Biosensors and Bioelectronics 46 (2013) 175–182

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Serum complement enhances the responses of genotoxin- and oxidative stress-sensitive *Escherichia coli* bioreporters

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ARTICLE INFO

Article history:
Received 24 August 2012
Received in revised form
27 December 2012
Accepted 19 February 2013
Available online 4 March 2013

Keywords:
Bioluminescence
Genotoxic
Oxidative stress
Serum
Complement

ABSTRACT

Bacterial bioreporters are limited in their abilities to detect large polar molecules due to their membrane selectivity. In this study, the activity of serum complement was used to bypass this undesired selectivity. Initially, the serum complement activity was assessed using the responses of a bacterial bioreporter harboring a *recA*::*luxCDABE* transcriptional fusion when exposed to the chemotherapy drug, mitomycin C (MMC). Using 50 °C-treated serum, the limit of detection for this bacterial sensor was lowered by nearly 450-fold, from 31 μg/L to 0.07 μg/L MMC. Real-time quantitative PCR demonstrated that serum-treated cultures responded more strongly to 100 μg/L MMC, with 3.1-fold higher *recA* expression levels. Subsequent experiments with other bioreporter strains also found enhanced sensitivities and responses. Finally, combining each of the above findings, tests were performed to demonstrate the potential application of the *recA*::*luxCDABE* bioreporter within a lab-on-a-CD platform as a point-of-care diagnostic to measure chemotherapeutic drug concentrations within blood.

1. Introduction

Numerous bioreporters have been developed for various applications, including wastewater treatment and environmental toxicity assessments, and these respond to a wide range of chemical effectors, such as genotoxins, oxidative radicals and phenolic compounds (Bechor et al., 2002; Belkin et al., 1996; Mitchell et al., 2006; Vollmer et al., 1997). Of the two general constructs, the first class, *i.e.*, constitutively-expressed sensors, commonly uses a strong promoter that shows little or no change in expression. In contrast, the second class incorporates an inducible promoter that is selectively expressed when the bacterial cell experiences a certain stress or stimulus and is therefore dependent entirely upon the activity of the promoter employed (Yagur-Kroll and Belkin, 2011). For many of these sensors to operate properly, it is essential that chemical effectors are able to enter the cell through its membrane where they can interact with the cellular components (*i.e.*, protein, lipids, and DNA) and elicit a response. This process is especially important for sensors with inducible promoters as they are often strongly affected by the stimuli. One major hurdle to overcome is the transport of the molecular effectors into the cytoplasm across the cellular membrane that is selectively permeable to molecules. Consequently, the transport of large molecules through a cellular membrane is difficult and requires long time periods.

Mitomycin C (MMC) has been used as a model genotoxin and inducer of the DNA-damage SOS response for many bacterial sensors (Ahn et al., 2009; Biran et al., 2011; Mitchell and Gu, 2004). It is a common chemotherapeutic agent and a potent DNA cross-linker. By cross-linking DNA, MMC blocks DNA replication, and has been shown to be lethal to bacteria (Tomasz, 1995). In response to DNA damage, the *recA* gene, which encodes for a protein involved in the repair and maintenance of the genomic DNA, is induced many fold in *Escherichia coli* (Min et al., 1999), making it useful for the development of a bioreporter that can sensitively detect DNA damage.

With regard to effectors, therefore, we hypothesized that the formation of pores in the bacterial membrane would be helpful for the rapid transport of bulky chemicals, resulting in both a reduced response time and higher sensitivity. In several studies, human complement activity against mammalian cells (HeLa cells)

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http://dx.doi.org/10.1016/j.bios.2013.02.038

has been shown using firefly luciferase (Corey et al., 1997; Sala-Newby et al., 1998). They found that complement activity leads to rapid lysis of the cell and the release of cellular ATPs. In those studies, the pores caused by the complement system acted as a passage for cytoplasm components to leak out of the cell. In contrast, we are more interested in establishing that the same pores also permit extracellular compounds to pass across the membrane and into the cytoplasm of bacterial cells. Two previous studies were conducted using a similar concept and reported that the influx of *D*-luciferin through the membrane is facilitated by complement attack (Virta et al., 1997, 1998).

In this work, therefore, we studied the responses of a DNA damage-sensitive *E. coli* bioreporter strain with various genotoxins, including MMC, in the presence of human serum and compared them to similar tests performed in growth media alone. In this manner, we determined that the pores formed by the serum complement system increase the sensitivity of a DNA damage-responsive bacterial bioreporter. Furthermore, the possibility of using this reporter within a CD-based microfluidic format for point-of-care diagnosis to determine the concentrations of chemotherapeutic agents within blood samples was evaluated.

2. Materials and methods

2.1. Cultivation of the bacteria

The bacterial strains used in this study are listed in Supplementary Table 1. These strains stored within a glycerol stock (20%) at −80 °C were struck out on a Luria–Bertani (LB, Difco, USA) agar plate containing 50 μg/ml ampicillin and grown for 16 h. From this plate, a single colony was inoculated into sterile LB media containing ampicillin at a concentration of 50 μg/ml. For growth and chemical exposure, an overnight culture of this strain was diluted with fresh LB media containing ampicillin at a ratio of 1:100 and grown until it reached an optical density (OD) of 0.1–0.15 measured at 600 nm with a BioPhotometer Plus (Eppendorf, Germany). After reaching this OD, the culture was mixed with human serum (AB Male, Sigma-Aldrich, USA), which was treated at a temperature of 50 °C or 56 °C for 30 min where noted, along with the chemical preparation as described below. All the experiments were performed at 30 °C.

2.2. Construction of the plasmids

The plasmids used in this study are listed in Supplementary Table 1. Plasmid pDEWMCS was constructed by inserting a linker containing the restriction sites *Eco*RI, *Not*I, *Xho*I, *BamH*I, *Xma*I, *Xba*I, *Eco*RV and *Kpn*I into plasmid pDEW201 after digestion with *Eco*RI and *Kpn*I. The promoter regions for the *katG* and *sodA* genes of *E. coli* BL21(DE3) were amplified with the primers listed in Supplementary Table 2. The products were inserted upstream of the *lux* operon within pDEWMCS after digestion with *BamH*I and *Kpn*I or *Eco*RI and *BamH*I for *katG* and *sodA*, respectively. The constructs were transformed into *E. coli* strain DH5α by electroporation and selected on LB agar supplemented with ampicillin (100 μg/ml). In all luminescence experiments, *E. coli* MG1655 was used as the host.

2.3. Chemical preparation

Several chemicals were selected based on the promoter used: for *recA*, mitomycin C (MMC) (Sigma-Aldrich, USA), ethidium bromide (EtBr) (Sigma-Aldrich, USA), methylmethane sulfonate (MMS) (TCI Chemicals, Japan), and ethane methylsulfonate (EMS) (TCI Chemicals, Japan); for *sodA*, benzyl viologen (Sigma-Aldrich, USA); and for *katG*, hydrogen peroxide (Sigma-Aldrich, USA). A 0.22 μM filter-sterilized stock solution of each was prepared using sterile deionized water.

2.4. Bioluminescence and viability tests

When the strains were ready, 100 μl cultures were added to the wells of white 96-well microplates (Greiner, USA) containing either LB media or human serum. Bioluminescence readings were taken every 20 min for 4 h using a GloMax Multi+detection system (Promega, USA).

For the serum concentration effect experiments, MMC was added to the Rec3 culture to a concentration of 100 μg/L just prior to mixing the culture with the serum, giving a final concentration of 50–100 μg/L depending on the serum volume tested. As a control, the Rec3 culture was mixed with the human serum without adding MMC. To determine the limit of detection (LOD), the test compounds were serially diluted within the wells of the plate using either serum or media before the culture was added.

To determine the *E. coli* viability, samples were prepared in 96-well plates using either 100 μl of LB, 40 μl of 50 °C-treated serum or 40 μl of 56 °C-treated serum. To these wells, 100 μl of fresh *E. coli* culture (OD 0.15) was added. This plate was then incubated at 30 °C with intermittent shaking for 2 h. At this time, samples were taken for bioluminescence (BL) (100 μl) and for viability (10 μl) determination. For the viability tests, the samples were serially diluted using LB media and plated on ampicillin LB agar plates. After being grown overnight at 30 °C, the number of colony-forming units (CFU) was counted.

2.5. Fluorescence image

Samples were visualized using an inverted epi-fluorescent microscope (Olympus IX71) at 10× and 20× objectives. An appropriate filter set was used according to the excitation/emission spectrum of the fluorescent dye (POPO-3) to ensure optimal signal recovery. Images were captured using the Metamorph Image Suite (version 7.07, Molecular Devices, USA).

2.6. Real-time quantitative PCR analysis

Bacterial strains *E. coli* MG1655 and *Bacillus subtilis* were used in the RT-qPCR analysis. A single colony was inoculated from the LB agar plate grown overnight as described above but was cultivated in 3 ml LB until the OD was 0.8, measured at 600 nm. At this time, the culture was diluted into fresh media at a ratio of 1:25 and grown further until the OD reached 0.1–0.15. When the culture was ready, 10 ml was transferred to a flask with an equal volume of sterile LB or 4 ml of 50 °C-treated serum so that the final MMC or benzyl viologen concentrations in all the cultures were 100 μg/L and 1 mg/L. These cultures were incubated for 40 min, after which the total cellular RNA was purified from 5 ml of these sample cultures using the ChargeSwitch RNA Purification Kit (Invitrogen, USA). We followed the methods described previously for RNA purification, cDNA synthesis and RT-qPCR (Lee and Mitchell, 2012), and the primers used to perform the RT-qPCR are listed in Supplementary Table 2. The 16s rRNA concentration was used to calculate the normalized concentration for each gene. For the relative expression levels, the *recA* and *sodA* RNA concentrations within each sample were compared with that of the unexposed LB alone control, allowing us to compare the relative quantities of each condition.