rate, and applied square pulse width. The evaluation is important to increase the eluted F₁-ATPase and the number of rotating beads in the assay channel. F₁-ATPase was diluted by buffer solution (50 mM MOPS-KOH, 50 mM KCl, 2 mM MgCl₂, pH7.0) injected from the buffer inlet. The molecular system for F₁-ATPase single molecule assay was achieved in the assay channel. The number of rotating beads by F₁-ATPase activity was measured according to x-direction in Fig. 2. The F₁-ATPase concentration diluted in Y-shape channel (Fig. 2) are calculated based on the general solution (Eq. 1) to the diffusion equation and its sum (Eq. 2).
The y-direction is eliminated in our analysis due to the shallow channel [4]. The initial interface between eluate and dilution buffer is assumed as $x=348\mu m$ and other parameters are summarized in Fig. 2.

**RESULTS AND DISCUSSION**

The amount of eluted F1-ATPase increased as field and pulse width increase as shown in Fig. 1a and Fig. 1c, respectively, which shows good agreement with the results obtained for the individual device [2]. However, Fig. 1b indicates that it is necessary to decrease the flow rate to elute enough F1-ATPase. Therefore, we chose the flow rate of 5 $\mu l/min$ for the following experiments and simulations.

Figure 3a shows the experimental results for the density of rotating beads in x-direction. The cell lysis condition was 0~250 V, pulse 50 $\mu s$, duty 0.01%, and 1200 total pulses. Arrows indicate the positions where the highest density was obtained, which means that the F1-ATPase concentration was optimized for the assay. The calculated concentrations depending on channel position and initial concentration ($C_0$) are obtained as expected (Fig. 3b), where $z$-direction is averaged down to $z=120$...

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*Figure 1*: Results of cell lysis in the integrated device: Relationship between absorbance ($OD_{280}$) and (a) field strength, (b) flow rate, and (c) pulse width. The absorbance represents the eluted protein concentration.

*Figure 2*: (Left) Y-shape channel model used to calculate F1-ATPase concentration gradient. Only x and y axes are considered as parameters. (Right) Parameters for numerical calculation using Eq. 1 and 2 to obtain concentration gradient in x and z direction as shown in Fig. 3a.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>Flow rate</td>
<td>5 $\mu l/min$</td>
</tr>
<tr>
<td>Flow velocity ($v$)</td>
<td>958 $\mu m/s$</td>
</tr>
<tr>
<td>Diffusion coefficient ($D$)</td>
<td>27 $\mu m^2/s$</td>
</tr>
<tr>
<td>Channel height ($h$)</td>
<td>150 $\mu m$</td>
</tr>
<tr>
<td>Channel width ($w$)</td>
<td>580 $\mu m$</td>
</tr>
<tr>
<td>Initial sample width</td>
<td>348 $\mu m$</td>
</tr>
</tbody>
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$C(x,t) = \frac{C_0}{\sqrt{4\pi Dt}} \exp \left( -\frac{x^2}{4Dt} \right)$ (Eq. 1)

$C(x,z) = \sum_{n=0}^{m} C_n(x,z)$ (Eq. 2)
mm from the mixing point \((z=0)\), and the F\(_1\)-ATPase concentrations are normalized by OD\(_{280}\) at 250 V. The result reveals that the concentration profiles in the assay channel mostly depends on the initial concentration. Arrows in Fig. 3b indicate the corresponding arrow position in Fig. 3a to determine the optimized F\(_1\)-ATPase concentration in the range of 0.1-0.6. The similar profile was also found in the simplified assay using a Y-shape channel without the cell lysis part (data not shown). Since the range is too large, we consider that our integrated device does not only dilute F\(_1\)-ATPase but optimize the ratio of F\(_1\)-ATPase to eluate for single molecule assay.

**CONCLUSIONS**

We have evaluated the cell lysis function in the integrated single molecule assay device. The comparison of our simulation for diffusing protein and experimental results reveals that assay efficiency of F\(_1\)-ATPase is not necessarily depend on its concentration only. It seems to be affected by the other eluate including contaminated proteins. However, results support that our device is applicable to dilute target proteins to the appropriate ratio including eluate from cells for the single molecule assay.

**ACKNOWLEDGEMENTS**

This research is supported by a research promotion program in Ritsumeikan University.

**REFERENCES**