Recombinant production of FRET-based mechanical sensor to visualize cell–material interaction

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Statement of Purpose: Physical behaviors of cells on materials, such as cell migration and cell adhesion force, are one of the important factors for scaffold designing. A large number of studies have evaluated mechanical properties of cells on materials by using engineering devices, including AFM and micropipette aspiration. However, most of these measurements are destructive and requires well-trained manipulations. Although elastic micropillar substrates have enabled the non-destructive, real-time measurement of cell traction force, adhesion area for cells is restricted and unnatural. Hence, there are limitations on the measurements of cell mechanical properties with engineering techniques. On the other hand, by using molecular biologic technologies, Grashoff et al. have developed a fluorescence resonance energy transfer (FRET)-based biosensor that measures forces across specific proteins in cells [1]. This sensor was fused with vinculin and sensed mechanical tension across the cell adhesion molecule. Here, we have aimed to produce a FRET-based mechanical sensor as a recombinant protein by reference to the report by Grashoff et al. [1]. To evaluate cell–material mechanical interaction, we have addressed to immobilize the sensor to a substrate.

Methods: The concept and principal of the FRET-based mechanical sensor is shown in Fig. 1. To prepare a vector for the expression of the sensor, 5′-Cys_3-mKusabira Orange (as acceptor; Ex, 548 nm; Em, 559 nm; MBL)-elastic linker ($GPGGA_8$)-mUmikinoko Green (as donor; Ex, 483 nm; Em, 500 nm; MBL)-$RGDS_2$-stop codon-3′ were constructed. This construct was cloned into the pTrcHis (Invitrogen) expression vector. The construct was verified by DNA sequencing. *E. coli* strain BL21 cells transformed with the vector were grown with expression induction by IPTG. Sensor proteins were extracted in a solubilized fraction according to the QIAexpressionist protocol [2]. The purification of the sensor protein was conducted by Ni chelate affinity and gel permeation chromatography. The resultant sensor protein solution was dialyzed against PBS.

The sensor protein concentration was quantified by a BCA assay and adjusted to 0.3 μM. Emission spectra of the sensor/PBS solution were measured with a fluorescence spectrophotometer (Hitachi). 483- or 548-nm lights were used as exciting lights. Protease K was added to the solution (final concentration, 0.2 μg/ml) to break the elastic linker. Emission spectra were obtained 0, 2, 5, 10, 20, 30, 45, and 60 after the addition of the enzyme.

Two μl of 4 μM sensor/PBS solution was spotted on an Au chip for a surface plasmon resonance (SPR) analysis to evaluate the immobilization of the sensor to the Au-coated substrate. PBS, 0.05%SDS/PBS, and PBS were streamed in this order in the flow channel of a SPR imager (GWC Instruments) for 5 min each at 0.1 ml/min, while reflection intensity was monitored every 5 sec. Sensor protein without Cys_3 was used as a comparison group.

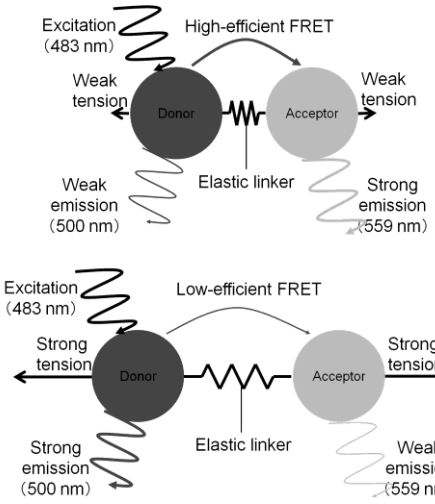

Fig. 1. Concept of the FRET-based mechanical sensor.

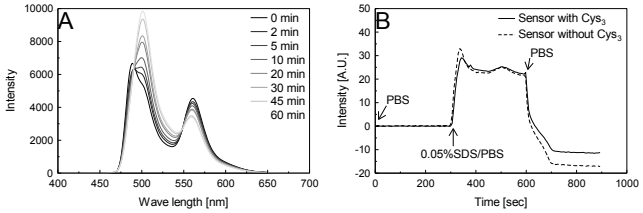

Fig. 2. Emission spectra of FRET-based mechanical sensor excited with 483-nm light (A) and SPR intensity as a function of time (B).

Results: The FRET-based sensor protein was successfully synthesized in recombinant *E. coli* and well purified.

Figure 2A shows the emission spectra of the sensor excited with 483-nm light. Two peaks were observed at 500 and 560 nm. Protease K treatment increased the 500-nm peak while decreased the 560-nm peak, suggesting that the donor and acceptor were separated from each other insufficient for FRET. Therefore, we confirmed the intramolecular FRET in the sensor.

Figure 2B shows the results of the SPR analysis. After washing with 0.05%SDS/PBS, the reflection intensity of the sensor without Cys_3 was lower than that of the sensor with Cys_3. This result indicates that the sensor with Cys_3 attached to the Au substrate more strongly than the sensor without Cys_3. As thiol group binds to Au coordinately, there is a possibility to immobilize and align sensor proteins on an Au substrate.

Conclusions: FRET-based sensors were successfully produced in recombinant E. coli and immobilized to a substrate. We are evaluating the performance of the sensor-fixed substrate with cells on it.

References: [1] Grashoff C et al. Nature. 2010;466:263-267. [2] http://web.mnstate.edu/provost/QiaExpressionist.pdf.