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SHORT COMMUNICATION

Spatial expression of a mercury-inducible green fluorescent protein within a nanoporous latex-based biosensor coating

Janet L. Schottel · Paul M. Orwin · C. Ron Anderson · Michael C. Flickinger

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Abstract Optimizing the reactivity of cell coatings developed as biosensors or biocatalysts requires measurements of gene expression in the immobilized cells. To quantify and localize gene expression within a latex-based mercury biosensor, a plasmid, p*mer*GFP, was constructed, which contains the green fluorescent protein (GFP) gene under transcriptional control of the mercury resistance operon regulatory sequences. When cells containing this plasmid were exposed to mercuric chloride, GFP synthesis was induced and could be quantified by fluorescence. *E. coli* strain JM109 (p*mer*GFP) was mixed with SF091 latex (Rohm & Haas), Tween 20, and glycerol, and coated as an approximate 20-μm thick nanoporous adhesive coating on a polyester substrate. The cell coat was overlaid with a nanoporous topcoat of latex, Tween 20, and glycerol. Different fluorescent microspheres were used to mark the topcoat and cell coat layers of the coating. Upon exposure to mercury(II), cells within the coating were induced to synthesize GFP, and laser scanning confocal microscopy was used to quantify expression spatially within the cell coat. GFP expression in the coatings increased with increasing mercury concentration (2–20 μM), temperature (21–37 °C), and time of incubation (0–39 h). There was a gradient of GFP expression through the cell coat with expression higher near the topcoat–cell coat interface relative to the bottom of the cell coat. The topcoat thickness did not significantly affect GFP expression indicating that diffusion of mercury(II) and oxygen through the topcoat was not limiting.

Keywords Mercury · Biosensor · Nanoporous latex · Laser scanning confocal microscopy · GFP

J. L. Schottel (✉) · M. C. Flickinger
Department of Biochemistry,
Molecular Biology and Biophysics,
University of Minnesota, 1479 Gortner Ave.,
St Paul, MN 55108, USA
e-mail: schot002@umn.edu

P. M. Orwin · C. R. Anderson · M. C. Flickinger
BioTechnology Institute,
University of Minnesota, St Paul, MN 55108, USA

Present Address:
P. M. Orwin
Department of Biology,
California State University at San Bernardino,
San Bernardino, CA 92407, USA

Present Address:
C. R. Anderson
Grain Value LLC, West St Paul, MN 55118, USA

Present Address:
M. C. Flickinger
Department of Microbiology,
Chemical and Biomolecular Engineering,
North Carolina State University,
Raleigh, NC 27695, USA

Introduction

Immobilized living cells or immobilized enzymes can be used to catalyze complex biochemical reactions that require energy or reduced coenzymes, and importantly, these transformations can be carried out under mild and controlled reaction conditions [14]. As opposed to immobilized enzymes, living cells can generate the expensive but critical reaction components such as ATP and NADPH, but the immobilization process must maintain cell viability and reactivity for the desired period of time. Adhesive latex has been used as an immobilization support for the development of biosensors and biocatalysts containing whole cells [8–10] and is available in a variety of polymer particle sizes and

formulations with different coalescence characteristics [20–23]. These coatings are typically composed of one or more cell coat layers in which the cells are mixed with latex and spread as a 10- to 70-μm thick sheet onto a polyester or metal substrate. After air-drying at high relative humidity (>50% RH) to partially arrest polymer particle coalescence of the latex, a nanoporous latex topcoat can be applied to entrap cells in the cell coat. To increase the permeability of the coatings and protect the cells from drying, pore-forming sugars such as sucrose, glycerol, or other osmoprotectants can be added to the latex [8, 20]. Previous studies indicated that the latex coatings can be stored dry and then rehydrated prior to use still retaining significant biological activity [22]. Model latex-based coatings have been developed for a number of applications including high temperature coatings with *Thermotoga maritima* [21], the production of hydrogen by *Rhodopseudomonas palustris* [13], oxidation of D-sorbitol to L-sorbose by *Gluconobacter oxydans* [8], and a mercury biosensor using *Escherichia coli* [22]. The high cell density, stability, adhesiveness, nanoporosity, and thinness of these coatings preserve cell viability of the latex-entrapped microorganisms, which are metabolically active but not dividing due to space limitations in the coating and nitrogen limitation in the incubation buffer. In addition to formulating coatings to preserve cell viability at ambient temperature, a major focus of coating development has been to optimize the expression of genes involved in the reactions of interest and to engineer coating microstructure to reduce diffusion limitations such that all the cells in the coating are reactive.

Green fluorescent protein (GFP) has been used for many applications as a reporter protein to monitor gene expression in plant, animal, and microbial cells [3, 4, 7]. In particular, GFP reporter constructs have been developed as biosensors to detect a variety of toxic compounds [12, 16, 18, 32]. Once GFP is synthesized and folded properly in the presence of oxygen [34], its fluorescence can be quantified by a number of methods including laser scanning confocal microscopy (LSCM) [5, 19, 26, 35]. Previous work developed a very sensitive latex-based mercury biosensor using Lux as the reporter protein [22]. However, to determine the spatial response of the cells within the coating to diffusion of mercury(II), the GFP reporter protein detected by LSCM is superior for precise localization of gene expression within the biosensor.

In this study, LSCM was used to spatially quantify GFP expression within the mercury biosensor when exposed to different amounts of the mercury inducer at various temperatures. Two different fluorescent microspheres incorporated into either the latex-only topcoat or into the cell coat were used to delineate the three-dimensional architecture of the coating. This method revealed the total amount of mercury-induced GFP produced by the biosensor as well as the spatial position of the cells within the latex coating that were induced.

Materials and methods

Strains, plasmids, and growth conditions

E. coli strain JM109 [F′ *traD36 proA*$^+$ *proB*$^+$ *lacI*^{q} *lacZΔM15/recA1 endA1 gyrA96*(Nal^r) *thi hsdR17 supE44 relA1* Δ(*lac-proAB*) *mcrA*] [36] transformed with p*mer*GFP was used for these experiments. p*mer*GFP was constructed by cloning an EcoRI-BamHI fragment from pRB28 [29], containing the promoter/operator sequences from the mercury resistance operon (*mer*) and the *merR* gene encoding the *mer* operon repressor protein, into pGFPemd-b (Packard Instrument Co, Meriden, CT, USA). pGFPemd-b contains a colE1 origin of replication, an ampicillin (Ap) resistance gene, and a promoterless green fluorescent protein (*gfp*) gene encoding E-GFP (488/509 nm excitation/emission) [6, 30]. The resulting plasmid has the *gfp* gene under transcriptional control of the mercury-inducible *mer* regulatory sequences.

Cultures were grown in Luria Bertani (LB) liquid medium (10 g/l tryptone, 5 g/l yeast extract, 10 g/l NaCl, pH 7.2) at 30 °C or on Antibiotic Medium 2 (Difco) solid medium at 37 °C. Ap was added at a final concentration of 25 μg/ml.

Cell immobilization in latex

Cultures of JM109 (p*mer*GFP) were grown in LB plus Ap at 30 °C with shaking at 250 rpm to stationary phase. Cells were harvested at 6k rpm at 4 °C for 10 min and washed twice in phosphate-buffered saline (PBS), pH 7.4 [1]. To prepare the latex-immobilized cell patches, the procedure of Lyngberg et al. [23] was used with modifications as follows. The cell pellet was weighed, and 0.6 g of cell paste was resuspended in a 150 μl solution containing 75 μl of 1.3 g/ml (wt/v) glycerol, 10 μl of 1% (v/v) Tween 20 (to prevent aggregation of the microspheres), 50 μl Flash Red fluorescent carboxylate-modified microspheres (0.19 μm diameter, 1% wt/v, 660/690 nm excitation/emission; Bangs Laboratories, Fishers, IN, USA), and 15 μl sterile distilled water. Immediately before preparing the cell coat, 0.5 ml of latex (SF091; 280 nm average polymer particle diameter, mono-dispersed acrylic/vinyl acetate copolymer emulsion; Rohm and Haas, Philadelphia, PA, USA) was mixed into the cell sample. The final cell–latex mixture contained approximately 50% (wt/v) of *E. coli* cells, 7.8% (wt/v) glycerol, 0.008% (v/v) Tween 20, and 0.04% (wt/v) Flash Red microspheres (1.3×10^{11} microspheres). The cell–latex mixture was coated onto an 89-μm thick pressure-sensitive clear vinyl adhesive template (ConTact, Stamford, CT) with 0.5-in. diameter holes, adhered to a 25-μm thick clear polyester substrate (3 M, St Paul, MN, USA), using a 26-mil-wire wound rod (Mayer bar, Paul N. Gardner

Company, Pompano Beach, FL, USA). The cell coat was dried at room temperature (21–24 °C) at 50 ± 5% relative humidity (RH) for 1 h. The dried cell coat was about 20-μm thick, and each patch contained approximately 5×10^8 cells [23].

After removing the template, a topcoat consisting of 0.9 ml SF091 latex, 50 μl of 1.3 g/ml (wt/v) glycerol, 10 μl of 1% (v/v) Tween 20, and 50 μl of Estapor@Y fluorescent carboxylate-modified microspheres (0.19 μm diameter, 1% wt/v, 555/570 nm excitation/emission; Bangs Laboratories) was spread over the cell coat using a 8-mil-wire wound Mayer bar (Paul N. Gardner Company, Pompano Beach, FL, USA). Final concentrations of topcoat mixture components were 6.4% (wt/v) glycerol, 0.01% (v/v) Tween 20, and 0.05% (wt/v) Estapor@Y microspheres (1.4×10^{11} microspheres). Spacers made of the adhesive template material (89-μm thick) were placed along the sides of the cell coat to prevent disruption of the cell–latex patches when applying the topcoat. The topcoat was dried at room temperature and at approximately 50% RH for 1 h. The dried cell coat plus topcoat was about 36-μm thick; after rehydration, the thickness increased to about 53 μm, which was consistent with the confocal microscopy measurements.

Thickness of the polyester substrate, the adhesive template, and the coatings was determined using a foot-pad constant-load micrometer (Model ID-C112GEB Mitutoyo USA Corp, Plymouth, MI, USA).

Mercury-induced GFP synthesis

Bilayer *E. coli* patches adhered to a polyester substrate were rehydrated in 5 ml pyruvate buffer [5 mM pyruvate, 34 mM sodium phosphate buffer pH 6.8, 33 mM potassium phosphate buffer pH 6.8 and 0.091 mM $(NH_4)_2SO_4$] [22, 28] for 30–60 min at room temperature in sterile glass scintillation vials covered with nongauze milk filters. Pyruvate buffer does not support significant culture growth; in a control experiment, the optical density at 650 nm of a stationary phase culture diluted 1:20 into pyruvate buffer and incubated at 30 °C with shaking at 250 rpm did not increase by more than 20% during a 3-day period. Mercuric chloride at 0–20 μM was added to the rehydration buffer to induce GFP synthesis, and the patches were incubated at 21, 30, or 37 °C with shaking at 100 rpm for various lengths of time.

Laser scanning confocal microscopy

Latex–*E. coli* patches were removed from the rehydration buffer and dried at 42 °C for 30 min to reduce moisture at the patch–air interface. The patches were mounted on a microscope slide, and the patches were topped with a drop of mineral oil, a cover slip, and another drop of mineral oil. The latex–cell patch was analyzed by LSCM using a Bio-Rad MRC1024 system equipped with a 15 mW Krypton/Argon ion laser with emission lines at 488, 568, and 647 nm and a CoolCamera 2000. The slide was placed on the Olympus 1X-70 inverted microscope and viewed with the 60× oil immersion objective lens. Fluorescence was measured simultaneously in three channels to detect GFP (488/509 nm excitation/emission; green channel), Flash Red microspheres (660/690 nm excitation/emission; blue channel), and Estapor@Y microspheres (555/570 nm excitation/emission; red channel). About 60–70 *X–Y* planes were analyzed for each latex–cell sample, and each *X–Y* plane was separated by 0.9 μm. *Z*-plane images were taken at three different locations within the sample. Images were processed and analyzed with the LaserSharp MCR-1024 and Image J (http://rsb.info.nih.gov/ij) suite of software. Relative fluorescence intensity was calculated by subtracting background fluorescence from the absolute fluorescence measurements.

Fluorescence microscopy

GFP production in the latex–cell patches was quantified by fluorescence microscopy. Patches were removed from the incubation buffer and mounted on a glass slide with a glass cover slip. Total GFP fluorescence of the sample was measured with a Nikon Eclipse E800 microscope with mercury lamp epi-fluorescence illumination. Excitation was at 450–490 nm with a 515-nm barrier filter. Images were captured with a CoolCam CCD camera and analyzed with Image Pro Plus software. Fluorescence was measured at five positions within the patch, and the average values with standard deviations were determined.

Results

Use of fluorescent microspheres as internal markers of latex-immobilized cell patches producing GFP

To examine spatial gene expression within a latex patch containing immobilized cells, two different fluorescent microspheres were used to label the cell coat and topcoat of the latex patch. Flash Red microspheres were mixed with the cells and latex before spreading the cell coat and were visualized in the blue channel by confocal microscopy. Estapor@Y microspheres were mixed with latex before applying the topcoat and were detected in the red channel. Cells containing the p*mer*GFP plasmid can be induced to synthesize GFP when exposed to mercuric chloride, and GFP production by the latex-immobilized cells was detected as green fluorescence. A latex patch containing

GFP-producing cells will have three fluorophores detectable by confocal microscopy: Flash Red microspheres in the cell coat, Estapor@X microspheres in the topcoat, and GFP in the cell coat. Each *X–Y* plane of the patch was analyzed for the presence of these three fluorescent molecules by LSCM, and the mean fluorescence intensities were quantified as a function of position within the patch (Fig. 1). The Estapor@X microspheres clearly marked the topcoat, and the Flash Red microspheres and GFP were confined to the cell coat. In this example, the cell coat–topcoat interface was at the *X–Y* plane number 32 from the air–topcoat boundary. Since the microspheres were uniformly distributed throughout the cell coat or topcoat, the thickness of each layer could be readily measured in *Z*-plane images.

Spatial expression of GFP by cells immobilized in latex patches as a function of mercury concentration

Cells immobilized in latex patches were exposed to varying mercuric chloride concentrations from 0 to 20 μM. While no GFP was detected in uninduced cells, the total amount of GFP produced in the presence of inducer increased with increasing concentrations of mercuric chloride (Fig. 2a). Cells positioned near to the cell coat–topcoat interface produced the highest levels of GFP fluorescence when compared to cells located closer to the bottom of the patch. As the mercuric chloride concentration increased, more cells toward the bottom of the patch were induced to produce GFP. The expression of GFP in each patch was quantified, and the profile of fluorescence within the patches at each mercury concentration is shown in Fig. 2b. In a control experiment, cells were preinduced with mercury before immobilization in latex and showed uniform expression of GFP in the cell coat (data not shown). This result indicated that the gradient of expression within the patches was a function of GFP induction by mercuric chloride rather than

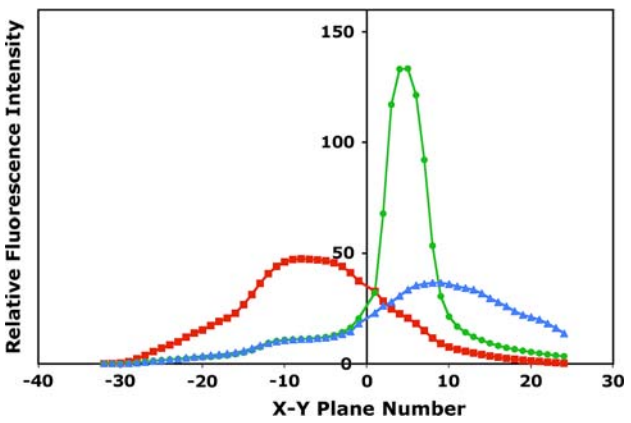

Fig. 1 Spatial detection of three fluorescent markers in the latex biosensor by confocal microscopy. The latex coating contained Estapor microspheres in the topcoat (*squares*) and Flash Red microspheres in the cell coat (*triangles*). *E. coli* strain JM109 (p*mer*GFP) was immobilized in the cell coat, and the patch was incubated in the presence of 10 μM mercuric chloride at 21 °C for 44 h to induce GFP synthesis (*circles*). The coating was analyzed by LSCM to measure the intensity of the three fluorescent markers in each successive 0.9 μm *X–Y* plane beginning at the topcoat–air side of the coating. The *negative numbers* refer to the topcoat planes, the *positive numbers* correspond to the cell coat, and *zero* represents the topcoat–cell coat interface

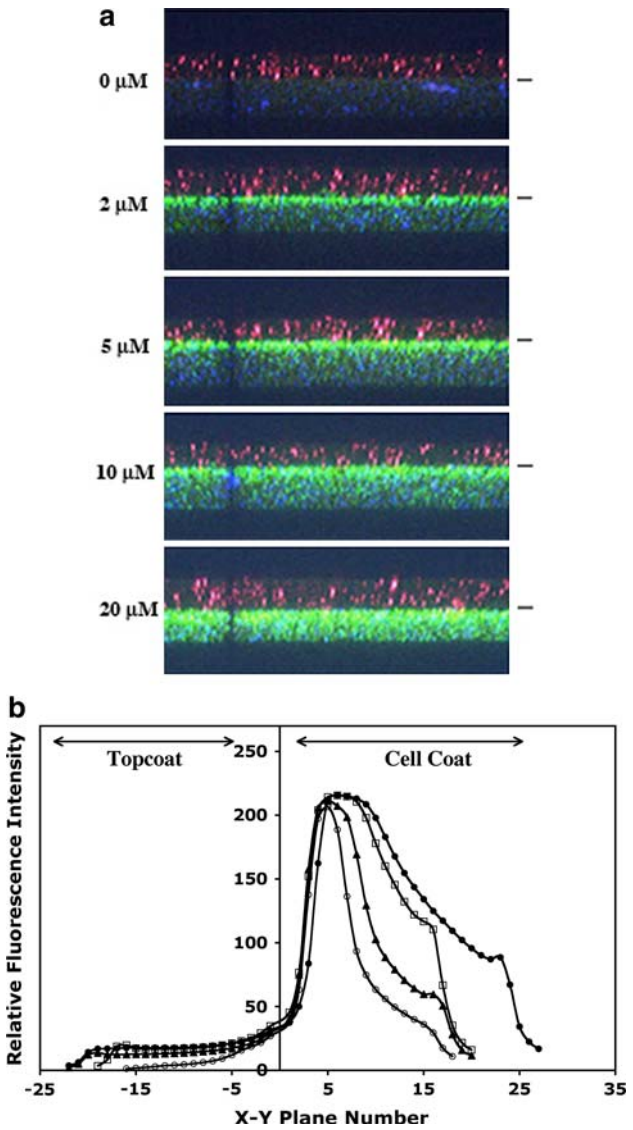

Fig. 2 Spatial expression of GFP in latex coatings in response to mercury concentration. *E. coli* strain JM109 (p*mer*GFP) was immobilized in latex coatings and incubated in the presence of 0–20 μM mercuric chloride at 37 °C for 44 h. **a** The *Z*-plane images were generated by LSCM. The layers with the *red* or *blue* colored microspheres are the topcoat and cell coat, respectively. The GFP produced by the induced cells in the cell coat is represented by the *green* color. The horizontal lines indicate the position of the topcoat–cell coat interface. **b** The amount of GFP produced at each mercury concentration was quantified in each *X–Y* plane of the coatings and plotted versus *X–Y* plane number relative to the topcoat–cell coat interface. Mercuric chloride concentrations: 2 μM (*open circles*), 5 μM (*closed triangles*), 10 μM (*open squares*), and 20 μM (*closed circles*)

a limitation of the confocal microscope to detect GFP fluorescence throughout the patch. To address whether the thickness of the topcoat layer would affect induction of GFP expression in the cell coat, three patches that had the same cell coat thickness but differed in topcoat thickness were analyzed for GFP expression (Fig. 3). No substantial difference in GFP induction was seen in patches with topcoats that were approximately 10-, 21-, or 37-μm thick as measured by LSCM. These results indicated that topcoat thickness did not affect GFP expression in the cell coat and that diffusion of neither the inducer nor oxygen through the topcoat was affected by topcoat thickness.

Effect of temperature and mercury concentration on GFP production in latex-immobilized cells

Latex patches were incubated at three different temperatures and four different mercury concentrations, and the total amount of GFP produced by the latex-immobilized cells was measured by LSCM (Fig. 4). At 37 and 21 °C, the total amount of fluorescence increased about two-fold when the mercury concentration was increased from 2 to 20 μM. At the 30 °C incubation temperature, the amount of GFP produced increased only about 25% over the range of mercury concentrations used for induction. At each mercury concentration, the total amount of GFP produced was positively correlated with the temperature of incubation. In particular, incubation of the coatings at 37 °C resulted in significantly more GFP production at the 10 and 20 μM mercury concentrations when compared to the other temperatures.

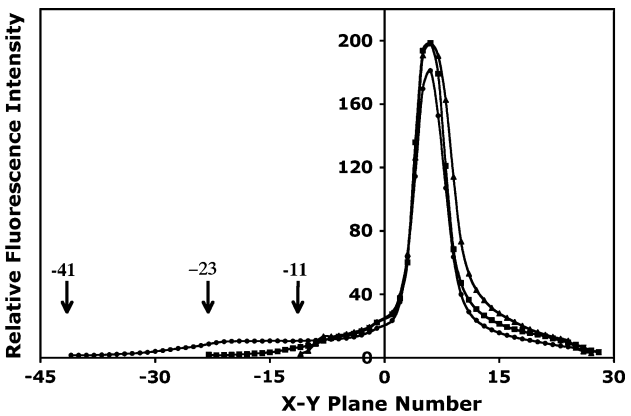

Fig. 3 GFP expression in the latex coatings with three different topcoat thicknesses. *E. coli* strain JM109 (p*mer*GFP) was immobilized in latex coatings and incubated in 10 μM mercuric chloride at 30 °C for 44 h. GFP fluorescence was quantified by LSCM. The cell coat was about 25-μm thick (*X*–*Y* planes 1–28) for all three coatings. The topcoat thickness was approximately 10-μm (*triangles*), 21-μm (*squares*), or 37-μm (*circles*) thick (indicated by *X*–*Y* plane numbers −11, −23, or −41, respectively). The *negative numbers* refer to the topcoat, the *positive numbers* correspond to the cell coat, and *zero* represents the topcoat–cell coat interface

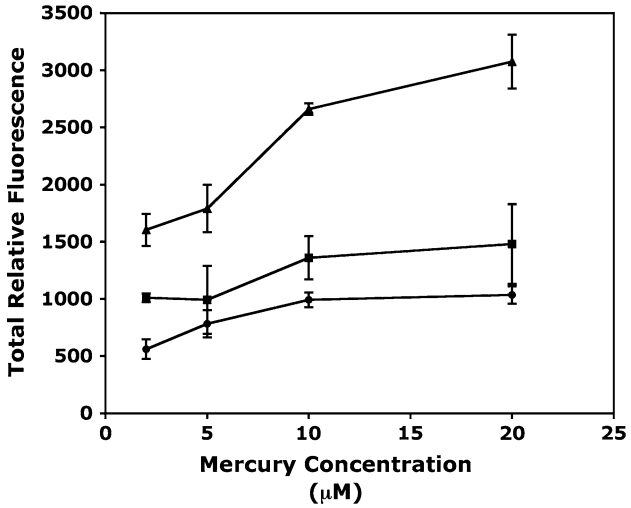

Fig. 4 GFP expression as a function of temperature and mercury concentration. *E. coli* strain JM109 (p*mer*GFP) was immobilized in latex coatings and incubated in 2–20 μM mercuric chloride at 21 °C (*circles*), 30 °C (*squares*), or 37 °C (*triangles*). GFP fluorescence was quantified by LSCM. The data represent the average (±SD) of three separate experiments

Time course of GFP production in latex-immobilized cells induced with different concentrations of mercury

GFP production in latex-immobilized cells was quantified by fluorescence microscopy. Patches were incubated at 37 °C in the presence of mercury concentrations ranging from 0 to 20 μM. At different times during the incubation, the patches were removed from the incubation buffer and assayed for GFP production over a 39-h period (Fig. 5). The

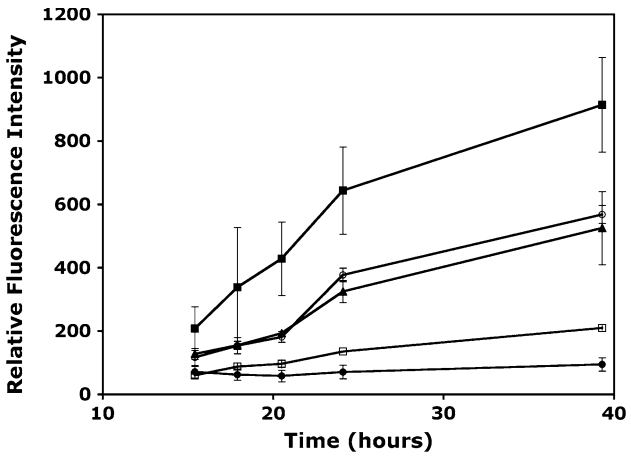

Fig. 5 Kinetics of GFP expression in latex coatings at different mercury concentrations. *E. coli* strain JM109 (p*mer*GFP) was immobilized in latex coatings and incubated in 0–20 μM mercuric chloride at 37 °C. At times during the incubation, the patches were removed from the incubation buffer, and the GFP fluorescence was quantified by fluorescence microscopy. The values shown are the average of five measurements (±SD). Mercuric chloride concentrations: 0 μM (*solid circles*), 2 μM (*open squares*), 5 μM (*triangles*), 10 μM (*open circles*), and 20 μM (*solid squares*)

highest rate of GFP production was seen with induction in the presence of 20 μM mercury. The rate of induction with 5 or 10 μM mercury (41.6 and 45.8 relative fluorescent units/h, respectively) was about twofold less than that with 20 μM mercury (88.3 relative fluorescent units/h), but it was about twofold or fourfold higher than the rate of GFP production when cells were incubated with 2 or 1 μM mercury (20.4 or 11.3 relative fluorescent units/h, respectively). In the presence of 5–20 μM mercury, the rate of GFP production was greater during the first 25 h of incubation compared to the last 15 h of incubation, even though GFP production continued to increase throughout the time course of the incubation at all mercury concentrations.

Discussion

Latex-immobilized living cells are important for high intensity biosensor and biocatalyst applications that require specificity, energy or reduced coenzymes, and involve complex reactions and transformations. These coatings can be constructed to contain a high volume fraction of cells and multiple layers of cells, which can be used to carry out successive reactions in a multistep process. However, cells trapped in these coatings may experience significant desiccation and mechanical stresses in addition to potential barriers to oxygen and nutrient diffusion through the coating. To assess the biological activity of cells in the latex coatings, mercury-inducible GFP expression in a mercury biosensor was analyzed by LSCM under different induction conditions. LSCM has been used as a valuable tool for localizing and quantifying specific gene expression within biofilms [24, 25, 37]. In this study, LSCM has been used to spatially quantify GFP expression within the three-dimensional structure of a latex–bacterial composite coating. The use of two different fluorescent microspheres to mark the topcoat and cell coat of the biosensor has provided a powerful and unique analytical tool for this analysis. Since LSCM can detect the three fluorescent molecules simultaneously, the topcoat and cell coat dimensions as well as the location of the GFP expressing cells within the cell coat could be determined.

Mercury as an inducer is challenging due to its affinity for binding nonspecifically to proteins and other molecules with sulfhydryl groups [33]. Even though there is a specific mercury transport system [2,15], the p*mer*GFP construct used in this biosensor does not contain the transport genes (*merTPC*) or the mercuric reductase gene (*merA*). Previous studies with a *mer-lux* biosensor reported decreased activity when a construct (pOS14) that contained the *mer*TPC genes was used [22, 29]; presumably mercury was transported into the cells more efficiently, but since the cells lacked *mer*A and were thus mercury sensitive, Lux expression at mercury concentrations above 10 nM was lower than that in cells lacking the transport genes. Constructs containing the transport genes as well as *merA* (pOS15) showed reduced Lux production probably due to reduction and volatilization of the mercury(II) inducer by the mercuric reductase. Based on these previous studies with the *mer-lux* biosensor, the *merTPCA* genes were not included in the p*mer*GFP construct.

With the p*mer*GFP-containing cells, the mercury must diffuse through the cell wall and membrane before interacting with the MerR repressor protein [27] to induce GFP synthesis. Considering this sequence of events, it is not surprising that increasing the temperature of incubation from 21 to 37 °C increased the reactivity of the coating. These properties of mercury may also explain the gradient of GFP expression observed in the coatings. The expression was always higher in cells near the topcoat–cell coat interface when compared to cells localized toward the bottom of the cell coat whether comparing GFP production with different mercury concentrations used for induction or different temperatures of incubation. When the mercury initially diffuses into the cell coat, it binds to the first layers of cells and then diffuses into the cells to induce GFP expression. Excess mercury or mercury released by the initial layers of cells can then diffuse into subsequent layers of cells within the coating. Consistent with this interpretation, the cell band of GFP induction was broader as the mercury concentration used for induction increased. Eventually, cells throughout the coating were induced. These results with mercury-induced GFP synthesis are in contrast to GFP induction by arabinose in natural biofilms of *E. coli* [11]. When arabinose was used, there was no gradient of GFP expression observed within the biofilm indicating unobstructed diffusion of the inducer and absence of an arabinose gradient. Therefore, the gradient of GFP synthesis observed in the mercury biosensor is likely due, at least in part, to the unique binding properties of mercury to cell surfaces and lack of uniform distribution of the inducer molecules throughout the cell coat. The mercury does not appear to bind nonspecifically to the latex particles as evidenced by the similar induction profiles in cell coats with different topcoat thicknesses (Fig. 3).

GFP expression in the latex coatings required higher mercury concentrations for induction and longer incubation times when compared to the *mer-lux*-based latex biosensor reported previously [22]. For example, 6×10^6 cpm of luciferase activity was achieved in 5 h with 100 nM mercuric chloride in the *mer-lux*-based latex sensor, whereas 20 μM mercury and 24 h of incubation was required to obtain 650 relative fluorescence units of GFP with the *mer-gfp*-based latex sensor. A similar difference in reactivity of Lux and GFP-based mercury biosensors was reported in a liquid culture system with *E. coli* containing pUT-Kn-res

plasmid derivatives with either the *lux* or *gfp* reporter genes [16]. In this system, 1×10^6 relative light units of Lux were achieved in 80 min with 3.7 nM mercuric chloride, whereas 16 h of incubation with 1.1 μM mercury was required to obtain 1,000 relative fluorescence units of GFP. These results have confirmed the sensitivity of Lux as a reporter protein for the mercury biosensor, but also emphasized the utility of GFP to monitor gene expression in situ within the latex biosensor in spite of its lower reactivity relative to Lux.

Even though *E. coli* is a facultative anaerobe, GFP fluorescence requires oxygen for protein folding [34]. Therefore, the lack of oxygen diffusion through the cell coat could contribute to the gradient of GFP expression observed in the mercury biosensor. However, at higher mercury concentrations, cells near the bottom of the cell coat expressed GFP suggesting that oxygen did indeed penetrate the entire cell coat, which was less than 30-μm thick. Constitutive GFP expression has been studied in natural biofilms of *Streptococcus gordonii* [17], and no gradient of expression was seen at 0.025–0.1 ppm dissolved oxygen concentrations indicating no barriers to oxygen diffusion throughout the biofilm. In addition, GFP expression studies with *Pseudomonas putida* biofilms revealed no oxygen limitation for biofilms less than 100-μm thick [31]. The results with these biofilm systems support the conclusion that the GFP gradient of expression observed in the mercury biosensor was not due to a limitation of oxygen availability within the cell coat but was instead influenced by the diffusion and binding properties of the mercury inducer.

GFP expression in combination with LSCM can be used to evaluate and optimize spatial gene expression in latex coatings engineered for biosensor and biocatalyst applications. In addition to determining overall coating reactivity, a spatial picture of specific gene expression can be developed to assess the effects of coating and topcoat thickness, diffusion of nutrients and inducers throughout the coating, the availability of oxygen to the immobilized cells as well as cell viability during coating preparation and storage.

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