

On-line monitoring of gene expression

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Gene expression in cultures of *Escherichia coli* has been determined *in situ* and on-line by the use of an electrochemical sensor. Intact bacteria were used to monitor the induction of the *lacZ* gene; the onset of stationary phase was also monitored, using a reporter gene fused to the RpoS-dependent promoter of the *osmY* gene. The technique described can in principle be used to determine the activity of any promoter, with a variety of reporter genes. This technology is non-intrusive, allows real-time monitoring of gene expression, and will be useful in the study of growth regulation and development.

Keywords: biosensors, reporter gene, on-line monitoring, β -galactosidase

INTRODUCTION

Reporter-gene systems are frequently used in gene-expression studies. Various analytical methods are available for monitoring the protein expressed by a reporter gene, including photometry, radiometry, fluorescence, colorimetry and immunoassays (Alam & Cook, 1990; Bronstein *et al.*, 1994; Groskreutz & Schenborn, 1997; Chalfie *et al.*, 1994). The predominant methods used to identify and quantify reporter-gene products in cell cultures involve repeated samplings of the culture and an assay for the enzymic activity, which often involves an additional step of lysis or permeabilization of the cells. These procedures usually perturb the culture and are time consuming – they may take several hours. An alternative procedure, that would overcome several of these difficulties, is on-line monitoring for *in situ* determination of gene (or reporter gene) products. Such a system is especially advantageous in the study of developmental or growth-phase-related genes. Recently, several publications have described the possibility of using reporter genes such as *lux* or *gfp* for on-line, or semi-on-line, non-invasive monitoring (Heitzer *et al.*, 1994; Kodikara *et al.*, 1991; Leibiger *et al.*, 1998; Valdez *et al.*, 1998).

This communication describes the development of a whole-cell biosensor for the on-line monitoring of gene expression. In the model system described, the determination was based on the electrochemical measurement of β -galactosidase activity, either expressed from

its own promoter or as a reporter gene transcriptionally fused downstream of promoters of interest.

The gene *lacZ*, encoding the *Escherichia coli* enzyme β -galactosidase, is one of the most widely used reporter genes (Silhavy & Beckwith, 1985). Standard assays are colorimetric (Miller, 1992), or use fluorimetry or chemiluminometry (Jain & Magrath, 1991). All these methods involve the permeabilization of the cells followed by a multi-step procedure. An alternative to these is the use of electroanalytical methods that are suitable for on-line measurements, even in turbid solutions.

The activity of β -galactosidase can be determined electrochemically by using the substrate *p*-aminophenyl β -D-galactopyranoside (PAPG). The product of the enzymic reaction, *p*-aminophenol (PAP), can be oxidized at an electrode as shown in Fig. 1. The electrochemical characteristics of PAP and PAPG have been studied before (Kulyts *et al.*, 1980), and several electroanalytical methods for PAP detection have been reported (Kulyts *et al.*, 1980; Masson *et al.*, 1995; Scott *et al.*, 1997). In previous studies we developed a simple and sensitive electrochemical immunoassay, in which a constant potential is applied at the electrode and the current generated by the oxidation of PAP is measured (Rosen & Rishpon, 1989). In the present study, this method was

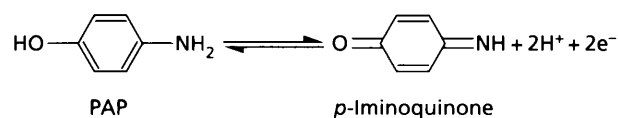


Fig. 1. Oxidation of PAP (the product of hydrolysis of PAPG, catalysed by β -galactosidase) to *p*-iminoquinone.

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Abbreviations: PAP, *p*-aminophenol; PAPG, *p*-aminophenyl β -D-galactopyranoside.

adapted for the *in situ* and on-line monitoring of enzymes, such as β -galactosidase. As an example, we monitored on-line the progress of *Escherichia coli* into stationary phase, using transcriptional fusion of *lacZ* to the RpoS-dependent *osmY* gene (Weichart *et al.*, 1993; Yim & Villarejo, 1992).

METHODS

Bacterial strains and growth. *E. coli* K-12 strain K10 (Hfr, *tonA22 ompF626 relA1 pit-10 spoT1 T2^h* CGSC [Coli Genetic Stock Center, New Haven, CT, USA] 4234) was from the CGSC collection. *E. coli* RO151 (MC4100 Φ (*csi-5::lacZ*) and *E. coli* RH99 (RO151 *rpoS359::Tn10*) were described by Weichart *et al.* (1993). All cultures were grown aerobically at 37 °C with vigorous shaking in LB medium (Miller, 1992), supplemented, when required, with 5 μ g tetracycline ml⁻¹ or 25 μ g kanamycin ml⁻¹.

Enzyme and chemicals. PAPG, isopropyl β -D-thiogalactopyranoside (IPTG) and β -galactosidase (β -D-galactoside galactohydrolase; EC 3.2.1.23) were obtained from Sigma.

The amperometric assay. Electrochemical measurements were performed using a disposable three-electrode cell, based on a screen-printed electrode (total volume 0.3 ml). The electrochemical cells were made of polystyrene tubes (Fig. 2). Graphite ink was used as the counter electrode and Ag/AgCl ink was used for the reference electrodes. Disposable graphite

electrodes in cylindrical form (made from pencil leads, HB 0.9 mm) were used as the working electrodes. The assay was performed directly in the electrochemical cells, which were vibrated to achieve mixing. The same screen-printed electrodes and graphite working electrode were also used for direct measurements in cultures. In this case the electrodes were placed inside the Erlenmeyer flask as shown in Fig. 2 and mixing was achieved by bubbling air. The graphite electrode was held at 220 mV versus the reference electrode and the substrate (PAPG) concentration was 0.4 mg ml⁻¹.

The experimental setup. The electrochemical measurements were performed using a PAR VersaStat potentiostat connected to an 8-channel PAR 314 model multiplexer (EG&G Princeton Applied Research). This system allows simultaneous measurements of eight samples with disposable electrochemical cells. The measurements were controlled by LABVIEW-based software using a Windows 95 operating system. The electrical current from the activity of β -galactosidase was visualized simultaneously in all eight samples in real-time on the computer screen (see Figs 3 and 4).

RESULTS

Configuration and optimization of the β -galactosidase amperometric monitoring system

A multiple-cell electrochemical device was used to optimize the detection of β -galactosidase activity. The

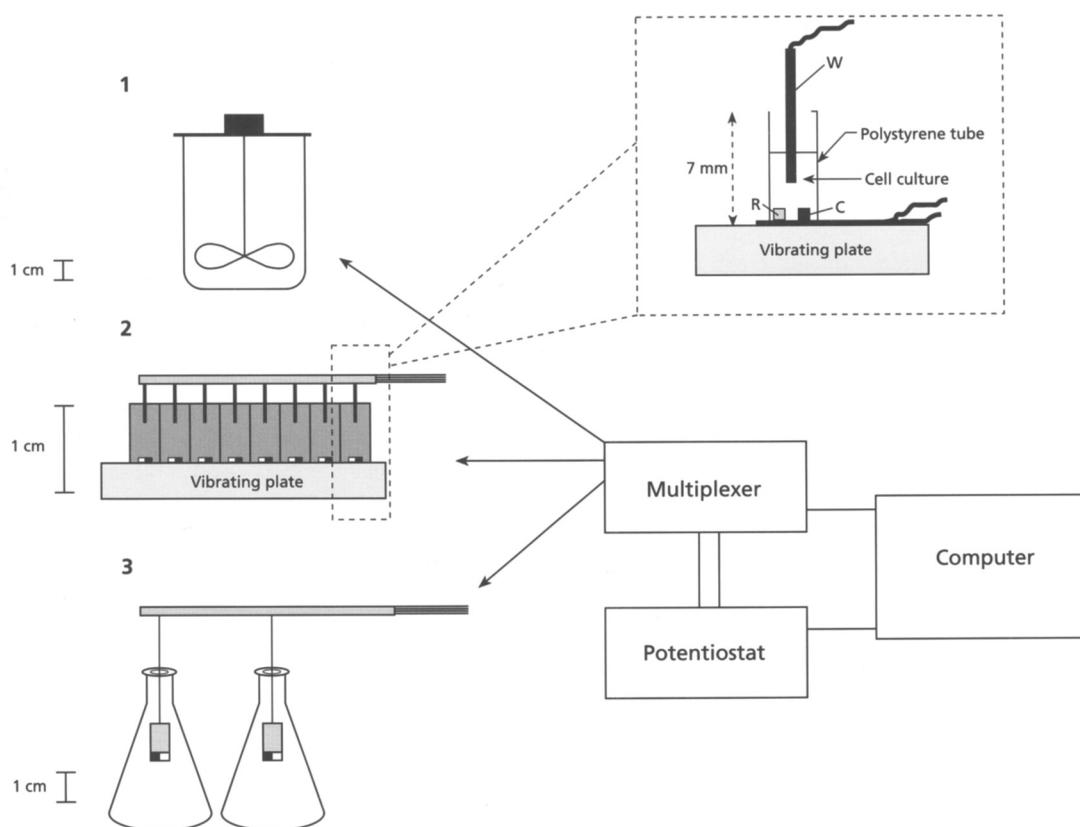


Fig. 2. The computerized electrochemical system. The electrochemical device is connected to a multiplexer and can be used for on-line monitoring in a fermenter (1), in multiple cells (volume 0.3 ml; eight electrodes are shown) (2), or directly in flasks (3). The electrodes used were disposable and sterilizable. The measurements were controlled by LABVIEW-based software using a Windows 95 operating system. (Inset: electrochemical cell. W, working electrode; C, counter electrode; R, reference electrode.)

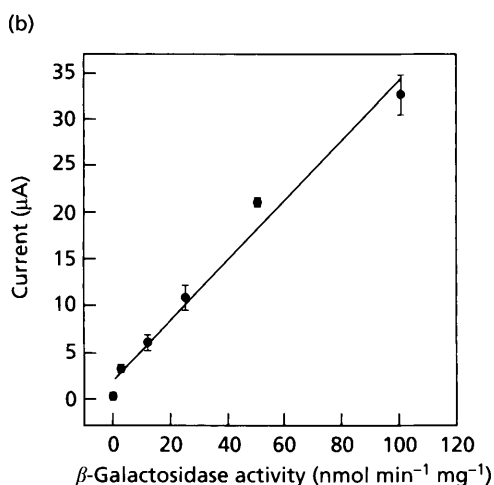
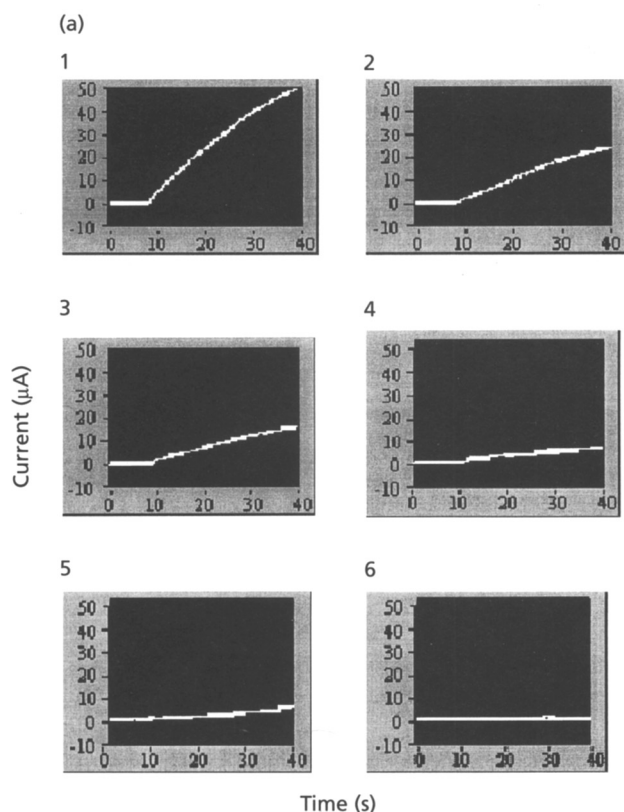


Fig. 3. On-line monitoring of β -galactosidase using the multiple-cell electrochemical device. The graphite electrode was held at 220 mV versus the reference electrode and the enzyme concentrations, in 0.1 M phosphate buffer containing 0.4 unit ml^{-1} PAPG and 1 mM MgCl_2 , were: (1) 100 units ml^{-1} , (2) 50 units ml^{-1} , (3) 25 units ml^{-1} , (4) 12 units ml^{-1} , (5) 6 units ml^{-1} , (6) no enzyme. (a) On-screen data; the current scales run from -10 to +50 μA and the time scales from 0 to 40 s. (b) Calibration curve calculated from the current obtained 10 s (lag time in the system) after the addition of the substrate.

results presented in Fig. 3(a) demonstrate the use of the multiple-cell device for determining the activity of purified β -galactosidase, using PAPG as a substrate. The results are presented as visualized on the computer

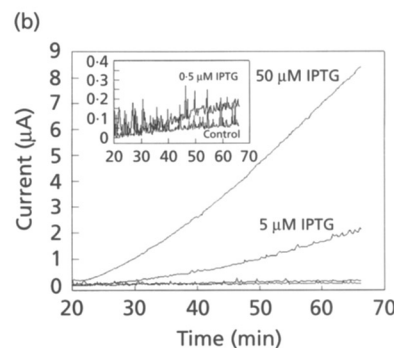
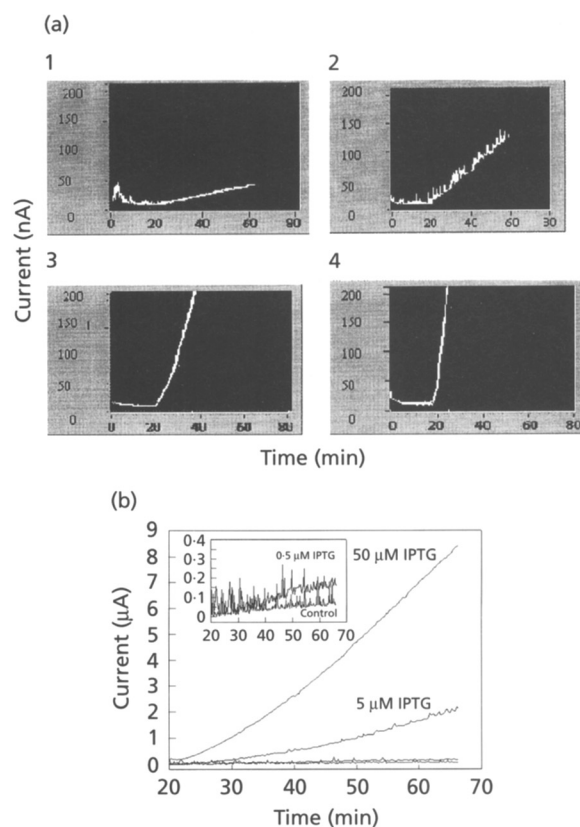


Fig. 4. β -Galactosidase activity of *E. coli* cultures treated with increasing concentrations of IPTG. The cells were grown in LB medium at 37 °C to a density of about 2×10^8 bacteria ml^{-1} . Samples of 0.3 ml were introduced in duplicate to the eight electrochemical cells. The inducer [(1) no IPTG, (2) 0.5 μM IPTG (3) 5 μM IPTG, (4) 50 μM IPTG] was added and monitoring started after 20 min with the addition of 0.4 unit ml^{-1} of the substrate (PAPG). (a) On-screen display of the data obtained from four electrochemical cells; the current scales run from 0 to 200 nA and the time scales from 0 to 80 min. (b) Curves of mean signal from duplicate samples.

screen and represent the signals from the enzymic reaction in six electrochemical cells containing increasing concentrations of the enzyme. In the calibration plot constructed from these and additional data (Fig. 3b), a linear correlation was observed in the range 3–100 units ml^{-1} and the detection limit was 1 unit β -galactosidase ml^{-1} .

On-line monitoring of β -galactosidase in intact bacteria

For many purposes it is preferable to monitor gene expression on-line. The electroanalytical assays are potentially suitable for such determinations, as they do not require permeabilization of the cells and are rapid and sensitive. To assess whether the electroanalytical assay for β -galactosidase, the *lacZ* reporter gene product, could be successfully adapted for on-line monitoring, we determined the enzymic activity following induction with IPTG. Increasing concentrations of the inducer were added to cultures of *E. coli* K10 (Annette &

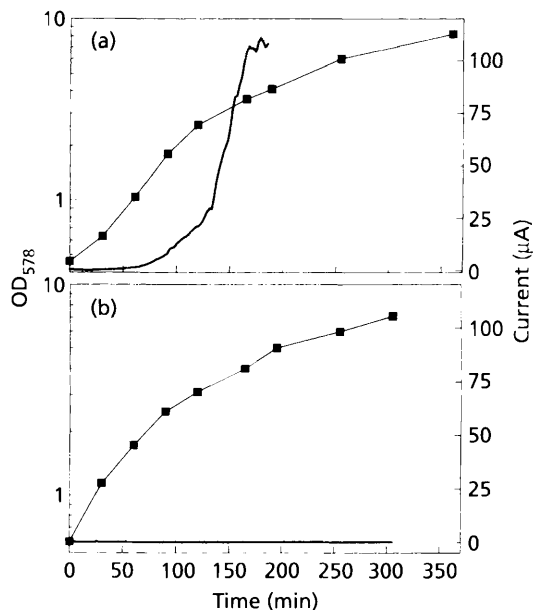


Fig. 5. On-line amperometric monitoring of the expression of the *osmY-lacZ* gene fusion in (a) *E. coli* RO151 (*rpoS*⁺) and (b) *rpoS* mutant *E. coli* RH99 (*rpoS359::Tn10*). Optical density (squares) and amperometric monitoring (line without symbols) of β -galactosidase, determined in cultures growing in LB medium at 37 °C. The substrate PAPG was added at the beginning of the experiment and the graphite electrode was held at 220 mV versus the reference electrode.

Anthony, 1987) and the current signal was monitored on-line in all the cultures simultaneously (Fig. 4a). The slope of the current signals with time was proportional to the concentrations of IPTG added (from 0.5 μ M IPTG) (Fig. 4b).

Monitoring the onset of stationary phase in *E. coli*

As a model for monitoring gene expression we used *E. coli* carrying a chromosomal *lacZ* fusion to the *osmY* promoter, which is positively regulated by the transcription factor RpoS (σ^S) and is therefore expressed only at the stationary phase (Weichart *et al.*, 1993; Yim & Villarejo, 1992). As a control we used an isogenic strain which carries an interruption of the *rpoS* gene (Weichart *et al.*, 1993). In this experiment the screen-printed electrodes and graphite working electrode were placed inside the Erlenmeyer flask (see Fig. 2) and the expression of *lacZ* was continuously measured. As shown in Fig. 5(a), a significant increase in the current signal was obtained in the transition to stationary phase. In parallel we examined the reporter gene in an *rpoS* mutant strain, where the signal was not obtained (Fig. 5b). These results are in agreement with previous results obtained with a colorimetric assay (Weichart *et al.*, 1993).

DISCUSSION

This paper describes the use of a new electroanalytical device for on-line and *in situ* monitoring of gene

expression. The on-line monitoring is facilitated by the ability to determine the enzymic activity in whole cells with no need for sample removal or changing the permeability of the cells. The *in situ* assay renders kinetic analysis of gene expression more convenient and accurate. Only a single sample of bacteria is needed to derive the time course of transcriptional activity of a gene and its response to transcriptional or environmental modulators. Moreover, the simultaneous monitoring allows a precise comparison of several strains or experimental conditions.

The on-line, *in situ*, amperometric monitoring of enzyme activity can be used to determine the expression of a variety of genes, as current molecular procedures facilitate the fusion of a variety of reporter genes to a limitless variety of promoters. One interesting application would be to monitor the expression of secreted proteins – periplasmic or extracellular – using fusion to reporter proteins such as chloramphenicol acetyltransferase or alkaline phosphatase (Manoil *et al.*, 1990), that are active only when secreted. The determination of a secreted protein would be even more sensitive than that of a cytoplasmic protein. An adaptation of our system for the use of alkaline phosphatase requires only minor modifications, since the same compound – PAP – is the product of both reactions. For other reporter genes the required modifications involve only identification of suitable substrates, but no alteration in the equipment.

Although it is conceivable to adapt other reporter genes, such as *lux* and *gfp*, for on-line or semi-on-line monitoring (Heitzer *et al.*, 1994; Kodikara *et al.*, 1991; Leibiger *et al.*, 1998; Valdez *et al.*, 1998), the amperometric biosensor reported here has several advantages. These include high sensitivity, simplicity, and the fact that the electrodes are disposable. Moreover the electrochemical reaction can be performed even in turbid solutions and under anaerobic conditions.

Whole-cell biosensors of the type described here can be developed for monitoring various aspects of growth and gene expression in prokaryotic and eukaryotic cells. This system is potentially useful for other on-line applications and can be applied to micro-organisms as well as for cell cultures. The potential uses include monitoring the availability of a nutrient in a fermenter, production of a protein of interest – natural or recombinant – and monitoring transfection efficiency in cell cultures (Hollon & Yoshimura, 1989). On-line monitoring of gene activity will be particularly useful for biotechnological applications, such as fermentation processes, where the exact state of the culture at any given time, and especially the concentration of the required fermentation product, are important parameters.

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