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Kun Yin

Design of Novel Biosensors for Optical Sensing and Their Applications in Environmental Analysis

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Design of Novel Biosensors for Optical Sensing and Their Applications in Environmental Analysis

Doctoral Thesis accepted by
Yantai Institute of Coastal Zone Research,
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Supervisor's Foreword

With the rapid development of industry and increasing anthropogenic influence, environmental pollution has already become one of the most serious problems on our planet. The growing risks of environmental accidents greatly threaten human beings, organisms as well as the ecological system. Therefore, considering severe long-term consequences of environmental toxic substances, how to rapidly and reliably detect them has already attracted great attention. Nowadays, various methods have been established to detect environmental pollutions, but realizing rapid, simple, convenient, highly sensitive, and selective detection for real samples is still a big challenge. In his thesis, Dr. Kun Yin focused on the development of novel optical biosensors to address this challenge. The selective recognition of target environmental pollutions can be achieved by special bioelements. After millions of years of evolution, some bioelements can recognize their target with astonishing accuracy and efficiency. In his study, fluorescent bioelement, bioreaction, bacterial surface-display system, and living cells have been utilized to establish optical biosensors with satisfactory performance, which can realize rapid detection of environmental pollutions in real samples. He successfully bridges gaps among different fields and opens up new opportunities for the development of novel biosensors. Compared with traditional analytical methods, these biosensors are much simpler with good sensitivity and selectivity, which have outstanding advantages in practical application.

Yantai, China
December 2018

Prof. Lingxin Chen

Abstract

Optical biosensors, based on special bioelements and optical transducers, have been widely applied for environmental analysis because of their outstanding advantages including excellent biocompatibility and high specificity. In our study, we have developed a series of novel biosensors based on different strategies such as new biomaterials and new bioreactions. These biosensors own satisfied optical properties, high sensitivity and selectivity, which can transform the recognition behavior of specific targets to optical signals and achieve the detection of target objects.

After millions of years of revolution, bioelement owns outstanding selectivity toward its target. Inspired by this phenomenon, pyoverdine, secreted by a *Pseudomonas aeruginosa* PA1, has been purified by us through affinity chromatography. As a metal-chelating peptide-derived bioelement, pyoverdine can selectively recognize copper ion and its fluorescence quenched obviously in the presence of copper ions through electron-transfer pathways. Therefore, we established a novel biosensor based on pyoverdine to detect copper ion. The biosensor owns good selectivity and sensitivity, which has been applied for detecting copper ion in real samples including water and biosamples, and the results agree well with those obtained by traditional methods. Therefore, the novel biosensor is creditable, which can be utilized to monitor copper pollution. Beside detecting copper ions, we find that furazolidone can also rapidly quench the fluorescence of pyoverdine within minutes. The electron-deficient nitro compound in furazolidone acts as strong quencher of pyoverdine, which can quench the fluorescence of pyoverdine via an electron-transfer mechanism. Based on this phenomenon, we have developed a simple method to detect furazolidone based on pyoverdine. The copper ion can be masked by EDTA, which will not influence the detection of furazolidone. The linear range of the detection is from 2 to 160 μM, and the limit of detection is about 0.5 μM. To our best knowledge, it is the first time to use pyoverdine as a biosensor for furazolidone detection. The biosensor owns satisfied properties such as good sensitivity and selectivity, which can be potentially applied for furazolidone detection in aquatic samples.

Considering that bioelements with good optical property are very rare, so I expect to develop biosensors based on the special optical bioreactions. In the next study,

I have developed a simple colorimetric sensor to ultra-sensitively detect copper ion. 1-chloro-2,4-dinitrobenzene and L-cysteine can be conjugated to yellow 2,4-dinitrophenylcysteine. Copper ions can catalytically oxidize cysteine to cystine in the presence of O_2, which inhibits the generation of yellow 2,4-dinitrophenylcysteine. Therefore, the colorimetric detection of copper ion can be established based on this phenomenon. The biosensor has been applied for detecting copper ion in drinking water, lake water, seawater, and biological samples, and the results agree well with those obtained by ICP-MS. The developed colorimetric biosensor for copper ion owns high sensitivity and selectivity, which is applicable for copper ion determination in environmental samples.

Additionally, I also try to establish biosensor based on bacteria. In this study, a biosensor based on carboxylesterase E2 secreted from *Pseudomonas aeruginosa* PA1 has been established for simultaneously adsorption and detection of mercury ion. The adsorption of mercury ion fits well with Langmuir adsorption model, which is followed by a physicochemical, saturatable, and equilibrated mechanism. Through regulating pH values, the system can be regenerated. Additionally, the activity of carboxylesterase E2 decreases in the presence of mercury ion, which can be used for mercury ion detection. Therefore, the simultaneous remediation and detection of mercury ion can be achieved.

In my last work, a near-infrared (NIR) ratiometric fluorescent probe has been developed for the detection of cysteine (Cys) in mitochondria to indicate oxidative stress, which used to monitor environmental toxin indirectly. In the presence of Cys, the polymethine π-electron system rearranges in the fluorophore, which leads to a remarkable absorption and emission spectrum shifts, which can be used for the detection of Cys. The detection limit is about 0.2 μM, which can be obtained within 5 min. With the help of living HepG2 cells and living mice model, the mitochondrial Cys level under different oxidative stress statuses has been successfully monitored by our probe Cy-NB.

Keywords Biomaterial · Optical sensing · Colorimetric detection · Fluorescence detection · Environmental analysis

Parts of this thesis have been published in the following journal articles:

Kun Yin, Min Lv, Qiaoning Wang; Yixuan Wu; Chunyang Liao*, Lingxin Chen. Simultaneous Bioremediation and Biodetection of Mercury Ion through Surface Display of Carboxylesterase E2 from *Pseudomonas Aeruginosa* PA1. ***Water Research***, 2016. 103: 383–390. (Reproduced with Permission. Copyright (2016) Elsevier).

Kun Yin, Yixuan Wu, Shasha Wang and Lingxin Chen*. A sensitive fluorescent biosensor for the detection of copper ion inspired by biological recognition element pyoverdine. ***Sensors and Actuators B: Chemical***, 2016, 232: 257–263. (Reproduced with Permission. Copyright (2016) Elsevier).

Kun Yin, Fabiao Yu*, Weiwei Zhang, and Lingxin Chen*. A near-infrared ratiometric fluorescent probe for cysteine detection over glutathione indicating mitochondrial oxidative stress in vivo. ***Biosensors and Bioelectronics***, 2015, 74: 156–164. (Reproduced with Permission. Copyright (2015) Elsevier).

Kun Yin, Bowei Li, Xiaochun Wang, Weiwei Zhang* and Lingxin Chen*. Ultrasensitive colorimetric detection of Cu^{2+} ion based on catalyticoxidation of L-cysteine. ***Biosensors and Bioelectronics***, 2015, 64: 81–87. (Reproduced with Permission. Copyright (2015) Elsevier).

Kun Yin, Weiwei Zhang and Lingxin Chen*. Pyoverdine secreted by Pseudomonas aeruginosa as a biological recognition element for the fluorescent detection of furazolidone. ***Biosensors and Bioelectronics***, 2014, 51: 90–96. (Reproduced with Permission. Copyright (2014) Elsevier).

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