Hydroxylated Polychlorinated Biphenyl Detection Based on a Genetically Engineered Bioluminescent Whole-Cell Sensing System

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Hydroxylated Polychlorinated Biphenyl Detection Based on a Genetically Engineered Bioluminescent Whole-Cell Sensing System

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The metabolites of polychlorinated biphenyls (PCBs), such as hydroxylated PCBs (OH-PCBs), have been identified as environmental contaminants. Various studies have shown that some OH-PCBs can potentially contribute to health problems. Detection of these compounds in environmental and biological samples could provide useful information about their levels and lead to a better understanding of their apparent toxicity. To that end, we have developed a whole-cell sensing system for the detection of OH-PCBs by taking advantage of the recognition of a group of related compounds, i.e., hydroxylated biphenyls, by the product of the *hbpR* gene in the *hbp* operon from *Pseudomonas azelaica* strain HBP1. By fusing the *luxAB* genes, encoding the reporter protein bacterial luciferase, to the *hbp* regulator−promoter sequence, a whole-cell sensing system was developed. Here, we describe the optimization and application of this whole-cell sensing system for the detection of a model compound, 2-hydroxy-3′,4′-dichlorobiphenyl. A detection limit of 1×10^{-8} M was achieved using this system. The detection of a broad range of individual OH-PCBs as well as an OH-PCB mixture was investigated. The system can detect OH-PCBs in whole serum samples in a trace amount, which is comparable to the detection of these analytes in medium alone. We envision that the method developed can potentially be employed as a rapid and sensitive way to monitor OH-PCBs for toxicological study in the laboratory, as well as a useful tool to evaluate the presence of bioavailable OH-PCBs in natural environments.

Since the 1970s, the apparent toxicities of polychlorinated biphenyls (PCBs) have been scrutinized in much detail. PCBs have been shown in numerous studies to contribute to negative health effects[1−4] and to be persistent in biological and environmental samples.[5] Hydroxylated PCBs (OH-PCBs) have also been more closely examined as potentially exhibiting significant toxic health effects. OH-PCBs have been detected in the environment at concentrations as much as 3.5 times that of PCBs, possibly originating from sewage treatment plants and byproducts of industrial-scale reactions involving biphenyl and biphenylol.[6] Additionally, OH-PCBs are also present in biological fluids as metabolites of PCBs.[5] Many OH-PCBs have been identified in human serum samples and may be present at concentrations comparable to the parent PCBs.[1] It has been shown that some OH-PCBs have estrogenic and antiestrogenic activities in various mammalian models,[1−4] inhibit gap junctional intercellular communication,[3] activate aryl hydrocarbon receptors,[3] and form DNA adducts leading to damage of DNA.[7]

Due to the toxicity of OH-PCBs, it is necessary to have efficient and economical methods to detect and quantify them. Currently, the methods of choice are GC/MS and LC/MS.[8] These methods have excellent detection limits and can effectively identify and quantify OH-PCBs. However, sample extraction, cleanup, and derivatization steps are required for analysis. These factors combined with the expenses associated with instrumentation and the need for trained laboratory technicians quickly drive up the cost and time required for analysis.

Alternatively, whole-cell sensing systems provide several advantages over traditional techniques. Most notably, the speed of analysis is increased due to the lack of need for extensive sample preparation steps and the ability to evaluate multiple samples in one analytical run. Whole-cell sensors, due to the inherent selectivity of the recognition/regulatory proteins involved, can be used in complex sample matrices without significant impact from interfering agents. In addition, a whole-cell sensing system could be developed into a field-portable assay and used as an on-site screening tool for both environmental and biological samples, allowing for a more effective selection of samples to be evaluated in more detail. Lastly, information on bioavailability of analytes is obtained with whole-cell sensing systems, which facilitates the

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(1) Moore, M.; Mustain, M.; Daniel, K.; Chen, I.; Safe, S.; Zacharewski, T.; Gillesby, B.; Joyeux, A.; Balaguer, P. *Toxicol. Appl. Pharmacol.* **1997**, *142*, 160−168.

(2) Connor, K.; Ramamoorthy, K.; Moore, M.; Mustain, M.; Chen, I.; Safe, S.; Zacharewski, T.; Gillesby, B.; Joyeux, A.; Balaguer, P. *Toxicol. Appl. Pharmacol.* **1997**, *145*, 111−123.

(3) Machala, M.; Blaha, L.; Lehmler, H.-J.; Pliskova, M.; Majkova, Z.; Kapplova, P.; Sovadinova, I.; Vondracek, J.; Malmberg, T.; Robertson, L. W. *Chem. Res. Toxicol.* **2004**, *17*, 340−347.

(4) Matthews, J.; Zacharewski, T. *Toxicol. Sci.* **2000**, *53*, 326−339.

(5) James, M. O. *PCBs, Recent Advances in Environmental Toxicology and Health Effects*; 2001; pp 35−46.

(6) Ueno, D.; Darling, C.; Alaee, M.; Campbell, L.; Pacepavicius, G.; Teixeira, C.; Muir, D. *Environ. Sci. Technol.* **2007**, *41*, 1841−1848.

(7) McLean, M. R.; Bauer, U.; Amaro, A. R.; Robertson, L. W. *Chem. Res. Toxicol.* **1996**, *9*, 158−164.

(8) Takasuga, T.; Senthilkumar, K.; Watanabe, K.; Takemori, H.; Shoda, T.; Kuroda, Y. *Organohalogen Compd.* **2004**, *66*, 2501−2506.

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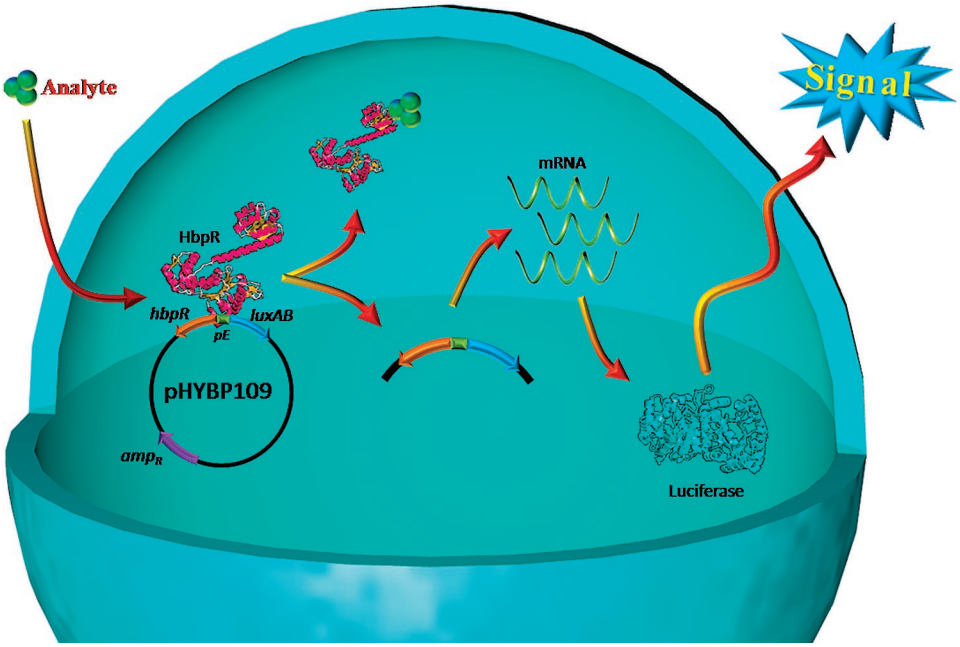

Figure 1. Schematic of the genetically engineered bioluminescent whole-cell sensing system. The analyte diffuses into the cell where it binds the regulatory protein HbpR which is bound to the reporter plasmid, pHYBP109. Upon binding, HbpR undergoes a conformational change, releases from the reporter plasmid, and allows expression of the reporter protein, LuxAB, which generates a bioluminescent signal.

prediction of fate and effect of the pollutants for toxicological studies.

A common strategy in the development of a whole-cell sensing system is placing a gene which encodes for a reporter protein under the control of a specific recognition element, such as a regulatory protein, for the analyte of interest as shown in Figure 1. Biosensors of this type have been developed for a variety of analytes. More in-depth information and examples can be found in a number of reviews.[9−11] Specifically, we have developed a whole-cell sensing system for the detection of OH-PCBs by employing the strain *Pseudomonas azelaica* HBP1. This bacterium contains the *hbpCAD* genes, which are responsible for the degradation of hydroxylated biphenyls. The expression of these genes is negatively regulated by a regulatory protein encoded by the gene *hbpR* located upstream from the *hbpCAD* genes. A strain of *Escherichia coli* carrying a recombinant plasmid consisting of the *luxAB* reporter gene, coding for bacterial luciferase, under control of the HbpR regulatory protein was constructed[12] and employed as a whole-cell sensing system in the present study. In the presence of analytes, such as OH-PCBs, the regulatory protein HbpR activates transcription through the *hbp* promoter resulting in the expression of the reporter gene. This expression can then be monitored by measuring bioluminescence emission after addition of decanal, a substrate for luciferase. Within a certain range of analyte concentrations, the expression of the reporter gene is dose-dependent; therefore, the intensity of the analytical signal is directly related to the amount of target compound.

Analyzing the concentration of OH-PCBs in serum samples as a biomarker of PCB exposure is essential for toxicological studies and remediation purposes. The hydrophilic nature of the hydroxyl group in OH-PCBs suggests that these chemicals may be readily excreted from the body. However, the most predominant PCB metabolites found in biological fluids contain between five to seven chlorines with one hydroxyl group in the biphenyl ring.[13,14] Therefore, the possibility of retention rather than excretion also exists due to the increased hydrophobicity contributed by physicochemical properties of chlorines attached to the biphenyl backbone. Moreover, their high lipophilicity and affinity to certain proteins such as the thyroxin-transporting protein transthyretin (TTR) lead to the retention of OH-PCBs in different body compartments, mainly in blood.[15]

In this study, we have employed a genetically engineered bacterium to develop a whole-cell sensing system to detect the presence of a variety of OH-PCBs in both environmental and biological samples. This sensing system has been optimized with respect to important assay conditions, and the feasibility of the application of this system as a screening tool has been discussed.

MATERIALS AND METHODS

Chemicals and Media. 2-Hydroxybiphenyl was obtained from Sigma-Aldrich Corp. (St. Louis, MO). In Table 1, OH-PCBs nos. 2, 5, 6, and 8−12 were obtained from Accustandard Inc. (New Haven, CT), OH-PCBs nos. 3 and 4 were obtained from Ultra Scientific Inc. (North Kingstown, RI), and OH-PCBs nos. 1, 7, and 13−27 were kind gifts from Hans Lehmler at the University of

(9) Daunert, S.; Barrett, G.; Feliciano, J. S.; Shetty, R. S.; Shrestha, S.; Smith-Spencer, W. *Chem. Rev.* **2000**, *100*, 2705−2738.
(10) van der Meer, J. R.; Tropel, D.; Jaspers, M. *Environ. Microbiol.* **2004**, *6*, 1005−1020.
(11) Feliciano, J.; Pasini, P.; Deo, S. K.; Daunert, S. *Photoproteins in Bioanalysis*; 2006; pp 131−154.
(12) Jaspers, M. C. M.; Schmid, A.; Sturme, M. H. J.; Goslings, D. A. M.; Kohler, H.-P. E.; Van der Meer, J. R. *J. Bacteriol.* **2001**, *183*, 270−279.
(13) Safe, S.; Washburn, K.; Zacharewski, T.; Phillips, T. *Chemosphere* **1995**, *31*, 3017−3023.
(14) Faengstroem, B.; Hovander, L.; Bignert, A.; Athanassiadis, I.; Linderholm, L.; Grandjean, P.; Weihe, P.; Bergman, A. *Environ. Sci. Technol.* **2005**, *39*, 9457−9463.
(15) Malmberg, T.; Hoogstraate, J.; Bergman, A.; Wehler, E. K. *Xenobiotica* **2004**, *34*, 581−589.

Table 1. Response of the Whole-Cell Sensing System to Various OH-PCBs

no.	compound	$\log(A/A_0)$[a]	$-\log ED_{50}$[b]	detection limit (M)[c]
1	2-hydroxy-4′-chlorobiphenyl	0.44	1.76 ± 0.37	1.0×10^{-6}
2	2-hydroxy-3′,4′-dichlorobiphenyl	0.88	5.66 ± 0.07	5.0×10^{-7}
3	2-hydroxy-3,5-dichlorobiphenyl	0.50	2.09 ± 0.24	5.0×10^{-5}
4	2-hydroxy-2′,3,5′-trichlorobiphenyl	0.56	3.86 ± 0.25	1.0×10^{-5}
5	2-hydroxy-2′,4′,6′-trichlorobiphenyl	0.37	3.21 ± 0.06	1.0×10^{-8}
6	2-hydroxy-2′,5,5′-trichlorobiphenyl	0.49	4.17 ± 0.03	5.0×10^{-6}
7	2-hydroxy-3′,4′,5-trichlorobiphenyl	0.24	4.84 ± 0.15	1.0×10^{-9}
8	2-hydroxy-3′,5,5′-trichlorobiphenyl	0.22	5.16 ± 0.32	1.0×10^{-7}
9	2-hydroxy-2′,3′,4′,5′-tetrachlorobiphenyl	0.47	3.21 ± 0.06	5.0×10^{-5}
10	2-hydroxy-2′,3′,5′,6′-tetrachlorobiphenyl	0.48	4.47 ± 0.11	5.0×10^{-6}
11	2-hydroxy-2′,4′,5,6′-tetrachlorobiphenyl	0.20	3.36 ± 0.62	1.0×10^{-6}
12	2-hydroxy-2′,3′,4′,5,5′-pentachlorobiphenyl	0.35	4.35 ± 0.76	1.0×10^{-6}
13	3-hydroxy-4′-chlorobiphenyl	0.46	2.29 ± 0.24	1.0×10^{-7}
14	4-hydroxy-4′-chlorobiphenyl	0.31	2.87 ± 0.19	5.0×10^{-5}
15	4-hydroxy-2′,3-dichlorobiphenyl	0.75	4.43 ± 0.09	1.0×10^{-8}
16	4-hydroxy-2′,5′-dichlorobiphenyl	0.61	3.46 ± 0.07	1.0×10^{-7}
17	4-hydroxy-3,3′-dichlorobiphenyl	0.64	4.58 ± 0.14	1.0×10^{-8}
18	4-hydroxy-3,4′-dichlorobiphenyl	0.60	3.75 ± 0.05	1.0×10^{-6}
19	4-hydroxy-3′,4′-dichlorobiphenyl	0.41	3.49 ± 0.69	1.0×10^{-8}
20	4-hydroxy-3,5-dichlorobiphenyl	0.63	3.49 ± 0.05	5.0×10^{-7}
21	4-hydroxy-2′,3,5-trichlorobiphenyl	0.32	3.75 ± 0.05	1.0×10^{-6}
22	4-hydroxy-3,3′,4′-trichlorobiphenyl	0.82	4.79 ± 0.05	1.0×10^{-7}
23	4-hydroxy-3,3′,5-trichlorobiphenyl	0.42	2.49 ± 0.11	1.0×10^{-6}
24	4-hydroxy-3,3′,5′-trichlorobiphenyl	0.50	4.21 ± 0.06	1.0×10^{-6}
25	4-hydroxy-3,4′,5-trichlorobiphenyl	0.38	3.40 ± 0.14	1.0×10^{-6}
26	4-hydroxy-3,3′,4′,5-tetrachlorobiphenyl	0.21	3.49 ± 0.30	1.0×10^{-5}
27	4-hydroxy-2′,3,4′,5-tetrachlorobiphenyl	0.12	4.42 ± 0.26	1.0×10^{-7}

[a] The response ratio (A/A_0) was calculated by dividing the maximum bioluminescence signals for each OH-PCB (A) by the bioluminescence signals for the blank (A_0). [b] ED_{50} was defined as the concentration at 50% maximum induction and was calculated by using GraphPad Prism 4.0 software. [c] Detection limit was defined as the analyte concentration that corresponds to a signal-to-noise ratio of 3.

Iowa. The purities (≥95.0%) of these synthesized OH-PCBs were verified by Agilent GC/MS 5975 prior to experiments.

Dimethyl sulfoxide (DMSO, ≥99.9%, for molecular biology), *n*-decanal (≥99%, for GC, liquid), ethanol (anhydrous, ≥99.5%, 200 proof), glycerin (meets USP testing specifications), and human serum (from clotted human male whole blood, sterile-filtered, mycoplasma tested, virus tested) were obtained from Sigma-Aldrich Corp. (St. Louis, MO). Deionized distilled water was produced by a Milli-Q water purification system (Millipore, Bedford, MA).

E. coli strains were grown at 37 °C on Luria−Bertani (LB) medium (BIO 101, Vista, CA) containing ampicillin (100 μg/mL, Sigma-Aldrich Corp., St. Louis, MO). For the luciferase induction assay, mineral medium (MM), defined by Gerhardt et al.,[16] was prepared and supplemented with 0.01% tryptone, 0.005% yeast extract, and 10 mM D-(+)-glucose before use, as described by Jaspers et al.[12] MM (per liter) was prepared from the ingredients: 1.00 g of NH_4Cl, 3.49 g of $Na_2HPO_4 \cdot 2H_2O$, 2.77 g of KH_2PO_4, 20 mL of Hunter's vitamin-free mineral base, and 2.0 mL of a vitamin solution, adjusted to pH 6.8. Hunter's vitamin-free mineral base contained the following (per liter): 10 g of nitrilotriacetic acid (neutralize with 6.00 g of KOH), 14.5 g of $MgSO_4 \cdot 7H_2O$, 3.33 g of $CaCl_2 \cdot 2H_2O$, 9.74 mg of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 99 mg of $FeSO_4 \cdot 7H_2O$, and 50 mL of the Metals 44 solution. The composition of Metals 44 (per 100 mL) was as follows: 387 mg of $Na_4EDTA \cdot 4H_2O$, 1.10 g of $ZnSO_4 \cdot 7H_2O$, 914 mg of $FeSO_4 \cdot 7H_2O$, 154 mg of $MnSO_4 \cdot H_2O$, 39.2 mg of $CuSO_4 \cdot 5H_2O$, 24.8 mg of $Co(NO_3)_2 \cdot 6H_2O$, 17.7 mg of $Na_2B_4O_7 \cdot 19H_2O$ and was neutralized with H_2SO_4. The vitamin solution (per 100 mL) was prepared by mixing 0.50 mg of biotin, 50 mg of nicotinic acid, and 25 mg of thiamine hydrochloride. MM was sterilized and stored at 4 °C before use. All chemicals for MM preparation were purchased from Sigma-Aldrich Corp. (St. Louis, MO) and were at least cell culture tested grade.

Preparation of *E. coli* Cells Harboring Plasmid pHYBP109. Cells were obtained as a frozen glycerol stock. A stab was taken from this stock and grown overnight at 37 °C in a culture tube containing 2 mL of LB medium and ampicillin (100 μg/mL). The following day, the 2 mL culture was transferred to a 1 L flask containing 250 mL of LB medium and grown at 37 °C to an optical density at 600 nm (OD_{600}) of approximately 0.90. The cells were then dispensed into 1.5 mL microcentrifuge tubes in 1 mL aliquots and stored at −80 °C until use. A volume of 50 μL of sterilized glycerin was added to each 1 mL stock of cells for preservation purposes. Unless otherwise specified, all the cells were grown in a rotary shaker at 200 rpm.

Detection of Compounds in Dimethyl Sulfoxide. Stock solutions of each compound were prepared at a concentration of 1×10^{-2} M by dissolving the appropriate mass of each compound in 1.0 mL of DMSO in a microcentrifuge tube and vortexing until dissolved. From these, solutions in a concentration range of 5.0×10^{-3} to 1.0×10^{-9} M were prepared by serial dilution of the original stock solution in DMSO. In addition, a blank was prepared containing only DMSO. Each set of solutions was prepared fresh, and the 1×10^{-2} M stock solutions were saved and frozen at −20 °C after use.

(16) Gerhardt, P., Murray, R. G. E., Costilow, R. N., Nester, E. W., Wood, W. A., Krieg, N. R., Phillips, G. B., Eds. *Manual of Methods For General Bacteriology*; 1981.

Immediately prior to use in each assay, a microcentrifuge tube containing a 1 mL aliquot of cells was removed from the −80 °C freezer and placed in a room-temperature water bath for 2 min and then on ice. For the final assay solution, 1.9 mL of MM, 33 μL of thawed cell suspension, and 20 μL of a compound at each concentration along with a blank as prepared above were dispensed into a series of 14 mL culture tubes in triplicate. These tubes were placed at 30 °C on an orbital shaker at 225 rpm for a 4 h incubation period.

Following incubation, a volume of 200 μL of the cell suspension from each culture tube was dispensed into the wells of a 96-well microtiter plate in triplicate. A volume of 100 μL of stock *n*-decanal substrate solution (2 mM in 1:1 ethanol/H_2O) was added into the reaction assay by automated injection for the bioluminescence signal measurement. Light output was integrated from 5 to 15 s after *n*-decanal injection for each of the wells in the plate using a POLARstar OPTIMA luminometer (BMG Labtech, Offenburg, Germany). The total light signal was expressed as relative light units (RLU).

For experimental optimization, the general protocol described above was employed, unless otherwise specified in the text. All assays were conducted in triplicate.

Detection of Model Compound in Human Serum. 2-Hydroxy-3′,4′-dichlorobiphenyl was used to evaluate the assay in human serum. This compound was prepared as above in a concentration range from 1×10^{-2} to 1×10^{-8} M in DMSO along with a blank containing only DMSO. In a separate set of microcentrifuge tubes, 10 μL of each of these solutions was dispensed into 90 μL of human serum. This resulted in serum samples with the compound of interest present in a range of 1×10^{-3} to 1×10^{-9} M along with a blank. Additionally, a set of samples was prepared using deionized water instead of human serum as a comparison. These solutions were then used to complete the assay as described above.

Detection of a Compound Mixture in Human Serum. A mixture of the following 10 OH-PCBs was prepared at a concentration of 1×10^{-3} M with respect to each individual compound in DMSO: 2-hydroxy-3′,4′-dichlorobiphenyl, 4-hydroxy-3,3′,4′-trichlorobiphenyl, 2-hydroxy-3′,4′,5-trichlorobiphenyl, 2-hydroxy-3′,5,5′-trichlorobiphenyl, 3-hydroxy-4′-chlorobiphenyl, 4-hydroxy-2′,3,4′,5-tetrachlorobiphenyl, 4-hydroxy-2′,5′-dichlorobiphenyl, 4-hydroxy-3′,4′-dichlorobiphenyl, 4-hydroxy-3,3′-dichlorobiphenyl, 4-hydroxy-2′,3-dichlorobiphenyl. To prepare this solution, 10 μL of the 1.0×10^{-2} M stock solution of each compound was dispensed into a single microcentrifuge tube. Serial dilutions were made using this solution to create a mixture in DMSO in a concentration range of 1×10^{-3} to 1×10^{-8} M with respect to each compound and a concentration of 1×10^{-2} to 1×10^{-7} M with respect to total OH-PCBs. In a separate set of microcentrifuge tubes, 10 μL of each of these solutions was dispensed into 90 μL of human serum, yielding samples of the mixture in human serum in a concentration range of 1×10^{-4} to 1×10^{-9} M with respect to each compound. These samples were then used to complete the assay as described above.

Statistical Analysis. The results presented are the averages of the values obtained in three independent experiments. GraphPad Prism 4.0 (GraphPad Software Inc., San Diego, CA) was used to generate nonlinear best-fit lines of the data.

RESULTS AND DISCUSSION

The strain *P. azelaica* HBP1 is able to use hydroxylated biphenyls as sole carbon and energy sources. The mechanism for the degradation of 2-hydroxybiphenyl by *P. azelaica* HBP1 has been well characterized.[17] The metabolism involves three enzymes encoded by the *hbpCAD* genes, which are negatively regulated by the HbpR protein. To study the P_{hbpC} promoter activity of *P. azelaica* HBP1, a plasmid (designated as pHYBP109) containing *hbpR*−P_{hbpC}−*luxAB* fusion was constructed and transformed to *E. coli* by Jaspers et al.[12] Induction experiments were carried out using 2-hydroxybiphenyl. Increasing bioluminescence signals upon induction with 0.2 mM of 2-hydroxybiphenyl for increasing induction times suggested the direct activation of P_{hbpC} promoter by the compound binding to the HbpR protein.[12] In our laboratory, initial experiments were conducted by treating these recombinant *E. coli* cells with varying concentrations of 2-hydroxybiphenyl. A preliminary detection limit of 1×10^{-6} M could be obtained (data not shown), under the experimental conditions reported by Jaspers et al.[12] The detection limit is defined as the minimum analyte concentration that corresponds to a signal-to-noise ratio of 3.

A whole-cell sensing system is a genetically modified system constructed in such a way that in the presence of an analyte, expression of a reporter protein is triggered. In the plasmid used in this study, *hbpR*, the gene encoding for the HbpR protein, is positioned upstream from the *luxAB* genes, which code for the bioluminescent reporter protein, luciferase. In this way, the expression of luciferase is placed under the control of the P_{hbpC} promoter, which is regulated by the HbpR protein. Thus, the expression of luciferase in *E. coli* harboring pHYBP109 is mediated by the HbpR regulatory protein from the same regulatory circuit. In the absence of hydroxylated biphenyls, the HbpR protein binds to the promoter and prevents the expression of *luxAB* genes. In the presence of hydroxylated biphenyls, the HbpR protein undergoes a conformational change upon binding of the inducer molecules, causing release of the protein−inducer complex from the promoter. As a result, expression of the reporter protein occurs.

In a study carried out by Jaspers et al. to evaluate the selectivity of the HbpR protein, a group of chemicals such as biphenylic compounds, polycyclic aromatic hydrocarbons, and monoaromatic hydrocarbons, among others, were screened using engineered bacteria.[12] It was found that only a few structurally similar chemicals containing the biphenyl backbone showed significant activity. In fact, when the signals were normalized to the response to 2-hydroxybiphenyl at a concentration of 2 mM, relative luciferase activities more than 32% were observed for only a narrow range of compounds. Additionally, some chemicals showing activity had a hydroxyl group at the ortho biphenyl ring position. On the other hand, biphenyl, chlorobiphenyl, or compounds with a monoaromatic structure failed to show activity. Because of the selectivity of the HbpR protein in recognizing compounds with biphenyl backbone and a hydroxyl group, we envisioned that employing engineered *E. coli* harboring plasmid pHYBP109 as a whole-cell sensing system would permit us to detect a group of structurally similar OH-PCBs. Moreover, the broad inducer range

(17) Kohler, H. P. E.; Kohler-Staub, D.; Focht, D. D. *Appl. Environ. Microbiol.* **1988**, *54*, 2683−2688.

of TbuT[18] and XylR,[19] which belong to the same NtRC family of proteins as HbpR, further supported the investigation of the inducer spectrum of HbpR protein by exposing the recombinant cells to OH-PCBs. As a pioneer study, cells were exposed to five randomly selected OH-PCBs. Significant increase of the bioluminescence signal as compared to a blank was observed for all five compounds tested. The maximum signal ratio of 1.72 ± 0.11 (mean ± standard deviation; $n = 3$) was found by using 2-hydroxy-3′,4′-dichlorobiphenyl at a concentration of 1×10^{-3} M after a 2 h incubation period. Thus, this chemical was used as a model compound for further optimization of assay parameters.

Because only the regulator and promoter region of the *hbp* operon was inserted into the luciferase expression vector, the degradation of OH-PCBs by the cells is unlikely. For this reason, the toxicity of OH-PCBs to the bacteria used in our assay was evaluated. Cell growth was monitored with/without the addition of 2-hydroxy-3′,4′-dichlorobiphenyl, at a final concentration of 1×10^{-5} M, to the medium containing cells harboring the pHYBP109 plasmid. Optical densities at 600 nm (OD_{600}) were checked every hour for 8 h. Observed differences in OD_{600} of less than 10% between the two cell growth curves indicate that the toxicity of the compound is negligible at the tested concentration.

Important assay parameters were optimized in order to improve the overall performance of the biosensing system. Among these were the growth stage at which the cells were harvested, the storage method of the harvested cells, and the length and temperature of the incubation of the cells with the analytes. The optimum biosensing conditions were found to be harvesting cells at an optical density of approximately 0.9 measured at 600 nm, storage of the cells at −80 °C until use, and incubating the cells at 30 °C for 4 h during the assay.

Once all biosensing system parameters were optimized, the genetically engineered whole cells were exposed to varying concentrations of 2-hydroxy-3′,4′-dichlorobiphenyl. An increase in the bioluminescence signal was observed with increasing concentration of the compound as shown in Figure 2. Data points were fitted with a sigmoidal dose−response curve, and a correlation coefficient R^2 of 0.9977 was obtained. The detection limit falls in the order of 10^{-8} M. When the analyte concentration was in the range of 1×10^{-7} to 1×10^{-3} M, a rapid increase of the bioluminescence signal was observed. Concentrations of the model compound higher than 1×10^{-3} M caused the bioluminescence signal to decrease significantly (data not shown). This observation is in agreement with the data published by Hay et al. for the detection of 2,4-dichlorophenol using a bioluminescent whole-cell reporter.[20] It is speculated that at higher concentrations of the analyte, there is a toxic effect, which leads to a reduced number of living cells and a subsequent significant decrease in the response.

The next step in our research aimed at evaluating the response of the whole-cell sensor to a variety of OH-PCBs. However, it is difficult to assess the most predominant OH-PCBs in human serum[4] because of the unavailability of these compounds for use as standards from commercial sources. For that, a range of 27 commercially available OH-PCBs at various concentrations were tested. A dose-dependent response was observed for all of these compounds. A representative example for 4-hydroxy-3,3′,4′-trichlorobiphenyl is reported in Figure 2. The data from each analyte was fit with a sigmoidal dose−response curve, and parameters such as log ED_{50} and $\log(A/A_0)$ were calculated and summarized in Table 1. ED_{50} was defined as the concentration at 50% of maximum induction and was calculated by using GraphPad Prism 4.0 software. The induction ratio (A/A_0) was calculated by dividing the maximum bioluminescence signal for each OH-PCB (A) by the bioluminescence signal for the blank (A_0). The maximum induction levels and the concentrations to achieve the maximum induction varied among the analytes tested. The dynamic range for the detection of OH-PCBs covered two to 5 orders of magnitude. The detection limits ranged from 1×10^{-9} to 1×10^{-5} M. These results are in agreement with previous studies which demonstrate differential light responses in whole-cell sensing systems to various members of a class of related compounds.[21,22] An attempt to explain the differences in the responses to the chemicals, based on their molecular structure, is ongoing at this stage.

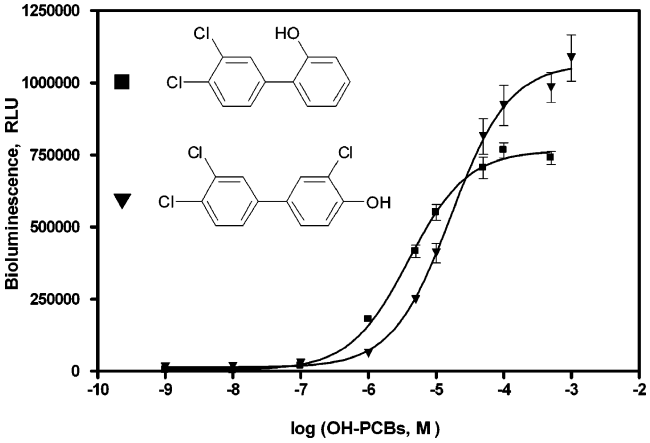

Figure 2. Dose−response curves of the whole-cell sensing system obtained with 2-hydroxy-3′,4′-dichlorobiphenyl (■) and 4-hydroxy-3,3′,4′-trichlorobiphenyl (▼) under optimized experimental conditions. The bioluminescence signals have been corrected with respect to the blank. Values represent the means ± standard deviation of triplicate determinations.

In order to validate the response of this luciferase-based whole-cell sensing system to the PCB metabolites in a biological matrix, human serum was spiked with a range of concentrations of the model compound. The assay was carried out under the optimized experimental conditions. As shown in Figure 3, a dose−response curve was obtained with a log ED_{50} of −5.29 and a detection limit of 5.0×10^{-8} M. Increased bioluminescence signals were observed for the serum samples spiked with 2-hydroxy-3′,4′-dichlorobiphenyl, as compared to the signals produced by the same analyte concentrations in the absence of the biological matrix. It is believed that this increased signal is due to the presence of proteins found in serum. These proteins may prevent the analyte from interacting with the walls of the plastic tube, thus making

(18) Byrne, A. M.; Olsen, R. H. *J. Bacteriol.* **1996**, *178*, 6327−6337.
(19) Abril, M. A.; Michan, C.; Timmis, K. N.; Ramos, J. L. *J. Bacteriol.* **1989**, *171*, 6782−6790.
(20) Hay, A. G.; Rice, J. F.; Applegate, B. M.; Bright, N. G.; Sayler, G. S. *Appl. Environ. Microbiol.* **2000**, *66*, 4589−4594.
(21) Applegate, B. M.; Kehrmeyer, S. R.; Sayler, G. S. *Appl. Environ. Microbiol.* **1998**, *64*, 2730−2735.
(22) Phoenix, P.; Keane, A.; Patel, A.; Bergeron, H.; Ghoshal, S.; Lau, P. C. K. *Environ. Microbiol.* **2003**, *5*, 1309−1327.

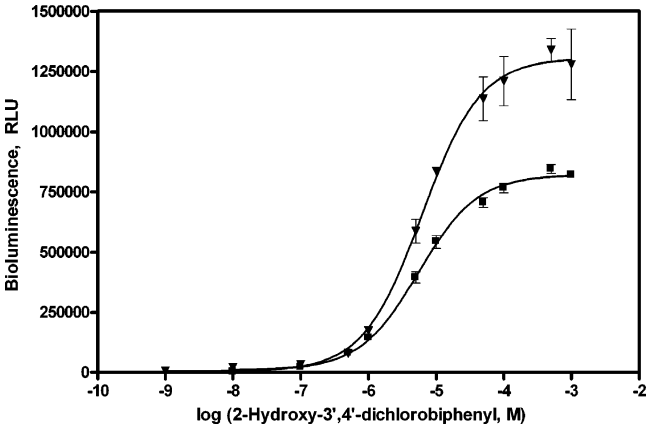

Figure 3. Dose−response curves of the whole-cell sensing system obtained with 2-hydroxy-3′,4′-dichlorobiphenyl in 90% water/10% DMSO (■) or 90% serum/10% DMSO (▼). The bioluminescence signals have been corrected with respect to the blank. Values represent the means ± standard deviation of triplicate determinations.

the analyte more available for diffusion into the cells. Overall, the resultant bioluminescence response of the whole-cell sensing system to the model compound in serum demonstrates the feasibility of direct detection of OH-PCBs in serum samples.

In real samples, PCB metabolites are present as mixtures. Various contamination patterns of OH-PCBs have been reported in the literature for fish plasma,[23] polar bear whole blood,[24] and human whole blood,[25] plasma,[26−29] serum,[14,30] and cerebrospinal fluid samples.[8] Additionally, the ratios of total OH-PCBs to total PCBs calculated were around 4−56% in human plasma[26] or even found to be 4−8 times higher in the whole blood of polar bears.[24] In our laboratory, the responses of the whole-cell sensing system to a model mixture of 10 OH-PCBs were tested. The obtained dose−response curve with a detection limit of 5.0×10^{-8} M suggests the potential for employing this sensing system as a screening method for multiple OH-PCBs contaminated samples. Notably, the application of this whole-cell sensing system to the detection of OH-PCBs in biological and environmental samples could remarkably reduce the analysis time and cost posed by currently used conventional methods, such as GC or LC methods. Further studies utilizing this whole-cell sensing system will enable us to monitor the bioavailable PCB metabolites in large pools of environmental and biological samples. This will provide a new insight into the pollution status at certain sites for better understanding of the current ecological conditions of the living environment.

CONCLUSIONS

In this work, a rapid and sensitive sensing system for the detection of OH-PCBs based on genetically engineered whole cells has been developed. Additionally, the performance of this whole-cell sensing system has been demonstrated in a biological sample matrix. The results achieved suggest that the sensing system may find applications in biomedical analysis as well as in the environmental monitoring of OH-PCBs. The ruggedness of the proposed sensing system can be further improved by employing lyophilized cells, which can be easily stored and transported to the field and reconstituted for later use. Upon miniaturization and integration into an appropriate assay platform, we envision this system being developed into a rapid, high-throughput, field-portable method for the detection of OH-PCBs in both environmental and biological samples.

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(23) Campbell, L. M.; Muir, D. C. G.; Whittle, D. M.; Backus, S.; Norstrom, R. J.; Fisk, A. T. *Environ. Sci. Technol.* **2003**, *37*, 1720−1725.
(24) Sandala, G. M.; Sonne-Hansen, C.; Dietz, R.; Muir, D. C. G.; Valters, K.; Bennett, E. R.; Born, E. W.; Letcher, R. J. *Sci. Total Environ.* **2004**, *331*, 125−141.
(25) Sandau, C. D.; Ayotte, P.; Dewailly, E.; Duffe, J.; Norstrom, R. J. *Environ. Health Perspect.* **2000**, *108*, 611−616.
(26) Sandau, C. D.; Ayotte, P.; Dewailly, E.; Duffe, J.; Norstrom, R. J. *Environ. Health Perspect.* **2002**, *110*, 411−417.
(27) Hovander, L.; Malmberg, T.; Athanasiadou, M.; Athanassiadis, I.; Rahm, S.; Bergman, A.; Wehler, E. K. *Arch. Environ. Contam. Toxicol.* **2002**, *42*, 105−117.
(28) Soechitram, S. D.; Athanasiadou, M.; Hovander, L.; Bergman, A.; Sauer, P. J. J. *Environ. Health Perspect.* **2004**, *112*, 1208−1212.
(29) Sandanger, T. M.; Dumas, P.; Berger, U.; Burkow, I. C. *J. Environ. Monit.* **2004**, *6*, 758−765.
(30) Faengstroem, B.; Athanasiadou, M.; Grandjean, P.; Weihe, P.; Bergman, A. *Environ. Health Perspect.* **2002**, *110*, 895−899.