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Mercury (II) sensor based on monitoring dissociation rate of the *trans*-acting factor MerR from *cis*-element by surface plasmon resonance

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ABSTRACT

Transcriptional switches regulate gene expression in response to environmental changes surrounding cell. Many studies have focused on two fundamentally different models of transcriptional control by bacterial metalloregulatory protein. Distortion of the DNA fragment including *cis*-element, to which the *trans*-acting factor MerR binds, is accepted as the mechanism of gene expression regulation by Hg (II) while, in cases of the other *trans*-acting factors ArsR and CadC, events of association to and dissociation from *cis*-element are known to control transcription in response to As (III) and Cd (II), respectively. In this study, interactions between green-fluorescent-protein-tagged *trans*-acting factor and immobilized *cis*-element were analyzed on solid surface. Fluorescent measurements and surface plasmon resonance (SPR) responses revealed that although the equilibrium dissociation constant (K_D) was much lower in MerR than in ArsR and CadC, the dissociation rate of MerR from DNA increased in response to Hg (II) at concentrations of 5–10^4 μg l^{-1}. These results firstly demonstrate an increase of K_D between MerR and its recognition site in DNA by Hg (II), and possibility of rapid Hg (II) quantification with the low detection limit (5 μg l^{-1}) and the high dynamic range (10^1–10^4 μg l^{-1}).

1. Introduction

Bacterial metalloregulatory proteins control transcription of genes encoding metalloenzymes that coordinate specific metals for their catalytic activities and genes responsible for conversion of toxic metal to its less-toxic form and transportation of toxic metal to outside of cell when the cell needs to adapt to environments including the toxic metal (Reyes-Caballero et al., 2011). Metalloregulatory proteins are generally recognized as *trans*-acting factors which are composed of two binding domains towards *cis*-element in DNA and metal ion. Their interactions support functional basis of transcriptional switch. The specific functional modes have been proposed towards metalloregulatory protein families. MerR, a member of MerR-family regulators (Brown et al., 2003; Outten et al., 1999), is encoded in plasmid R100 and transposon Tn501 (Misra et al., 1984), and regulates induction of the mercury resistance operon with inorganic mercury and the organomercurial compound (Nucifora et al., 1989). The conformational change of mercury-bound form causes the distortion of DNA around the *cis*-element where MerR can recognize to bind (Guo et al., 2010). On the other hand, ArsR and CadC are members of SmtB/ArsR family repressors and the conformational change of DNA-bound protein by As (III) and Cd (II) causes dissociation of the protein from the DNA, respectively (Busenlehner et al., 2003).

Toxic metals and metalloids in drinking water have caused worldwide health hazards (Hussam and Munir, 2007). Therefore, establishment of sensing devices to monitor such toxic materials in water and foods will contribute to prevention of chronic diseases caused by long term intake of polluted water. It has been demonstrated that surface plasmon resonance (SPR) of Au surface coated with thin chitosan film shows good responsivity to Fe (III)/Cu (II) (Praig et al., 2009) and Pb (II)/Hg (II) (Abdi et al., 2011). The molecular beacon design with fluorophore and silver nanoparticle modified at both ends of the single-stranded DNA molecule has been proposed to fluorescently detect mercuric ions (Zhou et al., 2014). Thus, biological materials with high sensitivity and selectivity towards toxic metals could be functional as sensing elements when their response signals would be converted to electrical signals by transducers. Functions of toxic metal-responsive transcriptional switch are also fundamental for the whole cell-based (Hynninen and Virta, 2010) and biopolymer-based (Nakanishi et al., 2013; Pellinen et al., 2004) toxic metal sensors.

It has been shown that the green fluorescent protein (GFP)-tagged *trans*-acting factors, ArsR-GFP and CadC-GFP, are applicable

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to detection of As (III)/Sb (II) and Cd (II)/Pb (II)/Zn (II) by measuring fluorescence intensity of the proteins bound to (Kawakami et al., 2010) and dissociated from the immobilized *cis*-elements (Siddiki et al., 2011, 2012a, 2012b). When application of the same strategy as established in ArsR and CadC to Hg (II) detection by MerR is considered, no significant quantitative changes of bound and dissociated proteins could be expected because in the mechanism of transcriptional regulation, both the apo and Hg-bound forms could associate to DNA. A conjugate of MerR and fluorescence-labeled duplex DNA including *cis*-element has been reported as the sensing element towards Hg (II) (Wegner et al., 2007). The principle of detection is based on a higher binding affinity of MerR to the duplex DNA and the DNA distortion mechanism that restores fluorescence from the quenching state. However, a lower limit of detection and a higher dynamic range would be required when practical use for detection and quantification of Hg (II) in drinking water and foods would be considered. In this study, the dissociation rates and dissociation constant changes of DNA-bound MerR in the presence and absence of Hg (II) were analyzed, and possibility of mercury detection and quantification by monitoring of MerR dissociated from *cis*-element was examined by comparing it with dissociation processes of DNA-bound ArsR and CadC.

2. Materials and methods

2.1. Materials

$HgCl_2$ 99.5% (Kanto chemical, Tokyo), $NaAsO_2$ 98%, and $CdCl_2 \cdot 2.5H_2O$ (both from Sigma-Aldrich, Tokyo) were dissolved in ultrapure water (Simplicity UV, Millipore-Japan, Tokyo) to prepare stock solutions of Hg (II), As (III), and Cd (II). Ca (II) and Mg (II) stock solutions were prepared from $CaCl_2 \cdot 2H_2O$ and $MgSO_4 \cdot 7H_2O$. Oligonucleotides with and without biotinylation were synthesized in Hokkaido system science (Japan).

2.2. Preparation of cis-elements and cell extracts containing trans-acting factor

P_{ars}-O_{ars}-50 (Kawakami et al., 2010) for ArsR-GFP, O_{ars}-30-down (Siddiki et al., 2011) for ArsR-GFP, and P_{cad}-O_{cad} (Kawakami et al., 2010) for CadC-GFP were used as *cis*-elements. A biotinylated oligonucleotide Pmer-F-3B and its complementary oligonucleotide Pmer-R (Table S1) were used to prepare the *cis*-element P_{mer} for MerR-GFP or MerR. Double stranded DNA including *cis*-element was immobilized on solid surface by making biotinylated double stranded DNA contact with streptavidin-coated surface (Kawakami et al., 2010).

All recombinant proteins were produced in *Escherichia coli* BL21 (DE3) pLysS (Novagen–Merck, Darmstadt, Germany) harboring pET-3a vector ligated to a *trans*-acting factor gene. *E. coli* extracts were prepared by culturing the cells in LB medium at 25 °C overnight, followed by disrupting those with an ultrasonic cell disruptor as described previously (Kawakami et al., 2010). The cell extracts were used to construct a DNA–protein complex on solid surface. ArsR-GFP and CadC-GFP were encoded in pETarsR-gfp and pETcadC-gfp, respectively (Kawakami et al., 2010).

For production of MerR-GFP, a DNA fragment encoding *merR* (GenBank accession number: AP000342.1) was synthesized by the polymerase chain reaction (PCR)-based total gene synthesis. Fusion of *merR* and *gfp* genes was carried out by replacing an *arsR* gene in pGEMarsR-gfp (Kawakami et al., 2010) with a *merR* gene fragment amplified with PCR using a primer set of SphI-NdeI-merR-F and BamHI-merR-R (Table S1) to form pGEMmerR-gfp (Fig. S1). A DNA fragment including merR-gfp was excised from pGEMmerR-gfp with *Nde*I digestion and inserted into the *Nde*I site of pET-3a to form pETmerR-gfp.

For production of recombinant MerR, a DNA fragment (merR-pET) composed of pET-3a and a *merR* gene, where the *gfp* gene was excluded, was amplified by the inverse PCR using a DNA template pETmerR-gfp and a primer set of BamHI-merRpET-F and BamHI-merRpET-R (Table S1). Purified merRpET was digested with *Bam*HI and self-ligated to form pETmerR.

2.3. Fluorescent assay

For immobilization of *cis*-element, 25 pmol per 100 μl biotinylated double-stranded DNA fragment in 25 mM Tris–HCl buffer (pH 7.4) was poured onto Reacti-bind streptavidin-coated high binding capacity black 96-well microplate wells (Thermo Fisher Scientific, Japan). After immobilization, unbound DNA was rinsed off three times by 25 mM Tris–HCl buffer pH 7.4. A mixture containing GFP-tagged *trans*-acting factor was prepared so as to include 50 μg ml^{-1} salmon sperm DNA, 40 mM NaCl, and 20 μg protein ml^{-1} in indicated buffer. Then, 100 μl of the mixture was poured to each well on which the double-stranded DNA fragment was immobilized, and incubated at room temperature for 30 or 60 min. Free proteins were washed off with 200 μl of 10 mM potassium phosphate buffer pH 6.0 containing 0.05% (w/v) Tween 20.

Water samples were prepared by adding different concentrations of toxic metals, 50 μg ml^{-1} salmon sperm DNA, and 40 mM NaCl in indicated buffer. The samples were added to the wells in which the solid surface was modified with DNA and protein, and incubated for the indicated time with orbital shaking at 120 rpm. Fluorescence intensity of the wells was measured with a microplate fluororeader at excitation/emission wavelengths of 490/530 nm (MTP-601, Hitachi High Technologies, Tokyo).

2.4. SPR assay

Interaction between *trans*-acting factor and *cis*-element in the presence and absence of toxic metals was analyzed using a commercially available SPR instrument Biacore X, a sensor chip SA, and running buffer HBS-N or HBS-EP (GE healthcare Japan, Tokyo). The sensor chip surface consists of carboxymethylated dextran pre-immobilized with streptavidin for immobilization of biotinylated interaction partners. The double stranded DNA including *cis*-element was prepared as describe above, and immobilized onto a sensor chip SA by injecting it with HBS-EP containing 500 mM NaCl. Recombinant protein was dissolved in running buffer and injected to the sensor chip at a flow rate of 20 μl min^{-1}. After finishing the injection of recombinant protein, water sample was injected at the protein-dissociating stage to obtain a signal response. After finishing the injection of water sample, protein remaining on the sensor chip was removed by injecting HBS-EP containing 1.0 M NaCl for the next protein injection. An assay using a real water sample was performed by injecting mineral water containing 4.2 mg l^{-1} Na, 1.5 mg l^{-1} K, 13.0 mg l^{-1} Ca, 4.7 mg l^{-1} Mg, and 0.05 mg l^{-1} V spiked with different concentrations of Hg (II).

For determination of kinetic parameters, running buffer containing or not containing toxic metal was sent to the sensor chip at a flow rate of 20 μl min^{-1} throughout the analysis. Concentrations of GFP-tagged *trans*-acting factor in cell lysate were determined by adding recombinant AcGFP1 (Takara Bio, Japan) at known concentrations as an internal standard to the aliquots of cell lysate, followed by measuring their fluorescent intensities. GFP-tagged *trans*-acting factors diluted to different concentrations indicated in Fig. S3 with running buffer were injected, and sensorgrams comprising association and dissociation stages were obtained.