

Detection of Hydroxylated Polychlorinated Biphenyls by Whole-Cell and Protein-Based Sensing Systems Employing the Regulatory Protein HbpR

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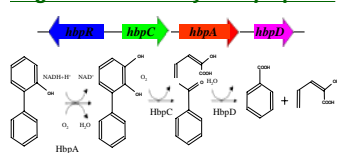
Introduction

Polychlorinated Biphenyls (PCBs) have been characterized as toxic, persistent pollutants and have been identified in environmental and biological samples including human serum, plasma, and whole blood. Hydroxyl-PCBs (OH-PCBs) are present in the environment as a result of past industrial pollution and are present in living organisms as a product of the metabolism of PCBs and as a result of exposure from the environment. The release of these compounds into the environment has caused global burden due to their persistent nature and tendency to bioaccumulate and biomagnify through the food chain. Extensive studies have focused on their environmental and biological distribution, toxic effects, health impact, and environmental remediation. Increasing interest has been shown to OH-PCBs, the main products in PCBs biodegradation pathway. Their estrogenic and thyroid-hormone-like activity in animal models, along with their potential health threat to humans are main concerns to scientists. The ubiquitous presence of PCB metabolites in environmental and biological samples dictates the need for a method able to rapidly detect and quantify these toxins. Standard analytical methods such as gas chromatography are costly, time consuming, and must be performed by trained technicians in a laboratory setting. Thus, a simpler and faster analytical method is needed.

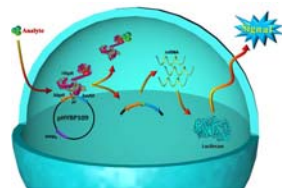
To that end, we are pursuing the development of whole-cell and protein-based sensing systems. Whole-cell sensing systems employ genetically modified cells constructed in such a way that, in the presence of a target analyte, the bacterial cells express a reporter protein in a concentration-dependent fashion. Consequently, the concentration of the analyte can be quantified by measuring the signal generated by the reporter protein. We have demonstrated the development of one such whole-cell biosensing system by employing the bioluminescent reporter protein, LuxAB and the regulatory protein, HbpR from *Pseudomonas azelaica*, which selectively recognizes OH-PCBs. Under optimum assay conditions, the detection limits of OH-PCBs are in the range of 10^{-4} to 10^{-9} M. The potential of using a whole cell sensing system to detect OH-PCBs in human serum samples was demonstrated by selecting 2-hydroxy-3',4'-dichlorobiphenyl as a model toxin. The validity of the assay in the detection of OH-PCBs in biological fluids was demonstrated by analysis of these compounds in serum samples.

Additionally, a protein-based sensing system for the detection of OH-PCBs is being developed. In this system, the HbpR protein undergoes a conformational change upon analyte binding which is employed in the development of a biosensing system. It has been shown that the isolated binding domain of related proteins are capable of binding their effector molecules and undergoing a conformational change *in vitro*. The effector-binding A-domain of HbpR has been expressed and labeled with an environmentally sensitive fluorescent label. Upon analyte binding and the resulting conformational change, the fluorescence signal from the fluorophore changes in a dose-dependent fashion, allowing the quantitation of OH-PCBs. Preliminary results indicate that the labeled protein is binding OH-PCBs and causing a change in the observed fluorescence signal. Further optimization of the sensing system is being pursued in the effort to develop an effective, field-portable sensing system for on-site quantitation of OH-PCBs.

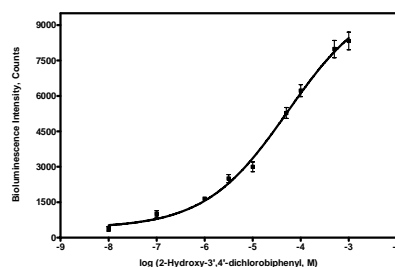
Degradation Pathway of *hbp* operon



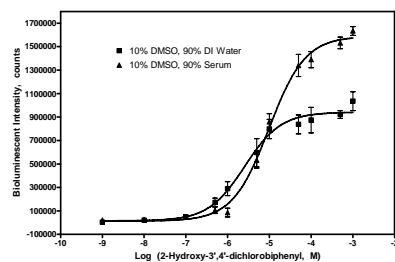
Whole-Cell Sensing System



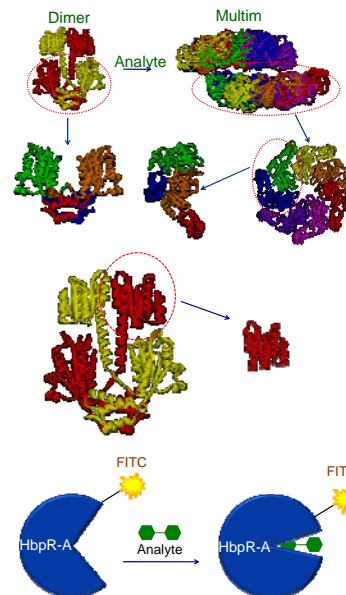
Calibration Curve for 2-Hydroxy-3',4'-dichlorobiphenyl



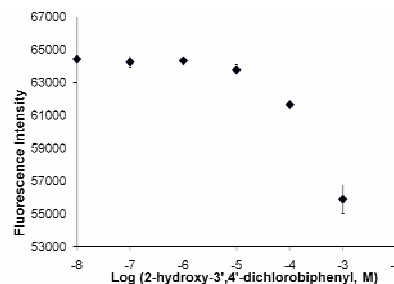
Detection of 2-Hydroxy-3',4'-dichlorobiphenyl in Human Serum Samples



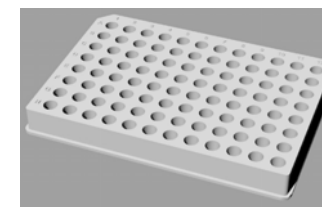
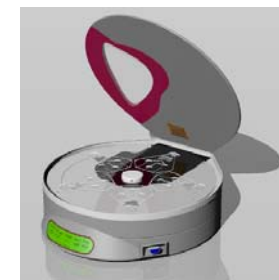
Protein-Based Assay Strategy



Pilot Assay of 2-Hydroxy-3',4'-dichlorobiphenyl



Miniaturization and High Throughput Assays



Conclusions

1. A rapid, sensitive, and selective biosensing system for the detection of hydroxylated PCBs based on genetically engineered cell as well as the truncated regulatory protein HbpR has been developed.
2. A wide selection of OH-PCBs were tested using the luciferase reporter gene-based whole cell sensing system. The dynamic range for the detection of OH-PCBs ranged from two to five orders of magnitude. The detection limits ranged from 10^2 to 10^7 M.
3. The luciferase reporter gene-based whole cell sensing system was demonstrated in biological samples by analyzing OH-PCBs in simulated human serum samples. Comparable detection limits were obtained in serum and in prepared standards.
4. Preliminary results demonstrate that the truncated binding domain of HbpR is binding its analyte and producing a change in fluorescence from FITC.
5. Further work is required to optimize the performance of these sensing system. To that end, several strategies will be pursued and investigated based on reproducibility, range of detection, and total assay time. Additionally, work is ongoing towards the miniaturization and packaging of these assays to render them portable for field and clinical studies.

Acknowledgments

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