Development of Surface-Engineered Yeast Cells Displaying Phytochelatin Synthase and Their Application to Cadmium Biosensors by the Combined Use of Pyrene-Excimer Fluorescence

Hideyuki Matsuura, Yosuke Yamamoto, Misa Muraoka, Kenji Akaishi, Yasuhisa Hori, Kanoko Uemura, Naoki Tsuji, Kazuo Harada, and Kazumasa Hirata
Graduate School of Pharmaceutical Sciences, Osaka University, Osaka 565-0871, Japan

Takeshi Bamba
Graduate School of Engineering, Osaka University, Osaka 565-0871, Japan

Hitoshi Miyasaka
The Kansai Electric Power Co., Environmental Research Center, Keihanna-Plaza, Hikaridai 1-7, Seikacho, Sourakugun, Kyoto 619-0237, Japan

Kouichi Kuroda and Mitsuyoshi Ueda
Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

DOI 10.1002/btpr.1789
Published online August 7, 2013 in Wiley Online Library (wileyonlinelibrary.com)

The development of simple, portable, inexpensive, and rapid analytical methods for detecting and monitoring toxic heavy metals are important for the safety and security of humans and their environment. Herein, we describe the application of phytochelatin (PC) synthase, which plays a critical role in heavy metal responses in higher plants and green algae, in a novel fluorescent sensing platform for cadmium (Cd). We first created surface-engineered yeast cells on which the PC synthase from Arabidopsis (AtPCS1) was displayed with retention of enzymatic activity. The general concept for the sensor is based on the Cd level-dependent synthesis of PC_2 from glutathiones by AtPCS1-displaying yeast cells, followed by simple discriminative detection of PC_2 via sensing of excimer fluorescence of thiol-labeling pyrene probes. The intensity of excimer fluorescence increased in the presence of Cd up to 1.0 μM in an approximately dose-dependent manner. This novel biosensor achieved a detection limit of as low as 0.2 μM (22.5 μg/L) for Cd. Although its use may be limited by the fact that Cu and Pb can induce cross-reaction, the proposed simple biosensor holds promise as a method useful for cost-effective screening of Cd contamination in environmental and food samples. The AtPCS1-displaying yeast cells also might be attractive tools for dissection of the catalytic mechanisms of PCS. © 2013 American Institute of Chemical Engineers *Biotechnol. Prog.*, 29:1197–1202, 2013
Keywords: cadmium biosensor, cell surface display, phytochelatin synthase, pyrene excimer, Saccharomyces cerevisiae

Introduction

Cadmium (Cd) is a heavy metal normally found in the earth's crust and as an environmental contaminant through natural occurrence, but its emanation into the environment has been greatly increased from the adoption of modern industrial and agricultural anthropogenic processes.[1,2] Contamination of the environment by emanated Cd leads to the subsequent contamination of food, especially plant-derived food. Consumption of contaminated food from plants becomes the main source of exposure to Cd for the general population, in addition to lesser levels of inhalation of contaminated ambient air and through drinking water.[1,3] Increasing lines of evidence suggests that chronic exposure to Cd at much lower levels than previously anticipated can cause adverse health effects through the damage of kidneys and bone, an increase in cancer risks, and disruption of reproductive functions.[4–7] Therefore, the detection and monitoring of foods and environmental contamination of Cd, with an aim to lower human Cd intake, is important for the risk management of food security and human health.

Conventional chromatographic and spectroscopic analytical techniques have recently undergone rapid development and been applied to food and environmental tests for potential Cd contamination, as they have for other environmental pollutants. Although these analytical techniques, such as graphite furnace atomic absorption spectrometry (GF-AAS) or inductively coupled plasma mass spectrometry (ICP-MS), are highly sensitive and selective, they suffer from several

Correspondence concerning this article should be addressed to H. Matsuura at hmhide@phs.osaka-u.ac.jp.

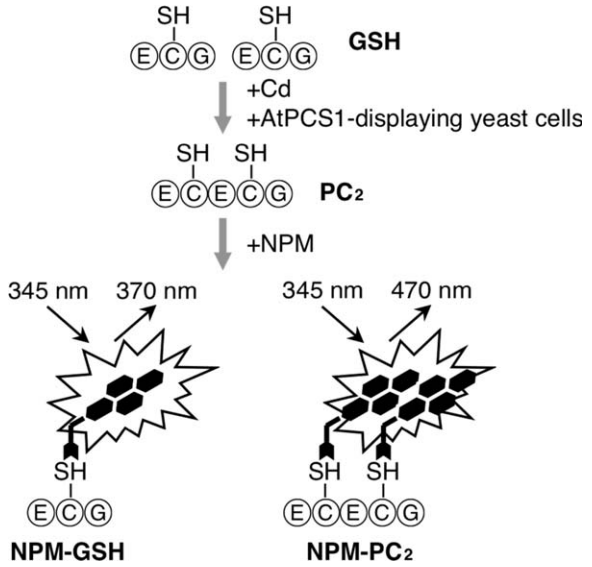

Figure 1. A schematic illustrating a Cd detection method based on excimer fluorescence derived from pyrene-labeled PC_2 as the product of AtPCS1-displaying yeast cells.

AtPCS1-displaying yeast cells mixed with GSH and the test sample can produce PC_2 in a Cd concentration dependent manner. The resulting PC_2 can be detected by derivatizing it with a thiol-labeling pyrene reagent, NPM. When the fluorophore is excited at 345 nm, the pyrene-excimer was monitored at 470 nm for discriminative detection of NPM-PC_2 from NPM-GST which emits fluorescence at 370 nm. E, L-glutamic acid; C, L-cysteine; G, glycine.

disadvantages including the use of expensive instruments with high running costs and expert operators, time-consuming processes, complicated sample preparation and techniques. Therefore, simple, portable, inexpensive, and rapid analytical methods for detecting Cd are required for primary comprehensive and on-site monitoring of Cd contamination for risk management of food and environment. One ideal way to fulfill these requirements is using biosensors, which most often consist of a biological recognition element that can detect the target analyte in conjunction with a transduction element that can sense and transmit recognition events.[8–10]

Enzymes are one of the recognition elements widely used for biosensors in which the target analyte is quantitated through the detection of the consumption or production of a species accompanied by an enzymatic reaction, or the detection of enzymatic activation or inhibition.[8] Phytochelatin (PC) synthase (PCS, EC 2.3.2.15) is an enzyme whose gene was first identified in plants and is known to exist in higher plants, and some algae, fungi, and invertebrates.[11–13] PCS, a unique enzyme that requires heavy metals as an activating factor, catalyzes the biosynthesis of PCs, $(\gamma\text{-Glu-Cys})_n\text{-Gly}$ polymers, from tripeptide glutathione (GSH; γ-Glu-Cys-Gly) and related thiols. PCs are cysteine-rich small peptides that accumulate under conditions of toxic levels of heavy metals.[11–13] They can form complexes with heavy metals, such as Cd, and hence play a role in their detoxification.[11–13] Previous studies have shown that the PCS activity of recombinant PCS enzymes or crude extracts of plant cells increases in a Cd concentration-dependent manner.[14–16] Given these properties of PCS, we speculated that PCS had the potential to be a recognition element of an activation-based enzyme Cd biosensor by detecting an increase in PC levels as the product of PCS.

Yeast cell surface engineering, the technology for displaying proteins including enzymes or peptides on living yeast cells as fusion proteins with cell surface proteins, has been widely used for the molecular breeding of whole-cell biocatalysts.[17,18] Yeast is useful as a host for cell surface engineering because it possesses typical eukaryotic-specific post-translational modification mechanisms, such as those for protein folding and glycosylation, and large proteins can be displayed.[19,20] A variety of functional enzymes, such as cellulases and lipases, have been successfully displayed on the yeast cell surface.[21–23] The display of enzymes on the yeast cell surface has two main potential merits relative to the use of the enzyme itself. First, immobilization on the cell surface offer proteins a physical support that is expected to improve their stability while often, natural proteins are too unstable to be used as a biosensor recognition element. Second, ease of preparation must be achievable because the displayed biocatalysts can be obtained only by culturing the yeast cells.

Herein, we describe a proof-of-concept study of a novel activation-based biosensor for detecting Cd in aqueous media based on the whole-cell biocatalyst, PCS-displaying yeast cells. The detection principle of the biosensor is shown in Figure 1. First, PC synthesis is allowed to proceed by mixing the recognition elements, PCS1-displaying yeast cells and Cd in an aqueous sample in the presence of GSH. Then, the thiols in the assay solution including GSH and PCs were derivatized with a maleimide-pyrene derivative, *N*-(1-pyrenyl)maleimide (NPM), and NPM-labeled PCs were discriminatively detected through excimer fluorescence.

Materials and Methods

Strains and media

Escherichia coli JM109 [*recA1 endA1 gyrA96 thi hsdR17*($r_K^- m_K^+$) *supE44 relA1* Δ(*lac-proAB*)/F′ (*traD36 proAB lacI*^{q} *lacZ*Δ*M15*)] was used as a host for cloning, and was grown in Luria-Bertani medium (1% [w/v] tryptone, 0.5% [w/v] yeast extract, 1% [w/v] sodium chloride) containing 50 μg/mL ampicillin. *Saccharomyces cerevisiae* strain BY4741/sed1Δ (*MAT*a, *his*3Δ1, *leu*2Δ0, *met*15Δ0, *ura*3Δ0, *YDR077*w::*KanMX*4), which was obtained from EUROSCARF (Frankfurt, Germany), was used for the expression of AtPCS1 on the surface of yeast cells. Transformed BY4741/sed1Δ cells were grown in synthetic dextrose (SD) medium with casamino acids (0.67% [w/v] yeast nitrogen base without amino acids, 2% glucose, 2% casamino acids, 0.003% L-histidine, 0.003% L-methionine, 0.003% L-leucine), or yeast peptone dextrose (YPD) medium (1% [w/v] yeast extract, 2% [w/v] glucose, and 2% [w/v] peptone) supplemented with or without 100 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, pH 7.8). For the plate medium, 2% (w/v) agar was added.

Expression of AtPCS1 on the surface of yeast cells

The plasmid for cell surface display of the *Arabidopsis thaliana PCS1* (At5g44070) gene was constructed as follows. The full-length open reading frame (ORF) of *AtPCS1* was amplified from the pET8c-AtPCS1 plasmid[24] by PCR using the forward primer 5′-GGAAGATCTTCCATGGCTATGGC-3′ (*Bgl*II site is underlined) and the reverse primer 5′-CCGCTCGAGCGGCTAATAGGCAGG-3′ (*Xho*I site is underlined). The PCR product was subcoloned into the pGEM-T

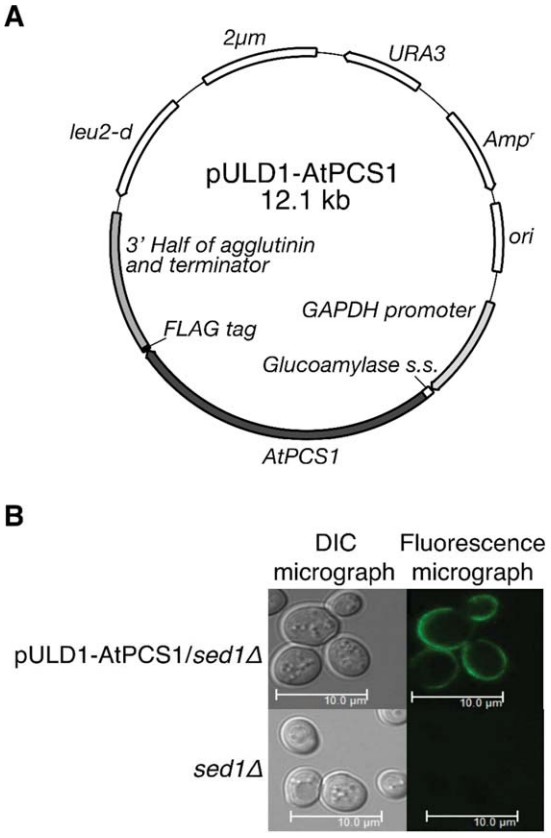

Figure 2. Creation of AtPCS1-displaying yeast cells.

(A) Map of the pULD1-AtPCS1 plasmid used for creating AtPCS1-displaying yeast cells. The *AtPCS1* gene was introduced into the pULD1 vector [20] and transformed into yeast cells (BY4741/*sed1*Δ). (B) Differential interference contrast (DIC) and fluorescence micrographs of AtPCS1-displaying and wild-type yeast cells after immunofluorescent labeling of the FLAG tag. The scale bar is 10 μm.

Easy Vector (Promega) and verified by DNA sequencing. The DNA fragment containing the ORF of AtPCS1 was introduced into the pULD1 plasmid[20] using the *Bgl*II and *Xho*I sites. The resulting plasmid was named as pULD1-AtPCS1 (Figure 2).

S. cerevisiae strain BY4741/sed1Δ was transformed with pULD1 or pULD1-AtPCS1 using a Frozen-EZ yeast transformation II kit (Zymo Research). Transformed cells were selected by plating and incubating at 30°C for 2 days on an SD medium plate. A single colony was inoculated into YPD medium supplemented with 100 mM HEPES (pH 7.8) at 30°C for 48 h, and the resulting cells were used for the subsequent analyses.

Immunofluorescence staining

Yeast cells harvested by centrifugation were rinsed with phosphate-buffered saline (PBS; pH 7.4), and resuspended in PBS (pH 7.4) containing 1% bovine serum albumin (BSA) for 30 min at room temperature. Mouse anti-Flag M2 monoclonal antibody (Sigma-Aldrich) was used as the primary antibody at a dilution rate of 1:300. A mixture of cells and antibody was incubated for 1.5 h at room temperature. Cells were washed with PBS and then reacted with Alexa Fluor 488-conjugated goat anti-mouse IgG antibody (Molecular Probes Inc., OR, USA) at a dilution of 1:300 for 1.5 h at room temperature. After washing with PBS, cells were examined with a confocal laser scanning microscope (TCS SP5; Leica).

Reaction for the synthesis of PC

Cultivated yeast cells were harvested by centrifugation (800*g*, 5 min, 4°C), washed, and resuspended in 200 mM Tris-HCl (pH 8.0) to an OD_{600} = 600. After centrifugation (18,500*g*, 5 min, 4°C) of 1.7 mL of the resuspended cells, 1.4 mL of the supernatant was removed, and the pellet was resuspended in the residual supernatant. The typical reaction mixture for PC synthesis consisted of 30 μL of the resuspended cells, 40 mM Tris-HCl (pH 8.0), 10 mM GSH, 600 μM tris(2-carboxyethyl)phosphine hydrochloride (TCEP), and 145 μL of heavy metal solutions in a total volume of 250 μL. The reaction mixture was incubated at 37°C with gentle rotation. The metal salts used were $CdCl_2{\cdot}2.5H_2O$, $ZnCl_2$, $PbCl_2$, $HgCl_2$, $NaHAsO_4$, $CuCl_2{\cdot}2H_2O$, $MnCl_2{\cdot}2H_2O$, $CaCl_2{\cdot}2H_2O$, $FeCl_2$, and $MgCl_2{\cdot}6H_2O$.

PC analysis using HPLC

For the HPLC analysis of PC, the reaction mixture was centrifuged (18,500*g*, 15 min, 4°C), and the supernatant was mixed with an equal volume of 3.6 N HCl to stop the PC-synthesis reaction. After centrifugation (18,500*g*, 15 min, 4°C), PC_2 levels in the supernatant were measured using a modified HPLC post-column system (LaChrome, Hitachi, Japan).[25] A reverse-phase column (Inertsil ODS-P; 5 μm particle size, 250 × 4.6 mm^2 id, GL Sciences, Japan) was equilibrated with 5 mM sodium 1-octanesulfonate solution containing 0.02% trifluoroacetic acid (TFA). PC_2 was eluted with a gradient using a 30% acetonitrile solution containing 0.02% TFA at a flow rate of 1.5 mL/min, according to a program established previously.[25] Separated thiols were mixed with 75.7 μM 5′-dithiobis(2-nitrobenzoic acid) (DTNB) in 50 mM phosphate buffer containing 10% acetonitrile at 50°C for derivatization and detected by absorbance at 412 nm. The PC_2 used as standards were obtained from AnaSpec (Fremont, CA).

PC analysis using NPM

Detection of PC_2 using NPM was conducted as follows. The reaction mixture for PC synthesis was centrifuged (18,500*g*, 5 min, 4°C), and 40 μL of the supernatant was mixed with 60 μL of NPM solution (5 mM NPM, 100 mM Tris-HCl, pH 8.0). The derivatization of thiols with NPM was allowed to take place at 30°C for 5 min. The solution was diluted 20-fold with 200 mM Tris-HCl (pH 8.0), and the fluorescent intensity at 470 nm was measured with an excitation at 370 nm using a SpectraMax M5e Microplate Reader (Molecular Devices).

Results and Discussion

Creation of surface-engineered PCS-displaying yeast cells

To create PCS-displaying yeast cells, an *Arabidopsis thaliana PCS* (*AtPCS1*; At5g44070) gene was introduced into an expression vector for yeast cell surface display, pULD1.[20] The pULD1 is a plasmid for the expression of the recombinant fusion protein with a glucoamylase secretion signal sequence, a FLAG tag, and an α-agglutinin cell-wall

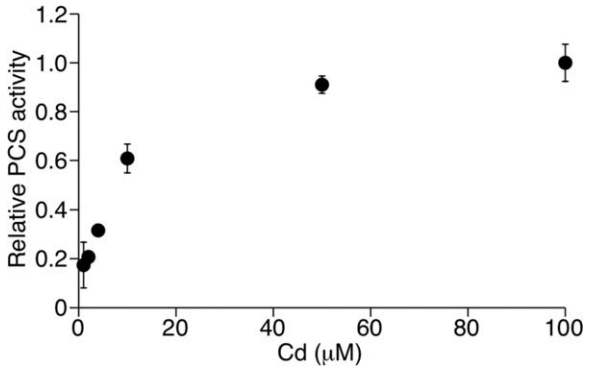

Figure 3. Effect of Cd concentration on the activity of yeast cell surface-displayed AtPCS1.

Enzymatic activity in the presence of different concentrations of Cd was plotted for the AtPCS1-displaying yeast cells. The *x*-axis shows the Cd concentration of the added sample (145 μL) to 105 μL of the assay solution (total final volume is 250 μL). The PC_2 level in the assay solution was determined using HPLC. The relative activity on the *y*-axis represents the fraction of enzyme activity when 100 μM Cd was added to the assay solution. The average and SD of three independent experiments are shown.

attachment domain under the control of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) promoter. The resulting pULD1-AtPCS1 plasmid (Figure 2A) was transformed into *S. cerevisiae* strain BY4741/*sed1*Δ. The display of AtPCS1 on the cell surface was examined by immunofluorescent labeling against the FLAG-tag fused to the C-terminus of AtPCS1. When immunolabeled with mouse anti-FLAG IgG and Alexa Fluor 488-conjugated goat anti-mouse IgG, yeast cells transformed with pULD1-AtPCS1 exhibited fluorescence from the corresponding fluorophore on the cell surface (Figure 2B). In contrast, no fluorescence was observed on wild-type yeast cells (Figure 2B), confirming that AtPCS1 was anchored on the yeast cell surface. In addition, PC_2 synthesis was confirmed in the reaction mixture containing 10 μM $CdCl_2$ and 10 mM GSH with AtPCS1-displaying yeast cells, but not with yeast cells transformed with the empty vector (pULD1) using liquid chromatography tandem mass spectrometry (LC-MS/MS) (data not shown). These immunofluorescent labeling results and confirmation of PC synthesis indicated that the AtPCS1 enzyme was successfully displayed as the active form on the yeast cell surface.

Cd-dependent activation of AtPCS1-displaying yeast cells

We next examined the effect of the Cd concentration on the enzymatic activity of the AtPCS1-displaying yeast cells to assess their potential as Cd biosensor recognition elements. The AtPCS1-displaying yeast cells were mixed with GSH and different concentrations of Cd and incubated at 37°C. The enzymatic activity was quantitated by measuring the amount of PC_2 produced during a given time period using high-performance liquid chromatography (HPLC) analysis after removing the yeast cells by centrifugation. The analyses revealed that the catalytic activity of the AtPCS1-displaying yeast cells for PC_2 synthesis increased in proportion to the Cd concentration in the range of 1–10 μM (Figure 3). These results prompted us to speculate that the AtPCS1-displaying yeast cells have the potential to be a Cd biosensor recognition element through the simple detection of PC_2.

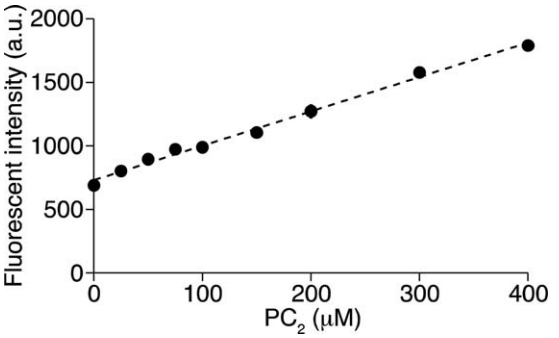

Figure 4. Fluorometric discriminative detection of PC_2 using pyrene-excimer fluorescence in the presence of GSH.

The intensity of excimer fluorescence at 470 nm is plotted against PC_2 concentration. Different concentrations of PC_2 (0–400 μM) were derivatized with NPM in the presence of 10 mM GSH, and the intensity of the excimer fluorescence emission was monitored. Excitation was at 345 nm. Values are the average of three independent measurements (SDs were within 5% for all points). The dotted line represents the regression line.

Fluorometric discriminative detection of PC_2 from GSH using a pyrene excimer

Given the potential of the AtPCS1-displaying yeast cells as the Cd recognition element of the biosensor, simple and rapid methods for detecting PC_2 were necessary as alternatives to conventional chromatographic analytical techniques such as HPLC or capillary electrophoresis. An approach using fluorometric or colorimetric labeling of thiols by SH labeling reagents should be suitable for high-throughput detection using a microplate reader. We should, however, take into consideration that the PC synthesis assay solution contains the substrate, GSH, a monothiol, and PC, a multithiol, thus utilization of any widely used SH labeling reagents like 5′-dithiobis (2-nitrobenzoic acid) or monobromobimane would not be possible.

In this study, we exploited the intramolecular excimer-forming fluorescence derivatization process that results from the use of a pyrene reagent.[26,27] Dipyrenylalkanes are known to form intramolecular excimers which emit fluorescence at longer wavelengths (450–500 nm) than the pyrene monomer (350–400 nm). When GSH and PC_2 were derivatized with the thiol-reactive pyrene reagent, NPM, the emission spectra of NPM-labeled GSH and PC_2 exhibited different wavelengths of fluorescence when excited at 345 nm: a peak at ~370 nm and ~470 nm for GSH and PC_2, respectively (data not shown). Therefore, we tested whether the PC_2 levels could be quantitatively determined in the presence of a larger amount of GSH compared with the amount of PC_2. The intensity of the excimer fluorescence (470 nm) from NPM-labeled PC_2 was linear ($R^2 = 0.99$) with respect to the PC_2 concentrations (0–400 μM) even in the presence of 10 mM GSH (Figure 4). These results indicated that this NPM-based method would be useful for PC_2 detection as a transduction element of the proposed biosensor using AtPCS1-displaying yeast cells.

Performance of the proposed biosensor based on AtPCS1-displaying yeast cells and pyrene-excimer fluorescence

To characterize the performance of the proposed Cd biosensor, we then constructed a calibration plot for Cd

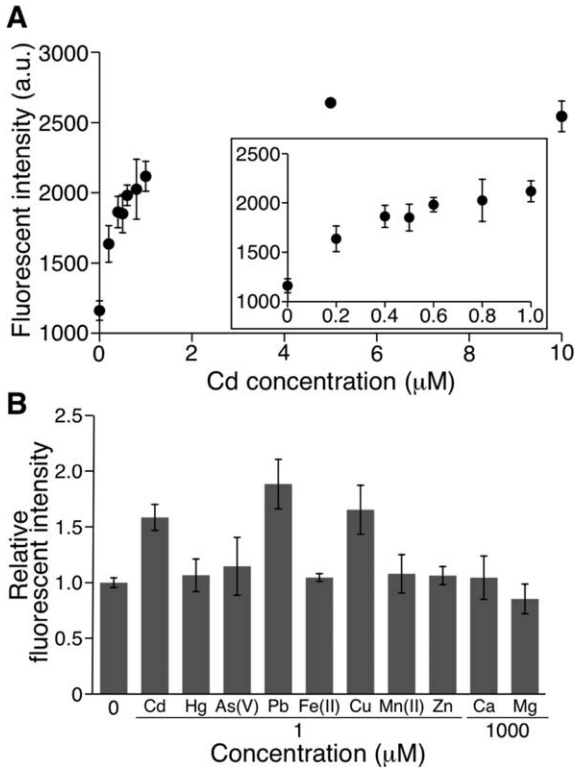

Figure 5. Response of the proposed biosensor to Cd and other metal ions.

Intensity of excimer fluorescence (470 nm) derived from NPM-labeled PCs produced by AtPCS1-displaying yeast cells in the presence of different concentrations of Cd (0, 0.2, 0.4, 0.5, 0.6, 0.8, 1.0, 5.0, and 10 μM) (A) or the tested metal ions at the indicated concentrations (B). (A) The *x*-axis shows the Cd concentration of the added sample. Inset: Enlarged version at low concentrations of Cd (0–1.0 μM). The average and SD of three independent experiments are shown. (B) Selectivity of the biosensor for the detection of Cd over other metal ions is shown. The relative fluorescent intensity on the *y*-axis represents the fraction of fluorescent intensity measured when no Cd was added to the assay solution. The average and SD of at least three independent experiments are shown.

concentrations. First, AtPCS1-displaying yeast cells were mixed with different concentrations of $CdCl_2$ (0–10 μM; concentration of the added sample) in the assay solution (40 mM Tris-HCl (pH 8.0), 0.6 μM TCEP, and 10 mM GSH), and the reaction was allowed to proceed at 37°C for 3 h. A reducing reagent, TCEP, was used because of its low reactivity with maleimide compared with the common reducing agent β–mercaptoethanol, which might interfere with the detection of NPM-labeled PC_2. After removing the yeast cells from the assay solution by centrifugation, NPM was added to the supernatant, and the excimer fluorescent intensity was measured using a microplate reader. The excimer fluorescent intensity was approximately linear with respect to the concentration of Cd between 0 and 1 μM (Figure 5A). The limit of detection of the sensor, which was defined as three times the standard deviation, was ~0.2 μM. This detection limit is well below the national effluent standards for Cd in Japan (0.89 μM) and the international standard for Cd in polished rice (3.6 μM) defined by Codex Alimentarius Commission. However, this detection limit was inferior to what was normally obtained using conventional instrumental analytical techniques.

The selectivity of the proposed biosensor was also investigated by exposing it to other metal ions including Hg, As(V), Pb, Fe(II), Cu, Mn(II), and Zn at 1 μM and Ca and Mg at 1 mM. The biosensor showed no cross-reactivity with most metal ions with the exception of 1 μM Pb and Cu (Figure 5). Because both Pb and Cu are heavy metals, which the national effluent standards are established in Japan, the cross-reactivity with the corresponding heavy metals might be acceptable in terms of practicality. Alternatively, a pretreatment of the samples with an ion exchange column that traps Cd selectively might be effective.[28] Overall, the proposed Cd biosensor had room for improvement in terms of its selectivity. Nevertheless, its low cost and proposed simple and rapid operation should make the proposed biosensor suitable for cost-effective preliminary screening of environmental and food samples suspected of Cd contaminations, before analysis using highly sensitive and selective but high cost, time-consuming, and complicated conventional analytical techniques.

Usefulness of the PCS-displaying yeast cells

In this study, we developed "arming yeasts"[17,29] which display PCS enzyme on their cell surfaces, and showed their potential as the recognition element of Cd biosensors when used with a simple pyrene-excimer fluorescence detection method for PC. PCS is one of the most important enzymes engaged in the response to nonessential heavy metals in vascular plants and some algae, fungi and invertebrates,[11] but its catalytic mechanism is not yet fully understood. The display of enzymes on the yeast cell surface has become an attractive tool for functional analysis of enzyme and/or protein evolution.[17,18,29,30] In that sense, the AtPCS1-displaying yeast cells created in this study might prove to be promising as a means to obtain a deeper understanding of PCS.

Acknowledgments

The authors thank Dr. Yoshihisa Nakazawa for his helpful technical advice. This work was supported in part by the Program for Enhancing Systematic Education in Graduate schools by Ministry of Education, Culture, Sports, Science and Technology, "Cultivating Professionals in Health and Environment Risk Management", Graduate School of Pharmaceutical Sciences, Osaka University.

Literature Cited

1. EFSA, Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on cadmium in food. *EFSA J*. 2009;980:1–139.
2. UNEP Final review of scientific information on cadmium. Available at: http://www.unep.org/hazardoussubstances/Portals/9/Lead_Cadmium/docs/Interim_reviews/UNEP_GC26_INF_11_Add_2_Final_UNEP_Cadmium_review_and_apppendix_Dec_2010.pdf
3. Clemens S, Aarts MG, Thomine S, Verbruggen N. Plant science: the key to preventing slow cadmium poisoning. *Trends Plant Sci*. 2013;18:92–99.
4. Jarup L, Akesson A. Current status of cadmium as an environmental health problem. *Toxicol Appl Pharmacol*. 2009;238:201–208.
5. Thompson J, Bannigan J. Cadmium: toxic effects on the reproductive system and the embryo. *Reprod Toxicol*. 2008;25:304–315.
6. Nordberg GF. Historical perspectives on cadmium toxicology. *Toxicol Appl Pharmacol*. 2009;238:192–200.

7. Satarug S, Garrett SH, Sens MA, Sens DA. Cadmium, environmental exposure, and health outcomes. *Environ Health Perspect*. 2010;118:182–190.
8. Wanekaya AK, Chen W, Mulchandani A. Recent biosensing developments in environmental security. *J Environ Monit*. 2008; 10:703–712.
9. Vo-Dinh T, Cullum B. Biosensors and biochips: advances in biological and medical diagnostics. *Fresenius J Anal Chem*. 2000;366:540–551.
10. Luong JH, Male KB, Glennon JD. Biosensor technology: technology push versus market pull. *Biotechnol Adv*. 2008;26: 492–500.
11. Rea PA. Phytochelatin synthase: of a protease a peptide polymerase made. *Physiol Plant*. 2012;145:154–164.
12. Hirata K, Tsuji N, Miyamoto K. Biosynthetic regulation of phytochelatins, heavy metal-binding peptides. *J Biosci Bioeng*. 2005;100:593–599.
13. Cobbett CS. Phytochelatins and their roles in heavy metal detoxification. *Plant Physiol*. 2000;123:825–832.
14. Ogawa S, Yoshidomi T, Yoshimura E. Cadmium(II)-stimulated enzyme activation of *Arabidopsis thaliana* phytochelatin synthase 1. *J Inorg Biochem*. 2011;105:111–117.
15. Klapheck S, Schlunz S, Bergmann L. Synthesis of phytochelatins and homo-phytochelatins in *Pisum sativum* L. *Plant Physiol*. 1995;107:515–521.
16. Zitka O, Krystofova O, Sobrova P, Adam V, Zehnalek J, Beklova M, Kizek R. Phytochelatin synthase activity as a marker of metal pollution. *J Hazard Mater*. 2011;192:794–800.
17. Kuroda K, Ueda M. Cell surface engineering of yeast for applications in white biotechnology. *Biotechnol Lett*. 2011;33:1–9.
18. Ueda M, Tanaka A. Genetic immobilization of proteins on the yeast cell surface. *Biotechnol Adv*. 2000;18:121–140.
19. Ueda M, Tanaka A. Cell surface engineering of yeast: construction of arming yeast with biocatalyst. *J Biosci Bioeng*. 2000;90: 125–136.
20. Kuroda K, Matsui K, Higuchi S, Kotaka A, Sahara H, Hata Y, Ueda M. Enhancement of display efficiency in yeast display system by vector engineering and gene disruption. *Appl Microbiol Biotechnol*. 2009;82:713–719.
21. Liu WS, Pan XX, Jia B, Zhao HY, Xu L, Liu Y, Yan YJ. Surface display of active lipases Lip7 and Lip8 from Yarrowia lipolytica on *Saccharomyces cerevisiae*. *Appl Microbiol Biotechnol*. 2010;88:885–891.
22. Shigechi H, Koh J, Fujita Y, Matsumoto T, Bito Y, Ueda M, Satoh E, Fukuda H, Kondo A. Direct production of ethanol from raw corn starch via fermentation by use of a novel surface-engineered yeast strain codisplaying glucoamylase and alpha-amylase. *Appl Environ Microbiol*. 2004;70:5037–5040.
23. Inaba C, Higuchi S, Morisaka H, Kuroda K, Ueda M. Synthesis of functional dipeptide carnosine from nonprotected amino acids using carnosinase-displaying yeast cells. *Appl Microbiol Biotechnol*. 2010;86:1895–1902.
24. Tsuji N, Nishikori S, Iwabe O, Matsumoto S, Shiraki K, Miyasaka H, Takagi M, Miyamoto K, Hirata K. Comparative analysis of the two-step reaction catalyzed by prokaryotic and eukaryotic phytochelatin synthase by an ion-pair liquid chromatography assay. *Planta*. 2005;222:181–191.
25. Hirata K, Tsujimoto Y, Namba T, Ohta T, Hirayanagi N, Miyasaka H, Zenk MH, Miyamoto K. Strong induction of phytochelatin synthesis by zinc in marine green alga, *Dunaliella tertiolecta*. *J Biosci Bioeng*. 2001;92:24–29.
26. Nohta H, Satozono H, Koiso K, Yoshida H, Ishida J, Yamaguchi M. Highly selective fluorometric determination of polyamines based on intramolecular excimer-forming derivatization with a pyrene-labeling reagent. *Anal Chem*. 2000;72: 4199–4204.
27. Yoshida H, Sudo M, Todoroki K, Nohta H, Yamaguchi M. Highly selective and simple method for determination of polythiols based on liquid chromatography with postcolumn excimer fluorescence derivatization. *Anal Sci*. 2009;25:829–832.
28. Abe K, Nakamura K, Arao T, Sakurai Y, Nakano A, Suginuma C, Tawarada K, Sasaki K. Immunochromatography for the rapid determination of cadmium concentrations in wheat grain and eggplant. *J Sci Food Agric*. 2011;91,1392–1397.
29. Shibasaki S, Maeda H, Ueda M. Molecular display technology using yeast—arming technology. *Anal Sci*. 2009;25:41–49.
30. Gai SA, Wittrup KD. Yeast surface display for protein engineering and characterization. *Curr Opin Struct Biol*. 2007;17: 467–473.

Manuscript received Apr. 22, 2013, and revision received Jun. 27, 2013.