Journal of Biotechnology 150 (2010) 246–250

Contents lists available at ScienceDirect

Journal of Biotechnology

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

Development of highly-sensitive microbial biosensors by mutation of the *nahR* regulatory gene

Hae Ja Shin*

Energy Environmental Engineering Major, Division of Energy Bioengineering, Dongseo University, Busan 617-716, Republic of Korea

ARTICLE INFO

Article history:
Received 14 May 2010
Received in revised form 27 August 2010
Accepted 9 September 2010

Keywords:
NahR
Mutagenesis
Highly-sensitive biosensors
Bioluminescence

ABSTRACT

NahR, a transcriptional regulator for naphthalene degradation in response to salicylate, is a central element in the microbial biosensor for detection of naphthalene and salicylate. To maximize the sensitivity of the biosensor, we have chosen a rational design of highly-sensitive microbial biosensors by introducing site directed mutagenesis to *nahR* gene. Eight single mutants (N169A, N169C, N169K, N169S, R248H, R248M, R248Q, and R248Y) were made at residues 169 and 248 known as the central inducer-recognition and the C-terminal multimerization domain. The effects of these mutations were examined by monitoring expression of a firefly luciferase (*luc*) reporter gene under the control of NahR. We found that all mutants at residues 248 and N169C show increased sensitivity (maximum ~50-fold) compared to wild type, respectively. R248M shows response even at toxic concentration, 5 mM. The results show the feasibility and potential versatility of mutational approach for the development of the highly-sensitive microbial biosensors.

1. Introduction

NahR activates transcription of the *nah* and *sal* operons for naphthalene degradation in response to the inducer salicylate (Henikoff et al., 1988; You et al., 1988; Schell and Sukordhaman, 1989), which makes NahR as a central element of the microbial biosensor for detection of naphthalene and salicylate. NahR belongs to the LysR-type transcriptional regulator (LTTR) family that is found in diverse prokaryotes. They are very similar to each other in size (300 ± 20 residues) and in the organization of their structure/function domains (Storz et al., 1990; Schell, 1993; Cebolla et al., 1997). Several LTTR X-ray structures reveal that these proteins consist of two major domains; an N-terminal helix-turn-helix DNA binding region (residues 1–58) and a much larger C-terminal regulatory domain (2 sub-domains, residues 88–294) involved in inducer binding (Mclver et al., 1989; Muraoka et al., 2003; Zaim and Kierzek, 2003; Smirnova et al., 2004). The X-ray structures also suggest that the family members could assemble as homodimers or homotetramers.

In the absence of salicylate, NahR binds a dyadic sequence (TTCAnnnnnnTGAT) around −65 of the *Psal* promoter. Salicylate binding is accompanied by a conformational change in the NahR protein, which induces further interaction with the promoter near the −35 region (the RNA polymerase binding site) (Schell and Poser, 1989). Evidence has suggested that direct contact between NahR and the RNA polymerase α subunit may be important for transcriptional activation (Schell et al., 1990; S.M. Park et al., 2003; W. Park et al., 2003). However, the molecular structure of NahR has not been determined, and the precise mechanism underlying the activation process initiated by inducer binding remains largely unknown (Huang and Schell, 1991).

Previous mutational studies (Schell et al., 1990; Cebolla et al., 1997) show two classes of residues in NahR: one defective in both activation and specific binding to the *Psal* promoter and one defective in activation but not DNA binding. Mutant forms of NahR with altered effector specificity were made by a random mutagenesis method (Cebolla et al., 1997): mutants N169D (Asn at 169 → Asp) and R248C (Arg at 248 → Cys) were particularly interesting in that they were responsive to the normal inducer, salicylate, but also to benzoate, which does not induce wild-type NahR. These results suggested that residues 169 and 248 might be involved in inducer binding.

A biosensor is a rapid, simple and low-cost analytical device that combines a biological sensing element with a transducer (Lei et al., 2006). Biological sensing elements include bio-molecules such as enzymes, antibodies, receptor and microorganisms as well as animal and plant cells or tissues. Among these, microorganisms can be an ideal alternative since microorganisms offer advantages of ability to detect wide range of chemicals, amenability to genetic modification, and adaptation to broad reaction condition. In fact, recombinant DNA technologies have inaugurated boundless possi-

* Tel.: +82 51 320 1791; fax: +82 51 320 1781.
E-mail address: hjshin@dongseo.ac.kr.

0168-1656/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jbiotec.2010.09.936

bilities of tailing the microorganisms for such purpose. However, microorganisms, with physical barriers of cell membrane and cell wall, have the drawback of long response time, poor selectivity, and low sensitivity in comparison with other sensing elements like enzymes or antibodies (Lei et al., 2006). In addition, microoganisms reveal cytotoxicity at high concentrations of detecting chemicals (Shin et al., 2005).

We constructed a microbial biosensor using NahR regulatory protein as a sensing element to detect salicylate. Our previous studies reveal that some NahR mutants respond to other inducer with altered binding affinity to *Psal* promoter (Park et al., 2005). To improve sensitivity of the microbial biosensors, here we further designed the NahR by generating additional mutations at residues 169 and 248. We then tested the mutant forms of NahR in terms of their responses to the inducers in comparison with wild type. The increased responses of NahR mutants have been challenged as an astute approach to extend the natural limits for developing highly-sensitive microbial biosensors in diverse biotechnological uses.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Escherichia coli DH5α (*hsdR*, *recA*, *thi-1*, *relA1*, *gyrA96*) was used for the maintenance of plasmids. *Pseudomonas putida* KCTC1768 was obtained from the KCTC (Korean Collection for Type Cultures) and grown in tryptic soy broth at 25 °C. *E. coli* BL21 (*hsdS*, *gal*, λ*clts*857, *ind*1, *sam*7, *nin*5, *lac*UV5-T7gene1) was used for maintenance of plasmids and expression of the NahR protein. *E. coli* DH5α and BL21 harboring plasmids were cultured at 37 °C in TYS media (1% tryptone, 0.5% yeast extract, and 0.5% NaCl) containing 30 μg ml^{-1} ampicillin.

2.2. Construction of plasmid pNRSAL

Genomic DNA was prepared from *P. putida* KCTC1768 with a genomic DNA isolation kit (Q-BIO gene, CA). The *nahR* gene was PCR amplified according to the Takara Ex Taq (Japan) manual, using primers (forward 5′-CGC GAG CTC TCA ATT CTC TCT ATC CTG CGA-3′ and reverse 5′-CCG CTC GAG GCT GTA CTC GTG ATG GCT TTA-3′) designed to introduce SacI and XhoI sites at each end. The resultant PCR products were gel-isolated and digested with SacI and XhoI (Takara, Japan). The *nahR/Pr/Psal* fragment was introduced upstream of the firefly luciferase (*luc*) gene in the pGL3 basic expression vector (Promega, WI) to generate pNRSAL (∼6 kb). Plasmids were isolated with a Qiagen spin column kit and transformed into *E. coli* DH5α by the $CaCl_2$ method. Cloning, ligation and transformation were carried out using standard techniques (Sambrook et al., 2001).

2.3. Site-directed mutagenesis and sequence determination

Mutagenesis was performed using the QuikChange Site-Directed Mutagenesis Kit (Stratagene, CA), according to the manufacturer's instructions. The primers used for site-directed mutagenesis are listed in Table 1. The nucleotide sequences of the cloned *nahR/Pr/Psal* fragment and the mutant *nahR* constructs were determined by the ABI PRISM BigDye™ terminator cycle sequencing system (Applied Biosystems, CA) and an ABI PRISM 310 sequencer, using custom-designed oligonucleotides (CTT TAT GTT TTT GGC GTC TTC CA, CTA GCA AAA TAG GCT GTC CC, and GCC ATA ACC GAC ATT GGC GAG ATC TAC TTC).

Table 1
Primers used for mutagenizing the NahR gene in pNRSAL.

Mutation	Primer[a]	Sequences[b]
N169A	F	CGC CGG CTG CTC CAG GCN CAC TAC GTG TGC CTA
	R	TAG GCA CAC GTA GTG NGC CTG GAG CAG CCG GCG
N169C	F	CGC CGG CTG CTC CAG TGY CAC TAC GTG TGC CTA
	R	TAG GCA CAC GTA GTG RCA CTG GAG CAG CCG GCG
N169K	F	CGC CGG CTG CTC CAG AAR CAC TAC GTG TGC CTA
	R	TAG GCA CAC GTA GTG YTT CTG GAG CAG CCG GCG
N169S	F	CGC CGG CTG CTC CAG TCN CAC TAC GTG TGC CTA
	R	TAG GCA CAC GTA GTG NGA CTG GAG CAG CCG GCG
R248H	F	GCC ACT GTG CCG ATA CAY TTA GCCAGC TGC TGC
	R	GCA GCA GTC GGC TAA RTG TAT CGG CAC AGT GGC
R248M	F	GCC ACT GTG CCG ATA ATG TTA GCCAGC TGC TGC
	R	GCA GCA GTC GGC TAA CAT TAT CGG CAC AGT GGC
R248Q	F	GCC ACT GTG CCG ATA CAR TTA GCCAGC TGC TGC
	R	GCA GCA GTC GGC TAA YTG TAT CGG CAC AGT GGC
R248Y	F	GCC ACT GTG CCG ATA ATA TTA GCCAGC TGC TGC
	R	GCA GCA GTC GGC TAA TAT TAT CGG CAC AGT GGC

[a] F and R refer to forward and reverse primers, respectively.
[b] Altered codons are underlined.

2.4. Treatment with salicylate and luciferase assay

E. coli DH5α cells harboring the expression plasmid were grown overnight at 37 °C in 4 ml of TYS medium containing 30 μg ml^{-1} ampicillin. Cells were subcultured and grown to the log phase (OD600 = 0.3–0.4), and then supplemented with sodium salicylate or other effectors (Sigma and Aldrich Chemical Co., MO) dissolved in distilled water at final concentrations ranging from 0.1 μM to 5 mM. After 2 h, 50 μl of cells from each sample were mixed with 5 μl of 1 M KH_2PO_4 and 20 mM EDTA (pH 7.8) and frozen at −70 °C. The frozen cells were lysed by incubation at room temperature for 10 min in 150 μl of lysis solution consisting of 1.25 mg ml^{-1} lysozyme, 2.5 mg ml^{-1} BSA and 1× CCLR (Promega, WI). Supernatants were obtained by centrifugation. For measurement of luciferase activity, 4 μl of supernatant was mixed with 20 μl of firefly luciferin solution (Promega, WI), and the bioluminescence was measured for 20 s with a LBP40 Mithras luminometer (Berthold, Germany).

2.5. Crude cell extract preparation

E. coli DH5α cells harboring wild-type or mutant plasmids were grown overnight at 37 °C in TYS media with 30 μg ml^{-1} ampicillin. Cells were subcultured and grown to the log phase (OD_{600} = 0.3–0.4), harvested (6000 rpm for 10 min), and washed with 10 mM KPO_4 (pH 7.8) buffer containing 5 mM EDTA, 0.2 mM PMSF and 10 mM β-mercaptoethanol. All procedures after cell culture were performed at 4 °C. Cells were sonicated, cell debris was removed by centrifugation, and supernatants were stored at −70 °C until use. Protein concentrations were determined by the microbiuret method (Legget-Bailey, 1968).

3. Results and discussion

3.1. Construction of assay system

Among the most abundant environmental pollutants, the aromatic compounds are of major concern because of their persistence and toxicity. The environmental problems caused by such tenacious pollutants have increased the demand for the development of sensitive pollutant and toxicity detection methods. The fusion of reporter genes to promoters that are induced when cell are exposed to chemicals are one promising approach that has been used to fabricate biosensor for such application (Lei et al., 2006).

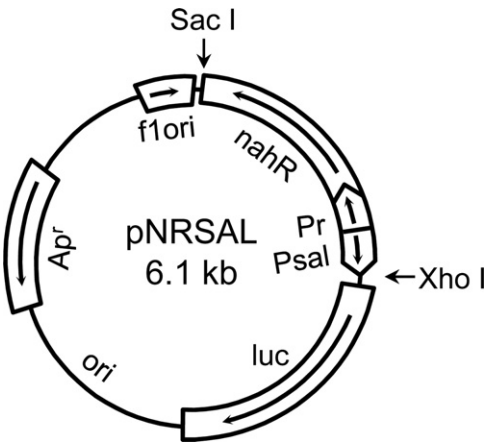

Fig. 1. Construction of plasmid pNRSAL. The *nahR* gene, its own expression promoter *Pr*, and the promoter of *Psal* were cloned from *P. putida* KCTC1768 as described in Section 2. These genes were digested with SacI and XhoI, and introduced upstream of the *luc* (luciferase gene) in the pGL3 basic expression vector (Promega, WI).

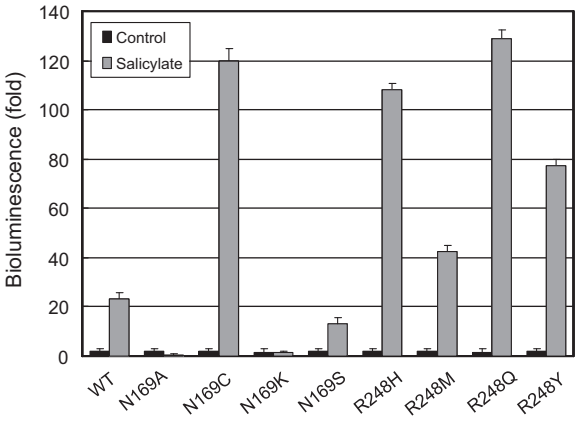

Fig. 2. Luciferase activity of *E. coli* cells harboring pNRSAL (wild-type and mutant NahR) after treatment with salicylate. *E. coli* cells harboring pNRSAL (which encoded wild-type and mutant NahR) were exposed to 100 μM of salicylate for 2 h. Cells were lysed and luciferase activities were measured. Basal activities represent luciferase activities in the absence of an inducer.

We constructed genetically engineered *E. coli* cells harboring a plasmid called pNRSAL (Fig. 1). The plasmid pNRSAL was constructed to act as an assay system by placing the *nahR* gene upstream of the *luc* reporter gene, along with the NahR promoter (*Pr*) and that of *Psal* promoter as well as NahR responsive sites such as a dyadic sequence at −65 and an interaction site at −35. The *Pr* and *Psal* promoters overlap at −35, and lead transcriptions in the opposite direction using different DNA strands. Transcription of *nahR* from *Pr* occurs constitutively, while transcription of genes under the control of *Psal* is inducible by an inducer (Schell, 1993). As a result, the concentration of the inducer salicylate can be quantitatively analyzed by detecting the bioluminescence intensity.

In this study, we introduced site-directed mutagenesis to the *nahR* transcriptional regulatory gene to increase the biological sensing capacity. Four mutants at residues 169, N169A (Asn → Ala), N169C (Asn → Cys), N169K (Asn → Lys), and N169S (Asn → Ser) as well as four mutants at residues 248, R248H (Arg → His), R248M (Arg → Met), R248Q (Arg → Gln), and R248Y (Arg → Tyr) were generated. Amino acids with different charges or sizes were substituted for the residues 169 and 248 known as the central inducer recognition and the C-terminal multimerization domain. Wild-type or mutant *nahR* genes were inserted upstream of the *luc* reporter gene of pNRSAL. *E. coli* cells harboring pNRSAL containing the wild-type or mutant NahR were tested for their activation in the presence of the inducer salicylate. The cells showed a dramatic, concentration dependent response to the normal inducer salicylate indicating the system worked as intended.

3.2. Responses of wild type and mutant NahR to salicylate

The effects of these mutations were examined by the response properties to the inducer salicylate and compared with wild type. Cells harboring wild type or mutant NahR were treated with 100 μM salicylate (preliminary experiments indicated that mutant NahR responded best to ~100 μM salicylate) and their luciferase activities were measured (Fig. 2). Bioluminescence was shown as fold activities with ratios calculated for each response versus the corresponding response in the absence of any inducer (basal activity). Mutants showed altered responses to the normal inducer salicylate, with both loss and gain in degree of sensitivity. All the mutants at the residues 248 and N169C among the mutants at residue 169 showed increased responses to salicylate compared with wild type (Fig. 2). Among them, N169C, R248H, and R248Q showed more than 5–6-fold increased responses at 100 μM salicylate. Especially R248Q showed the most increased response (about 6-fold). However, N169A, N169K and N169S showed no or little responses.

Substitution of amino acid with similar size and uncharged polar side chain (Cys) for Asparagine at the residue 169 showed increased response to salicylate, whereas amino acid with smaller size and uncharged polar side chain (Ser) did not. Given that Cystein might form a possible bond, we presume that structural change by the bond formation might cause the inducer binding pockets of N169C mutant be more flexible so that more inducer might fit better in. However, it is unclear what interaction causes this increased sensitivity. Substitution of either amino acid with charged polar side chain (Lys) or nonpolar side chain (Ala) showed decreased responses to salicylate, indicating that substitutions of amino acids with different sizes or charges might be detrimental at the residue 169 for the function of NahR. The results argue that amino acid at the residue 169 might play a pivotal role in NahR protein-inducer binding during transcriptional activation. Indeed, the X-ray structure of DntR (an LTTR family member 60% homologous to NahR) revealed that the carboxyl group of the salicylate forms direct hydrogen bonds with the side chains of T104, H169 (corresponding to N169 of NahR), and H206. The resulting inducer binding pocket is largely hydrophobic, as it is lined by the side chains of residues I106, Y110, F111, G152, F167, R248, and I273 (Smirnova et al., 2004). Therefore, it has been postulated that residue N169 of NahR seems to directly participate in inducer binding, while residue R248 is involved in stabilization of the inducer-binding cavity (Smirnova et al., 2004).

All substitutions of amino acids with nonpolar side chain (Met), uncharged polar side chains (Gln, Tyr), and charged polar side chain (His) for Arginine at residue 248 showed increased responses to salicylate. Substitutions of amino acids with polar side chain (R248H, R248Y, and R248Q) show dramatically increased responses. The results suggest that their mutations did not alter inducer binding specificity, but instead facilitated the multimerization cycles required for activation. Thus, we presume that the mutants at the residue 248 might be involved in the change of NahR regulatory protein in a way for better binding with other proteins or transcription of downstream reporter gene. Further experiments on the structural and functional mechanism of NahR regulatory protein with inducer might be needed for the precise explanation. In conclusion, the results indicate that N169C, R248H, R248Q and R248Y could be the candidates for the highly-sensitive microbial biosensors.

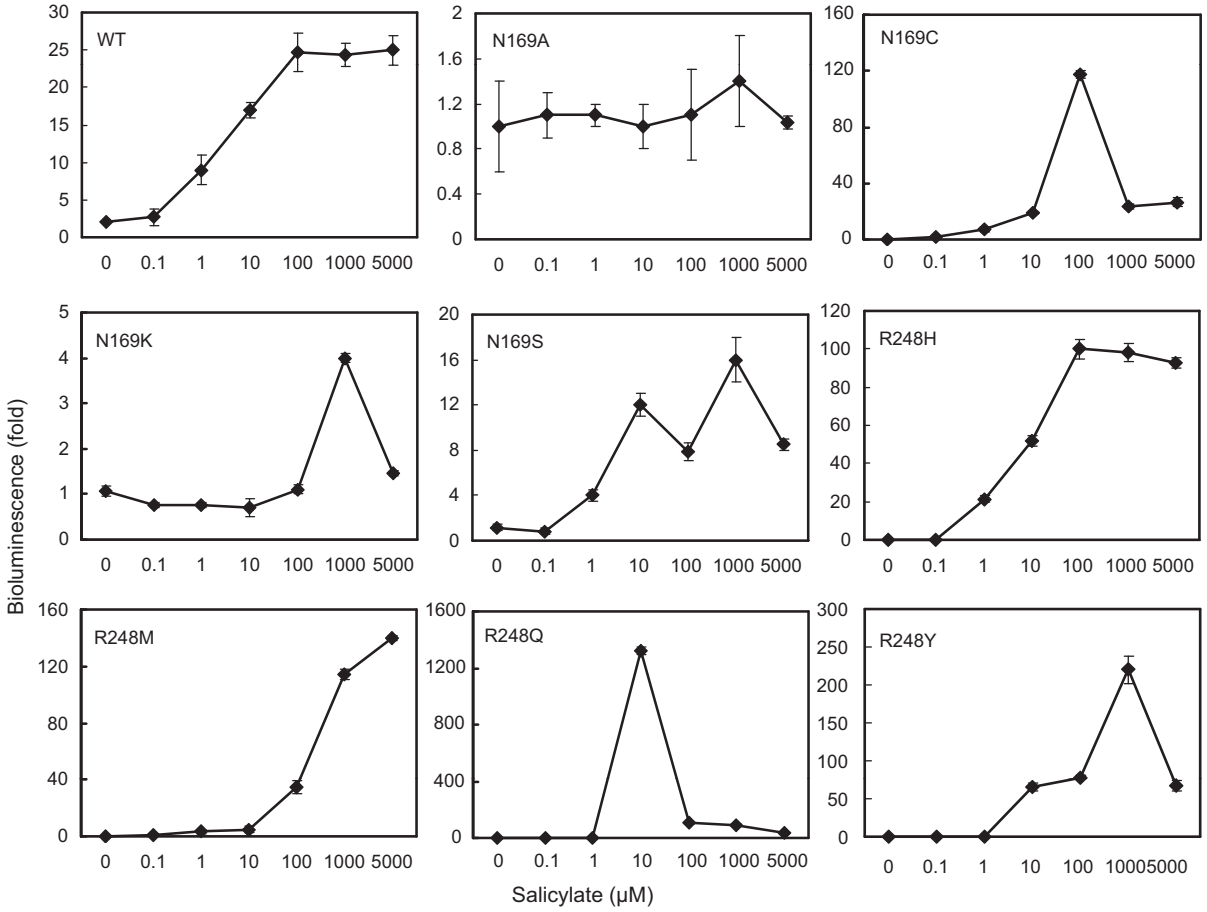

Fig. 3. Luciferase activities of cells expressing wild-type and mutant NahR after treatment with various concentrations of salicylate. Cells harboring the wild-type (WT) or mutant NahR were treated with various concentrations of salicylate, and their bioluminescence responses were measured. Fold activities represent the ratio of non-induced bioluminescence activities.

3.3. Sensitivity of wild type and mutant NahR

Sensitivity is a major concern for a good biosensor system. To examine the sensitivity of our system, the luciferase activities of the wild-type and mutant proteins were examined further at various concentrations of salicylate (Fig. 3). Wild type began to response to salicylate above 0.1 μM and showed best activity at 100 μM. Mutants N169C, R248H, R248M, R248Q and R248Y began to response above 0.1–10 μM and showed different best responses at 10 μM for R248Q, 100 μM for N169C and R248H, 1 mM for R248Y, and 5 mM for R248M. No increased responses were shown with mutants N169A, N169K, and N169S, in comparison with wild type. Mutants N169C, R248H, R248M, R248Q and R248Y showed about 4–50-fold maximum increased activities in comparison with wild-type. Especially R248Q showed about ~50-fold increase in activity at 10 μM salicylate while about ~6-fold at 100 μM as shown in Fig. 2. R248Y revealed best response about ~10-fold increase at 1 mM, whereas R248H and R248M about 4- and 6-fold at 100 μM and 5 mM, respectively. As the only mutant showing increased sensitivity among the series of residue 169 mutants, N169C showed about 5-fold increased response at 100 μM. Although their sensitivities and best response concentrations vary, they are highly-sensitive microbial biosensors that can detect salicylate with wide range of concentrations from 1 μM to above 5 mM. A chemical standard spectrophotometric method for salicylate, based on the formation of a complex between Fe(III) and salicylate, exhibits reliable responses usually between 60 μM and 4 mM (Loh et al., 2006). An enzyme-mediated colorimetric assay of salicylate using salicylate monooxygenase showed a detection range of 0–5 mM (Morris et al., 1990). Most analytical methods for salicylate have their sensitivity in sub μM–mM, even though there was a report that a coupled enzymatic assay using hydroxylase and tyrosinase could detect it at sub-nanomolar levels (6.5 nM up to 1.4 μM) (Bouvrette and Luong, 1996). Therefore, our biosensors presented here fall in a sensitivity range comparable to those analytical methods nowadays used in the detection of salicylate. It is noteworthy that microbial biosensors could also provide information about biological effects such as bioavailability and toxicity of salicylate in addition to its concentration.

We previously observed that the luciferase activity increased ~500 μM salicylate, but tended to decrease thereafter, and that cell turbidity decreased above ~1 mM salicylate (Park et al., 2005). Therefore, there was some cytotoxicity at high inducer concentrations, perhaps leading to decreased responses. However, R248M showed increased or maximum activity at 5 mM without observed cytotoxicity. This result shows that a chronic cytotoxicity problem of microbial biosensors could be alleviated by introducing mutation to the sites where protein binding with inducer and/or interaction with other transcriptional factor(s) might occur. As mentioned before, transcriptional activation by NahR requires its interaction with RNA polymerase in addition to *Psal* promoter DNA binding. It is believed that this might occur via multimerization by domain interactions among different proteins (Schell, 1993). Therefore, mutations in the inducer-binding site might influence the DNA binding capability by disrupting the interaction with RNA polymerase and/or multimerization (Schell, 1993). Overall, the results suggest that mutational approach could increase the sensitivity of microbial biosensors.

Table 2
Responses of wild type and mutants to various inducers.

Types	Salicylate	Benzoate	Phenol	Toluene	o-Xylene
Wild type	+++	–	–	–	–
N169A	–	–	–	–	–
N169C	+++++	+	–	–	–
N169K	–	–	–	–	–
N169S	++	–	–	–	–
R248H	++++	–	–	–	–
R248M	+++	–	–	–	–
R248Q	+++++	+	–	–	–
R248Y	++++	–	–	–	–

+, responses detected with the inducers; –, no response detected under the same conditions used.

3.4. Specificity of wild type and mutant NahR

We examined the specificity of wild type and the mutants by testing their responses to various aromatic compounds including benzoate, phenol, toluene, and o-xylene (Table 2). Wild type and mutant (except N169C and R248Q) NahR regulatory proteins did not show any responses to benzoate, phenol, toluene, and o-xylene, indicating that NahR cannot be induced by these compounds. The results also prove that NahR regulatory protein is different from the regulatory proteins induced by phenol, toluene, and o-xylene such as CapR (S.M. Park et al., 2003; W. Park et al., 2003) or XylR (Kim et al., 2005). However, N169C and R248Q show weak responses to benzoate as well, although their responses to salicylate were much higher than benzoate, implying that salicylate might fit better in the inducer binding pockets of the NahR mutants. These two mutants seem to have more flexible binding pocket for expanded inducers in comparison with wild type or other mutants. This notion is not without precedent, as it has been shown that mutants N169D (Asn at 169 → Asp) and R248C (Arg at 248 → Cys) were responsive to the normal inducer, salicylate, but also to benzoate and other structural analogs (Cebolla et al., 1997). However, these mutants, unlike our data, do not show such increased responses to the normal induer salicylate compared with wild type.

Previously, several microbial biosensors have been demonstrated in field-contaminated samples (Shin et al., 2005). Here we propose a model of highly-sensitive microbial biosensors designed by mutation of NahR regulatory protein for many biotechnological applications.

4. Conclusions

Microbial biosensor is a simple, rapid, and effective analytical device that combines a biological sensing element with a transducer. As a sensing element, microorganism has not only advantages of easy manipulation and adaptation to environment but also disadvantages of poor selectivity, low sensitivity and cytotoxicity problems. Here we challenged one promising strategy to address the problems by tailoring microorganism through mutagenesis to the *nahR* regulatory gene. Eight single mutants were made at residues 169 and 248 known as the central inducer-recognition and the C-terminal multimerization domain in *nahR* regulatory gene. Amino acid replacements resulted in more dramatic effects on inducer responses. All mutants at residues 248 and N169C show increased sensitivity (maximum ~50-fold) compared to wild type, respectively. R248M shows increased response even at 5 mM known to be toxic concentration. Thus, the mutagenesis method could open endless possibilities of microorganisms as excellent biosensing elements by overcoming the drawback of low sensitivity and cytotoxicity in the development and application of microbial biosensors for prospective future advances.

Acknowledgment

This study was supported by the Korean Ministry of Science and Technology as an R&E Project.

References

Cebolla, A., Sousa, C., Lorenzo, V., 1997. Effector specificity mutants of the transcriptional activator NahR of nahphthalene degrading *Pseudomonas* define protein sites involved in binding of aromatic inducers. J. Biol. Chem. 272, 3986–3992.
Bouvrette, P., Luong, J.H.T., 1996. A coupled enzymatic assay for salicylate and acetylsalicylate using salicylate hydroxylase and tyrosinase. Anal. Chim. Acta 335, 169–175.
Henikoff, S., Haughn, G.W., Calvo, J.M., Wallace, J.C., 1988. A large family of bacterial activator proteins. Proc. Natl. Acad. Sci. U.S.A. 85, 6602–6606.
Huang, J., Schell, M.A., 1991. In vivo interactions of the NahR transcriptional activator with its target sequences. J. Biol. Chem. 266, 10830–10838.
Kim, M.N., Park, H.H., Lim, W.K., Shin, H.J., 2005. Construction and comparison of *Escherichia coli* whole-cell biosensors capable of detecting aromatic compounds. J. Microbiol. Meth. 60, 235–245.
Legget-Bailey, J., 1968. Techniques in Protein Chemistry. Elsevier Scientific Publishing Co., New York.
Lei, Y., Chen, W., Mulchandani, A., 2006. Microbial biosensors. Anal. Chim. Acta 568, 200–210.
Loh, H.C., Ahmad, M., Taib, M.N., 2006. Usage of artificial neural network (back propagation) in optimizing salicylic acid determination with ferric (III) nitrate. Anal. Lett. 39, 221–229.
Mclver, J., Djordjevic, M.A., Weiman, J.J., Bender, G.L., Rolfe, B.G., 1989. Extension of host range of *Rhizobium leguminosarm* caused by point mutations in *nodD* that result in alterations in regulatory function and recognition of inducer molecules. Mol. Plant Microbe. Interact. 2, 97–106.
Morris, H.C., Overton, P.D., Ramsay, J.R., Campbell, R.S., Hammond, P.M., Atkinson, T., Price, C.P., 1990. Development and validation of an automated, enzyme-mediated colorimetric assay of salicylate in serum. Clin. Chem. 36, 131–135.
Muraoka, S., Okumura, R., Ogawa, N., Nonaka, T., Miyashita, K., Senda, T., 2003. Crystal structure of a full-length LysR-type transcriptional regulator, CbnR; unusual combination of two subunit forms and molecular bases for causing and changing DNA bend. J. Mol. Biol. 328, 555–566.
Park, H.H., Lee, H.Y., Lim, W.K., Shin, H.J., 2005. NahR: effects of replacements at Asn 169 and Arg 248 on promoter binding and inducer recognition. Arch. Biochem. Biophys. 434, 67–74.
Park, S.M., Park, H.H., Lim, W.K., Shin, H.J., 2003. A new variant activator involved in the degradation of phenolic compounds from a strain of *Pseudomonas putida*. J. Biotechnol. 103, 227–236.
Park, W., Jeon, C.O., Madsen, E.L., 2003. Interaction of NahR, a LysR-type transcriptional regulator, with the α subunit of RNA polymerase in the naphthalene degrading bacterium, *Pseudomonas putida* NCBI 9816-4. FEMS Microbiol. Lett. 213, 159–169.
Sambrook, J., Fritsch, E.F., Maniatis, T., 2001. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
Schell, M.A., 1993. Molecular biology of the LysR family of transcriptional regulators. Annu. Rev. Microbiol. 47, 597–626.
Schell, M.A., Brown, P.H., Raju, S., 1990. Use of saturation mutagenesis to localize probable functional domains in the NahR protein, a LysR-type transcription activator. J. Biol. Chem. 265, 3844–3850.
Schell, M.A., Poser, E.F., 1989. Demonstration, characterization, and mutational analysis of NahR protein binding to *nah* and *sal* promoters. J. Bacteriol. 171, 837–846.
Schell, M.A., Sukordhaman, M., 1989. Evidence that the transcription activator encoded by the *Pseudomonas putida nahR* gene is evolutionarily related to transcriptional activators encoded by the *Rhizobium nodD* genes. J. Bacteriol. 171, 1952–1959.
Shin, H.J., Park, H.H., Lim, W.K., 2005. Freeze-dried recombinant bacteria for on-site detection of phenolic compounds by color change. J. Biotechnol. 119, 36–43.
Smirnova, I.A., Dian, C., Leonard, G.A., McSweeney, S., Birse, D., Brzezinski, P., 2004. Development of a bacterial biosensor for nitrotoluenes: the crystal structure of the transcriptional regulator DntR. J. Mol. Biol. 9, 405–418.
Storz, G., Tartaglia, L.A., Ames, B.N., 1990. Transcriptional regulator of oxidative stress-inducible genes: direct activation by oxidation. Science 248, 189–194.
You, I.S., Ghosal, D., Gunsalus, I.C., 1988. Nucleotide sequence of plasmid NAH7 gene nahR and DNA binding of the nahR product. J. Bacteriol. 170, 5409–5415.
Zaim, J., Kierzek, A.M., 2003. The structure of full-length Lys-type transcriptional regulator: modeling of the full-length OxyR transcription factor dimer. Nucleic Acids Res. 31, 1444–1454.