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On-chip electrochemical measurement of β-galactosidase expression using a microbial chip

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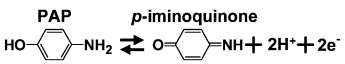
β-Galactosidase expression in a small number of *Escherichia coli* cells has been measured in real time with an electrochemical sensor chip. *E. coli* cells were embedded using collagen gel within a micropore which was microfabricated onto a chip. The activity of the expressed β-galactosidase was determined using *p*-aminophenyl β-D-galactopyranoside (PAPG) as a substrate.

Reporter-gene systems are frequently used in gene-expression studies by incorporation into a vector plasmid or fusion with a promoter-gene of interest. The gene *lac*Z, encoding the *Escherichia coli* (*E. coli*) enzyme β-galactosidase, is one of the most widely used reporters.[1] Standard assays for monitoring the reporter proteins are based on colorimetric, fluorimetric, or chemiluminometric detection.[2] All these methods involve repeated sampling from the culture and an additional step of lysis or permeabilization of the cells, making the assay procedures time-consuming and possibly perturbing natural cell growth. Electroanalytical methods, on the contrary, are expected to overcome the problems mentioned above. The activity of the expressed β-galactosidase can be determined by using *p*-aminophenyl β-D-galactopyranoside (PAPG) as a substrate. The product, *p*-aminophenol (PAP), can be oxidized by the following electrode reaction:[3]



Recently, several studies have been reported to electrochemically detect β-galactosidase activity in whole cell microorganisms, either as a reporter, genetically fused downstream of a certain promoter, or expressed from the *lac* operon originally existing in *E. coli* cells.[4] Daunert *et al.* have investigated highly selective sensing of antimonite and arsenite using voltammetry.[5] Rishpon *et al.* have constructed a multititer plate system monitoring β-galactosidase expression on-line.[6]

We have developed the microfabrication of living microorganisms on a solid support and a system for measuring the function of microorganisms and mammalian cells with scanning electrochemical microscopy (SECM).[7–9] In the present study, we investigated β-galactosidase expression in a small number of *E. coli* cells by utilizing an electrochemical microdevice with on-chip incubation and on-chip monitoring. To the best of our knowledge, this is the first study to realize the whole cell sensing of gene expression in a miniaturized system.

Escherichia coli K-12 (IFO3301) strains were obtained from NCIMB Japan. All cultures were preincubated aerobically for 16–18 hours at 37 °C with vigorous shaking in the nutrient medium (pH 7.0) containing 5 g of meat extract, 10 g of peptone, 5 g of NaCl, and 5 g of KH_2PO_4 in 1000 ml of water. PAPG, isopropyl β-D-thiogalactopyranoside (IPTG), and *o*-nitrophenyl β-D-galactopyranoside (ONPG) were purchased from Sigma and used without further purification.

Basic photolithographic and chemical etching techniques were used to microfabricate the chip which has a micropore and a microelectrode on the same glass substrate. The platinum pattern was fabricated by sputtering two thin layers, titanium and platinum, onto the photolithographic patterned substrate. After lift-off resist polymer, the micropore was fabricated near the Pt pattern by wet glass etching through the chromium mask. The micropore has the geometry of 300 μm squares and 50 μm depth, and the distance between the Pt pattern and the micropore was 30 μm. A layer of photo-definable polyimide was used to define the electrode shape, 20 μm squares (Fig. 1).

The incorporation of *E. coli* cells using collagen gel microstructure has been reported previously.[7,8] We prepared a mixed solution with collagen type I (Nitta Gelatin Inc.), *E. coli* suspension, and a 10-fold concentrated PBS buffer solution in a proportion of 8 : 2 : 1. The number of embedded *E. coli* in the collagen gel microstructure was 10^3 cells.

Figure 1 shows a cyclic voltammogram (a) of 4.0 mM ferrocyanide measured with the electrode on the chip along with a photograph (b) of the chip. The voltammogram appears to have an ideal sigmoidal shape, indicating that the electrochemical nature of the square-shaped electrode is practically identical to that of a microdisk electrode with a 10-μm radius, and that the polyimide resist on the electrode seen in the left part of the photograph effectively shields the working electrode. As the electrode is located very close to the reaction zone, a rapid response and a large dynamic range are expected. In comparison with the SECM detection scheme reported previously,[7,8] on-chip detection significantly reduces processes of preparation and measurement setup in the incubator, because the alignment of the detector and the sample is done before the recording.

To assess whether the electroanalytical assay for expressed β-galactosidase could be successfully adapted for on-line monitoring, we determined the enzymatic activity following induction with IPTG. The potential of the Pt microelectrode was held at 0.30 V *vs.* Ag/AgCl to detect the PAP oxidation current. All the electrochemical measurements were conducted in a nutrient medium containing 1.0 mM IPTG and 2.0 mg ml^{-1} PAPG, in a 37 °C incubator. As IPTG functions to activate *lac* operon including *lac*Z, β-galactosidase expression of *E. coli* microfabricated on the chip can be electrochemically monitored in real time. Figure 2 shows the on-chip amperometric responses of the PAP with (a) and without (b) 1.0 mM IPTG. In the presence of IPTG, two peaks were observed in the current response: the first around 5 h, and the second around 10 h after the addition of IPTG. In the absence of

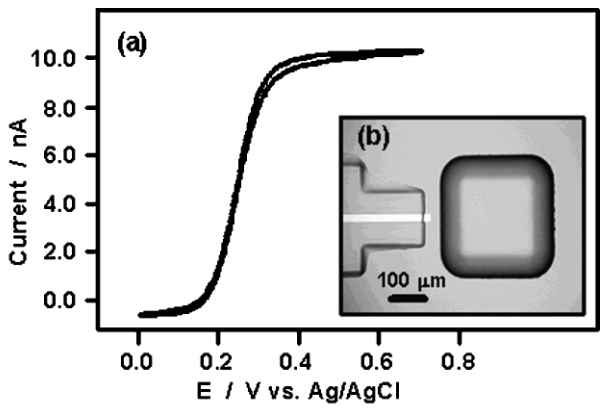


Fig. 1 (a) Cyclic voltammogram of 4.0 mM ferrocyanide at a Pt microelectrode fabricated on glass substrate. Scan rate: 0.02 V s^{-1}. (b) An optical microscopic image of micropore and microelectrode on the glass substrate.

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IPTG, a single peak was observed at 10 h after the on-chip incubation. Clearly, as the first peak was observed only when IPTG was present, the increase in current corresponds to the β-galactosidase activity induced by IPTG. The timescale (~ 5 h) tends to be longer compared with that for the gene-expression in a solution of 300 μl volume reported by the other group.[6] On the contrary, the second peak that appeared around 10 h after incubation did not depend on IPTG, suggesting that the increase in current reflects an increase in the number of *E. coli* cells. Generally, the monad of the *lac* operon product exists in *E. coli* cells before the induction with IPTG. The response around 7 h to 10 h after incubation is explained by the growth rate of *E. coli* on the chip, which has been previously monitored by a growth assay based on respiration activity using SECM.[8] The decline after the peak current at 10 h after incubation causes the inactivation of the Pt microelectrode by the *E. coli* cells that over-flowed from the collagen gel microstructure.

To compare the current response with a conventional technique, β-galactosidase expression was monitored by a colorimetric method using ONPG as shown in Fig. 2 (inset, ○). In the colorimetric assay, the activity of expressed β-galactosidase was measured by the optical density of *o*-nitrophenol, the product of the substrate ONPG (final concentration 2.6 mM) at 420 nm (OD_{420}). The amperometric (▲) and the colorimetric responses (○) matched completely, especially for the starting time of the response. The colorimetric method needs a significant amount of microorganisms

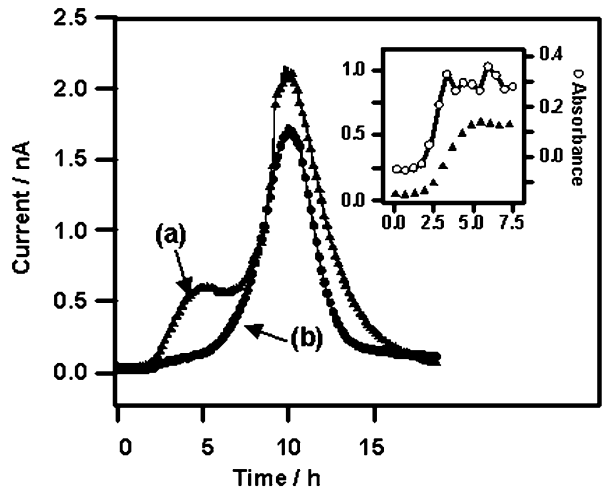

Fig. 2 The current responses of *E. coli* cells in the collagen gel microstructure detected at the Pt microelectrode on the glass substrate with (a, ▲) and without (b, ●) 1.0 mM IPTG. The measurement solution contained 2.0 mg ml^{-1} PAPG. The inset shows the time course curves of β-galactosidase expression, absorbance measurement of *o*-nitrophenol (○, Y-axis on right side) together with the electrochemical measurement of *p*-aminophenol (▲, Y-axis on left side).

(~ 10^8 cells) and involves the permeabilization of the cells followed by a multi-step procedure. Thus, this method cannot be applied to a case in which the available sample with microorganisms is limited in volume, or a case in which real-time measurements are necessary. On the other hand, the on-chip monitoring system described in the study is capable of determining the enzymatic activity in whole cells and does not require sample removal or changing the permeability of the cells. Moreover, the present system is potentially useful for screening DNA cloning systems based on bacterial culture. The miniaturization and integration of the microstructures can be used to fabricate arrays of cells or microbial chips for drug screening and environmental monitoring.

In conclusion, a microbial chip was fabricated by embedding *E. coli* cells in a collagen gel microstructure and by placing a detection electrode close to the patterned *E. coli* zone. The IPTG-induced expression of β-galactosidase from the embedded *E. coli* cells was monitored continuously by the detection electrode on the microbial chip. The validity of this system was shown by comparing it with the colorimetry as a conventional method. The whole cell biosensor system described in this study can be developed for monitoring various mutagens and environmental hormones.

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