Accepted Manuscript

Colorimetric biosensor for the assay of paraoxon in environmental water samples based on the iodine-starch color reaction

Liangqia Guo, Zhihong Li, Huan Chen, Yarong Wu, Ling Chen, Zhiping Song, Tianran Lin

PII: S0003-2670(17)30245-3

DOI: 10.1016/j.aca.2017.02.028

Reference: ACA 235088

To appear in: *Analytica Chimica Acta*

Received Date: 16 November 2016

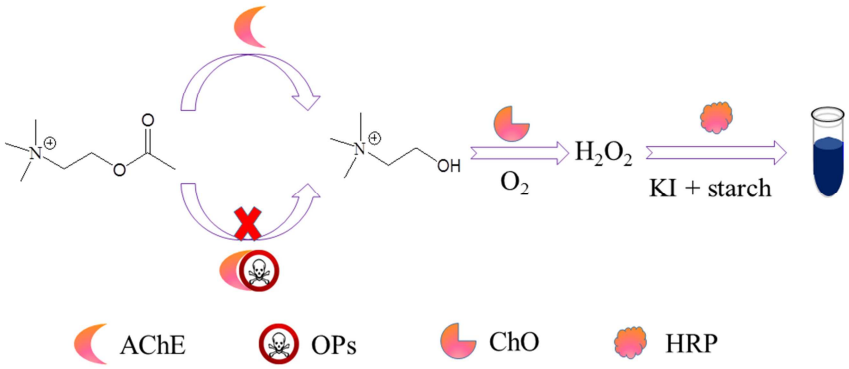
Revised Date: 24 February 2017

Accepted Date: 25 February 2017

Please cite this article as: L. Guo, Z. Li, H. Chen, Y. Wu, L. Chen, Z. Song, T. Lin, Colorimetric biosensor for the assay of paraoxon in environmental water samples based on the iodine-starch color reaction, *Analytica Chimica Acta* (2017), doi: 10.1016/j.aca.2017.02.028.

Graph Abstract

In the presence of acetylcholine chloride, the enzymes acetylcholinesterase (AChE) and choline oxidase (ChO) catalyzed the formation of H_2O_2, which then activated horseradish peroxidase (HRP) to catalyze the oxidation of KI to produce an iodine-starch color reaction. Upon exposure to paraoxon, the catalytic activity of AChE was inhibited and less H_2O_2 generated, resulting in a decrease in the production of I_2 and a drop in the intensity of the color of the solution. By employing the conventional iodine-starch color reaction, this biosensor has the potential of on-site assay of OPs residues in environmental samples.



Colorimetric biosensor for the assay of paraoxon in environmental water samples based on the iodine-starch color reaction

Liangqia Guo*, Zhihong Li, Huan Chen, Yarong Wu, Ling Chen, Zhiping Song, Tianran Lin

Ministry of Education Key Laboratory of Analysis and Detection for Food Safety, Fujian Provincial Key Laboratory of Analysis and Detection Technology for Food Safety, College of Chemistry, Fuzhou University, Fuzhou 350116, China

Abstract:

In this work, a new colorimetric biosensor for the assay of paraoxon was developed via the conventional iodine-starch color reaction and multi-enzyme cascade catalytic reactions. In the presence of acetylcholine chloride, acetylcholinesterase (AChE) and choline oxidase (ChO) catalyzed the formation of H_2O_2, which then activated horseradish peroxidase (HRP) to catalyze the oxidation of KI to produce an iodine-starch color reaction. Upon exposure to paraoxon, the catalytic activity of AChE was inhibited and less H_2O_2 generated, resulting in a decrease in the production of I_2 and a drop in the intensity of solution color. This colorimetric biosensor showed high sensitivity for the assay of paraoxon with a limit of detection 4.7 ppb and was applied for the assay of paraoxon in spiked real samples. By employing the conventional iodine-starch color reaction, this biosensor has the potential of on-site

assay of OPs residues in environmental samples.

Keywords: Organophosphorus pesticides; Acetylcholinesterase; Colorimetric method; Biosensor; Paraoxon

1. Introduction

Organophosphorus pesticides (OPs) are the most extensively used pesticides in modern agriculture because of their relatively low persistence and high effectiveness against insects. However, OPs residues in food products and the environment pose great threat to public health due to their high neurotoxicity [1]. Acetylcholinesterase (AChE) is a key enzyme in the central and peripheral nervous system. OPs can irreversibly inhibit the activity of AChE by phosphorylating the serine residue in the active site of AChE [2], resulting in the accumulation of the neurotransmitter acetylcholine (ACh), thus interfering with muscular responses and causing respiratory and myocardial impairment and even death [3]. Therefore, it is of great significance to develop a fast, reliable and cost-effective method to detect OPs residues for the purpose of human safety and environmental protection.

Over the past few decades, several analytical techniques such as high-performance liquid chromatography [4], gas chromatography [5], mass spectrometer [6], enzyme-linked immunosorbent assay [7], capillary electrophoresis [8], surface enhanced Raman scattering spectroscopy [9], have been developed for the detection of OPs residues in water and food samples. Although these techniques are selective and sensitive, the disadvantages such as expensiveness, requirement for well-trained personnel and time-consuming pre-treatment, limit their application in on-site analysis, especially for emergency cases such as accidental release of pesticides, acute poisoning, and so on. Therefore, simple, sensitive, portable, and

low-cost methods for on-site detection of OPs residues are on great demand.

In the past few decades, biosensors based on AChE, a commercially available enzyme, have been extensively investigated due to their advantages such as simplicity, rapidity, specificity, cost effectiveness, and user-friendly operation, which are helpful for on-site analysis of OPs residues [10]. By taking advantage of the inhibition of AChE activity by OPs, they can be quantitatively measured indirectly through the detection of thiocholine or H_2O_2 with colorimetric [11-13], fluorescent [14-16], chemiluminescent [17], electrochemiluminescent [18], photoelectrochemical [19], and electrochemical [20-22] signals.

Among these biosensors, the colorimetric assay showed great advantages such as low cost, simplicity and practicality in on-site analysis, for the color related to the concentration of target can be observed directly by naked eyes, without the need of expensive or sophisticated instruments [23]. The most widely used colorimetric assay for OPs based on AChE was the Ellman method [11, 12]. However, this method showed low sensitivity and might result in false-positive effect [13]. Noble metal (e.g., Au and Ag) nanoparticles (NPs) are attractive as colorimetric reporters because of their particular local surface plasmon resonance property [24, 25]. Therefore, the integration of enzymatic reaction with Au or Ag NPs has recently emerged for the colorimetric assay of OPs [23, 26, 27]. However, uncontrolled aggregation of Au or Ag NPs in real samples might result in a poor specificity for the targets.

Starch is a biodegradable natural polymer of α-D-glucose contained in plants such as grain, rice, and potatoes [28]. Amylose, a main constituent of starch, can form inclusion complexes with hydrophobic guest molecules by encapsulating them within the helical cavity. An intensely blue-colored iodine-starch complex appears upon encapsulation of iodine by amylose when starch is mixed with iodine in an aqueous

solution [29-31]. This phenomenon is known as the iodine-starch reaction, which was first discovered by Colin and de Claubry in 1814 [32]. Besides, as a sensitive test for iodine, the iodine-starch reaction has been used for the colorimetric detection of adenosine, glucose, α-amylase, hydrogen sulphide, and single nucleotide polymorphisms [33-37]. However, to the best of our knowledge, the conventional iodine-starch color reaction has not been applied for the environmental assay.

Herein, we proposed a colorimetric biosensor for on-site assay of paraoxon (a model of OPs) in environmental water samples by conventional iodine-starch color reaction. AChE and choline oxidase (ChO) catalyzed the formation of H_2O_2 in the presence of acetylcholine. Then the resulting H_2O_2 oxidized KI to I_2, and subsequently formed blue-colored starch-iodine complexes via the iodine-starch reaction. When paraoxon was introduced, the inhibition of AChE activity resulted in a decrease of iodine-starch reaction. Therefore, the color of iodine-starch complexes is inversely proportional to the concentration of paraoxon residues. The assay of paraoxon can be performed in homogenous solution without the need of reagent synthesis and enzyme immobilization. Finally, this colorimetric biosensor was applied for the assay of paraoxon in environmental water samples.

2. Experimental

2.1. Chemicals and materials

ACh was obtained from Tokyo Chemical Industry. AChE from Electrophorus electricus (137 U mg^{-1}) and ChO from Alcaligenes sp. (15 U mg^{-1}) were obtained from Sigma-Aldrich. A certified reference material (CRM) of paraoxon methanol solution (101.6 μg mL^{-1}) with a CRM uncertainty of ±5% was purchased from AccuStandard. H_2O_2 (AR, 30%), Na_2HPO_4, and NaH_2PO_4 were purchased from

Sinopharm Chemical Reagent Co., Ltd. (China). Horseradish peroxidase (HRP, Rz>1.5) was obtained from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (China) and dissolved with deionized water. Potassium iodide (KI) was purchased from Shantou Chemical Factory of Guangdong Province (China). Soluble starch was purchased from Tianjin Bodi Chemical Factory (China). A starch solution (0.5% w/v) was prepared by dispersing the required amount of starch in 5 mL deionized water and then adding the mixture to a boiling water dropwise with constant rapid stirring to avoid the aggregation of starch. AChE and ChO were dissolved in phosphate buffer solution (PBS, pH 7.4, 10 mmol L^{-1}). All chemicals used were of analytical grade and all components of the assay unless stated otherwise were dissolved in deionized water (18.2 MΩ•cm) prepared by a Millipore system.

2.2. Colorimetric assay of paraoxon

Paraoxon was selected as a model of OPs to carry out the experiments. 50 μL of AChE (0.03 U mL^{-1}), 50 μL of PBS (pH 7.4, 10 mmol L^{-1}) were firstly mixed with 50 μL of various concentrations of paraoxon at room temperature (25°C) for 30 min. The reaction solution was then mixed with 100 μL of ACh (500 μmol L^{-1}) and 50 μL of ChO (2 U mL^{-1}) for further 30 min. Finally, 50 μL of KI (40 mmol L^{-1}), 50 μL of HRP (2 μg mL^{-1}), and 50 μL of starch (0.5%) were added to the mixture solution. The absorption spectrum of mixture solution was recorded by a Lamda 750 UV-Vis-NIR spectrophotometer (PE, USA) over 400 to 850 nm. The relative absorbance at 572 nm (ΔAbs) was used to quantitatively detect paraoxon, where ΔAbs was the difference between the absorbance of mixture solution without paraoxon and that with the

inhibition of paraoxon. Photos of mixture solution were also taken with a Nikon D7000 digital camera.

3. Results and discussion

3.1. Principle of colorimetric assay of OPs

The colorimetric biosensor for the assay of OPs was based on the conventional iodine-starch reaction and multi-enzyme cascade catalytic reactions (Scheme 1). In a normal condition, AChE catalyzes the hydrolysis of the substrate ACh to produce choline, and ChO catalyzes the oxidation of choline to generate H_2O_2 in the presence of oxygen. After HRP catalyzes the oxidation of KI to I_2 by H_2O_2, blue iodine-starch complexes are produced in the coexistence of starch. Although the structure of iodine-starch complexes remains elusive, it is believed that linear polyiodide ions encapsulated in the coil of the starch molecules cause the blue color [28-32]. However, when OPs are present, the activity of AChE is (partially) inhibited via the formation of phosphorylated cholinesterase [3]. Thus, the generation of choline and H_2O_2 is impeded, resulting in a decrease in the solution color or no color in the solution. The blue color in the mixture solution, which is very sensitive to naked eyes, is positively related to the concentration of H_2O_2 that positively depends on the activity of AChE in the solution. The absorbance of iodine-starch complexes in the solution is inversely proportional to the concentration of OPs residues. Therefore, the iodine-starch color reaction coupled with multi-enzyme cascade catalytic reactions is expected for the colorimetric assay of OPs residues.

The preferred position for Scheme 1.

To validate the iodine-starch reaction-based colorimetric biosensor for the assay of OPs, the following experiments were carried out. As shown in Figure 1, after AChE was incubated with ACh and ChO for 30 min, following by the addition of KI, HRP and starch, a strong absorption peak was observed at 572 nm (curve a), which is the characteristic absorption of iodine-starch complexes. However, no color reaction was observed in the absence of HRP (curve b) or AChE (curve c). These phenomena indicated that the iodine-starch color reaction resulted from the oxidation of KI by H_2O_2, which was generated by the AChE and ChO enzyme catalytic reactions. To investigate the potential application for the assay of OPs, AChE was first incubated with paraoxon for 30 min, and then mixed with ACh and ChO for 30 min, followed by the addition of KI, HRP and starch, no color reaction was observed either (curve d). This result indicated that paraoxon inactivated AChE, thus impeding the generation of H_2O_2.

The preferred position for Figure 1.

3.2. Optimization of assay conditions

In order to develop a highly effective colorimetric biosensor for the assay of paraoxon, the reaction conditions were carefully optimized. It was found that the iodine-starch color reaction can be greatly enhanced by HRP due to its catalytic effect on the production of I_2. As shown in Figure S1, the absorbance of iodine-starch complexes increased rapidly with the increase in the concentration of HRP within 0-1.0 μg mL^{-1} and reached a maximum at 2 μg mL^{-1}. The absorbance levelled off when the concentration of HRP further increased. KI is the precursor for the

production of I_2 and polyiodide ions. Amylose in starch is responsible for the blue polyiodide complexes [29, 30]. The absorbance of iodine-starch complexes increased quickly with the increase in the concentration of KI within 0-40 mmol L^{-1}, the absorbance increased slowly as the concentration of KI further increased (Figure S2). The absorbance of iodine-starch complexes increased with the increase in the concentration of starch until it reached 0.5%. The absorbance started to decrease as the concentration of starch further increased (Figure S3). Therefore, 2 μg mL^{-1} HRP, 40 mmol L^{-1} KI and 0.5% starch were chosen in the iodine-starch color reaction for further experiments.

The catalytic hydrolysis reaction of AChE towards ACh was also investigated. The absorbance of iodine-starch complexes increased with the increase in the concentration of ACh until ACh reached 500 μmol L^{-1}. As ACh concentration further increased, the absorbance showed a little decrease on the contrary (Figure S4). The absorbance of iodine-starch complexes increased with the increase in the concentration of AChE within 0-0.05 U mL^{-1} (Figure S5). Taking the absorbance and amount of AChE into account, 0.03 U mL^{-1} AChE was chosen. The absorbance of iodine-starch complexes increased with the increase in the concentration of ChO until ChO reached 2.0 U mL^{-1} (Figure S6). As the concentration of ChO further increased, the absorbance did not increase remarkably. Effect of incubation time for the catalytic hydrolysis reaction of AChE towards ACh was also examined. As shown in Figure S7, the incubation time was optimized at 30 min. Therefore, 500 μmol L^{-1} ACh, 0.03 U mL^{-1} AChE, 2.0 U mL^{-1} ChO, and 30 min incubation time were chosen for further

experiments.

3.3. Colorimetric assay for paraoxon

Paraoxon, one of the most commonly used OPs [23], was chosen as a model of OPs to explore the feasibility of colorimetric biosensor for the assay of OPs residues. Prior to the assay, paraoxon with different concentrations was incubated with aliquots of AChE solution at room temperature (25°C) for 30 min. ACh and ChO were then introduced into each mixture solution, which was further incubated for 30 min. After KI, HRP and starch solution were introduced into each mixture solution in sequence, it could be seen that the solution color turned blue, which was inversely proportional to paraoxon concentration. The relative absorbance of mixture solution at 572 nm without and with the inhibition of paraoxon could therefore be used to quantitatively detect paraoxon and the solution color could be used for semi-quantitative assay of paraoxon by naked eyes.

As shown in Figure 2A, the apparent absorption as well as the blue color of mixture solution gradually decreased with the increase in paraoxon concentration, revealing a dependence of the color reaction on OPs concentration. Upon exposure to paraoxon, the catalytic activity of AChE was inhibited and less H_2O_2 generated, resulting in the production of less I_2 and a drop in the intensity of the color of iodine-starch complexes. The blue color of mixture solution almost faded when paraoxon concentration exceeded 100 ppm. The relative absorbance of mixture solution at 572 nm (ΔAbs) increased with the increase of paraoxon concentration within 10 - 400 ppb (Figure 2B). There was a linear relation between ΔAbs and

paraoxon concentration over 10 to 80 ppb (i.e., 36-290 nmol L^{-1}). The limit of detection (3σ/k) for paraoxon was found to be 4.7 ppb (17 nmol L^{-1}), which was lower than the maximum residue limits (MRL) in the European Union pesticides database (10 ppb) [23]. The limit of detection is also lower than that of previous colorimetric biosensors for the assay of OPs [12, 38-41].

The preferred position for Figure 2

In order to validate the feasibility of this colorimetric biosensor for the assay of OPs residues in real samples, environmental water samples from vegetable irrigation water were tested by a standard spike and recovery experiment. Real samples were first filtered through a 0.22 μm membrane twice, and the filtrate was collected. Different concentrations of paraoxon were spiked into the samples, and then the colorimetric biosensor was employed to detect the spiked paraoxon concentration. The color of mixture solution decreased with the increase in the spiked concentration of paraoxon (Figure S8). The recoveries of spiked paraoxon were obtained in the range from 88% to 110% (Table 1). These results indicated that the colorimetric biosensor could be potentially applied for on-site assay of OPs in real environmental samples.

The preferred position for Table 1

4. Conclusion

In summary, a new colorimetric biosensor for the assay of paraoxon has been developed via the conventional iodine-starch color reaction coupled with multi-enzyme (AChE, ChO and HRP) cascade catalytic reactions. Paraoxon could

inhibit AChE activity, resulting in the production of less H_2O_2. Therefore, the formation of iodine-starch complexes with blue color could be suppressed; the absorbance and the color intensity were inversely proportional to the concentration of paraoxon. This colorimetric biosensor showed high sensitivity for the assay of paraoxon residues with a relatively short reaction time (about 60 min). The limit of detection for paraoxon was 4.7 ppb, which was lower than the MRL of the European Union. Finally, the colorimetric biosensor was successfully applied for the assay of paraoxon in vegetable irrigation water samples with good recoveries. Although it was unable to discriminate each individual OP in the same sample as most AChE-based biosensors reported, this simple, sensitive, and portable colorimetric biosensor is expected to be applied for the rapid on-site pre-screening of OPs residues in environmental samples where traditional analytical instrumentation is not readily accessible.

ACKNOWLEDGMENT

This work was financially supported by NSFC (21577017), Program for New Century Excellent Talents in Fujian Province University and Program for Changjiang Scholars and Innovative Research Team in University of Ministry of Education of China (IRT15R11).

References

[1] F. Sánchez-Santed, F. Cañadas, P. Flores, M. López-Grancha, D. Cardona, Long-term functional neurotoxicity of paraoxon and chlorpyrifos: behavioural and pharmacological evidence, Neurotoxicol. Teratol. 26 (2004) 305-317.

[2] D.A. Dougherty, D.A. Stauffer, Acetylcholine binding by a synthetic receptor: implications for biological recognition, Science 250 (1990) 1558-1560.

[3] Y. Miao, N. He, J.-J. Zhu, History and new developments of assays for cholinesterase activity and inhibition, Chem. Rev. 110 (2010) 5216-5234.

[4] C.C. Leandro, P. Hancock, R.J. Fussell, B.J. Keely, Comparison of ultra-performance liquid chromatography and high-performance liquid chromatography for the determination of priority pesticides in baby foods by tandem quadrupole mass spectrometry, J. Chromatogr. A 1103 (2006) 94-101.

[5] B. Albero, C. Sanchez-Brunete, J.L. Tadeo, Determination of organophosphorus pesticides in fruit juices by matrix solid-phase dispersion and gas chromatography, J. Agric. Food Chem. 51 (2003) 6915-6921.

[6] M. Gómez-Ramos, C. Ferrer, O. Malato, A. Agüera, A. Fernández-Alba, Liquid chromatography-high-resolution mass spectrometry for pesticide residue analysis in fruit and vegetables: screening and quantitative studies, J. Chromatogr. A 1287 (2013) 24-37.

[7] J. Mehta, P. Vinayak, S.K. Tuteja, V.A. Chhabra, N. Bhardwaj, A. Paul, K.-H. Kim, A. Deep, Graphene modified screen printed immunosensor for highly sensitive detection of parathion, Biosens. Bioelectron. 83 (2016) 339-346.

[8] M. Lecoeur-Lorin, R. Delépée, P. Morin, Simultaneous enantioselective determination of fenamiphos and its two metabolites in soil sample by CE, Electrophoresis 30 (2009) 2931-2939.

[9] B. Liu, G. Han, Z. Zhang, R. Liu, C. Jiang, S. Wang, M.-Y. Han, Shell thickness-dependent Raman enhancement for rapid identification and detection of pesticide residues at fruit peels, Anal. Chem. 84 (2011) 255-261.

[10] N. Prabhakar, H. Thakur, A. Bharti, N. Kaur, Chitosan-iron oxide nanocomposite based electrochemical aptasensor for determination of malathion, Analytica Chimica Acta 939 (2016) 108-116.

[11] G.L. Ellman, K.D. Courtney, V. Andres, R.M. Featherstone, A new and rapid colorimetric determination of acetylcholinesterase activity, Biochem. Pharmacol. 7 (1961) 88-95.

[12] J. Guo, J.X. Wong, C. Cui, X. Li, H.-Z. Yu, A smartphone-readable barcode assay for the detection and quantitation of pesticide residues, Analyst 140 (2015) 5518-5525.

[13] M. Liang, K. Fan, Y. Pan, H. Jiang, F. Wang, D. Yang, D. Lu, J. Feng, J. Zhao, L. Yang, Fe_3O_4 magnetic nanoparticle peroxidase mimetic-based colorimetric assay for the rapid detection of organophosphorus pesticide and nerve agent, Anal. Chem. 85 (2012) 308-312.

[14] J. Hou, Z. Tian, H. Xie, Q. Tian, S. Ai, A fluorescence resonance energy transfer sensor based on quaternized carbon dots and Ellman's test for ultrasensitive detection of dichlorvos, Sens. Actuators, B 232 (2016) 477-483.

[15] S. Liao, W. Han, H. Ding, D. Xie, H. Tan, S. Yang, Z. Wu, G. Shen, R. Yu, Modulated dye retention for the signal-on fluorometric determination of acetylcholinesterase inhibitor, Anal. Chem. 85 (2013) 4968-4973.

[16] Q. Long, H. Li, Y. Zhang, S. Yao, Upconversion nanoparticle-based fluorescence resonance energy transfer assay for organophosphorus pesticides, Biosens. Bioelectron. 68 (2015) 168-174.

[17] Y. He, B. Xu, W. Li, H. Yu, Silver nanoparticle-based chemiluminescent sensor array for pesticide discrimination, J. Agric. Food Chem. 63 (2015) 2930-2934.

[18] S.S. Miao, M.S. Wu, L.Y. Ma, X.J. He, H. Yang, Electrochemiluminescence biosensor for determination of organophosphorous pesticides based on bimetallic Pt-Au/multi-walled carbon nanotubes modified electrode, Talanta 158 (2016) 142-151.

[19] H. Mao, Y. Yan, N. Hao, Q. Liu, J. Qian, S. Chen, K. Wang, Dual signal amplification coupling dual inhibition effect for fabricating photoelectrochemical chlorpyrifos biosensor, Sens. Actuators, B 238 (2017) 239-248.

[20] H. Dzudzevic Cancar, S. Soylemez, Y. Akpinar, M. Kesik, S. Göker, G. Gunbas, M. Volkan, L. Toppare, A Novel Acetylcholinesterase Biosensor: Core-Shell Magnetic Nanoparticles Incorporating a Conjugated Polymer for the Detection of Organophosphorus Pesticides, ACS Appl. Mater. Interfaces 8 (2016) 8058-8067.

[21] G. Yu, W. Wu, Q. Zhao, X. Wei, Q. Lu, Efficient immobilization of acetylcholinesterase onto amino functionalized carbon nanotubes for the fabrication of high sensitive organophosphorus pesticides biosensors, Biosens. Bioelectron. 68 (2015) 288-294.

[22] H. Zhao, X. Ji, B. Wang, N. Wang, X. Li, R. Ni, J. Ren, An ultra-sensitive acetylcholinesterase biosensor based on reduced graphene oxide-Au nanoparticles-β-cyclodextrin/Prussian blue-chitosan nanocomposites for organophosphorus pesticides detection, Biosens. Bioelectron. 65 (2015) 23-30.

[23] R. Zhang, N. Li, J. Sun, F. Gao, Colorimetric and phosphorimetric dual-signaling strategy mediated by inner filter effect for highly sensitive assay of organophosphorus pesticides, J. Agric. Food Chem. 63 (2015) 8947-8954.

[24] P.D. Howes, S. Rana, M.M. Stevens, Plasmonic nanomaterials for biodiagnostics, Chem. Soc. Rev. 43 (2014) 3835-3853.

[25] K.M. Mayer, J.H. Hafner, Localized surface plasmon resonance sensors, Chem. Rev. 111 (2011) 3828-3857.

[26] L. Lu, Y. Xia, Enzymatic reaction modulated gold nanorod end-to-end self-assembly for ultrahigh sensitively colorimetric sensing of cholinesterase and organophosphate pesticides in human blood, Anal. Chem. 87 (2015) 8584-8591.

[27] K.D. Nanda, S.A. Alex, N. Chandrasekaran, A. Mukherjee, Acetylcholinesterase (AChE)-mediated immobilization of silver nanoparticles for the detection of organophosphorus pesticides, RSC Adv. 6 (2016) 64769-64777.

[28] Z. Zaheer, E.S. Aazam, Reversible encapsulation of silver nanoparticles in to the helix of amylose (water soluble starch), RSC Adv. 6 (2016) 60513-60521.

[29] S. Madhu, H.A. Evans, V.V. Doan‐Nguyen, J.G. Labram, G. Wu, M.L. Chabinyc, R. Seshadri, F. Wudl, Infinite polyiodide chains in the pyrroloperylene-iodine complex: insights into the starch-iodine and perylene-iodine complexes, Angew. Chem. Int. Ed. 55 (2016) 8032-8035.

[30] E. Redel, C. Röhr, C. Janiak, An inorganic starch-iodine model: the inorganic-organic hybrid compound $\{(C_4H_{12}N_2)_2[Cu^{I}I_4](I_2)\}_n$, Chem. Commun. 16 (2009) 2103-2105.

[31] R.C. Teitelbaum, S.L. Ruby, T.J. Marks, On the structure of starch-iodine, J. Am. Chem. Soc. 100 (1978) 3215-3217.

[32] J. Colin, H. de Claubry, On the reactions of iodine with vegetable and animal materials, Ann. Chim 90 (1814) 87-100.

[33] N. Alagirisamy, S.S. Hardas, S. Jayaraman, Novel colorimetric sensor for oral malodour, Anal. Chim. Acta 661 (2010) 97-102.

[34] S. Dutta, N. Mandal, D. Bandyopadhyay, Paper-based α-amylase detector for point-of-care diagnostics, Biosens. Bioelectron. 78 (2016) 447-453.

[35] K. Kawai, H. Kodera, T. Majima, Photocatalytic formation of I-I bonds using DNA which enables detection of single nucleotide polymorphisms, J. Am. Chem. Soc. 132 (2010) 14216-14220.

[36] J. Nie, T. Brown, Y. Zhang, New two dimensional liquid-phase colorimetric assay based on old iodine-starch complexation for the naked-eye quantitative detection of analytes, Chem. Commun. 52 (2016) 7454-7457.

[37] Y. Zhang, D. Gao, J. Fan, J. Nie, S. Le, W. Zhu, J. Yang, J. Li, Naked-eye quantitative aptamer-based assay on paper device, Biosens. Bioelectron. 78 (2016) 538-546.

[38] M.-P.N. Bui, A. Abbas, Simple and rapid colorimetric detection of p-nitrophenyl substituent organophosphorous nerve agents, Sens. Actuators, B 207 (2015) 370-374.

[39] T. Liu, X. Zhang, J. Hao, W. Zhu, W. Liu, D. Zhang, J. Wang, Acetylcholinesterase-free colorimetric detection of chlorpyrifos in fruit juice based on the oxidation reaction of H_2O_2 with chlorpyrifos and $ABTS^{2-}$ catalyzed by hemin/G-quadruplex DNAzyme, Food Anal. Methods 8 (2015) 1556-1564.

[40] A. Mishra, J. Kumar, J.S. Melo, An optical microplate biosensor for the detection of methyl parathion pesticide using a biohybrid of Sphingomonas sp. cells-silica nanoparticles, Biosens. Bioelectron. 87 (2017) 332-338.

[41] J.V. Rohit, H. Basu, R.K. Singhal, S.K. Kailasa, Development of p-nitroaniline dithiocarbamate capped gold nanoparticles-based microvolume UV-vis spectrometric method for facile and selective detection of quinalphos insecticide in environmental samples, Sens. Actuators, B 237 (2016) 826-835.

Caption:

Scheme 1. Principle of colorimetric biosensor for the assay of organophosphorus pesticides

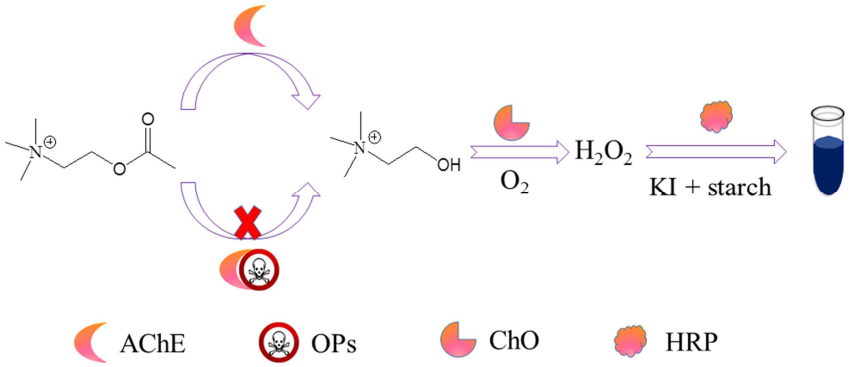
Figure 1. Absorption spectra and photos of mixture solutions

Reaction conditions: (a) 50 μL AChE (0.04 U mL^{-1}), 50 μL PBS (pH 7.4, 10 mmol L^{-1}) and 50 μL H_2O were mixed and incubated for 30 min, then 100 μL ACh (500 μmol L^{-1}) and 50 μL ChO (2 U mL^{-1}) were added and further incubated for 30 min, followed by adding 50 μL KI (100 mmol $L^{-1)}$, 50 μL HRP (3.6 μg mL^{-1}) and 50 μL starch (1%); (b) and (c) were under the same reaction conditions as (a) but without addition of HRP, AChE, respectively. (d) was under the same reaction condition as (a) but incubation AChE with 50 μL paraoxon (1 mg L^{-1}) in PBS buffer in advance.

Figure 2. (A) Absorption spectra of mixture solution in the presence of different concentrations of paraoxon (inset: corresponding photo of each mixture solution) and (B) the relative absorbance (ΔAbs) versus paraoxon concentration (inset: the linear calibration curve)

Table 1. Recovery experiments of spiked paraoxon in vegetable irrigation water samples (each result was the average of three determinations)

Sample No.	Paraoxon (ppb)		Recovery (%)
	Add	Found (Mean ± SD)	
1	10.0	11.0 ± 1.7	110%
2	40.0	40.7 ± 5.2	101%
3	80.0	70.5 ± 0.5	88%



Scheme 1.

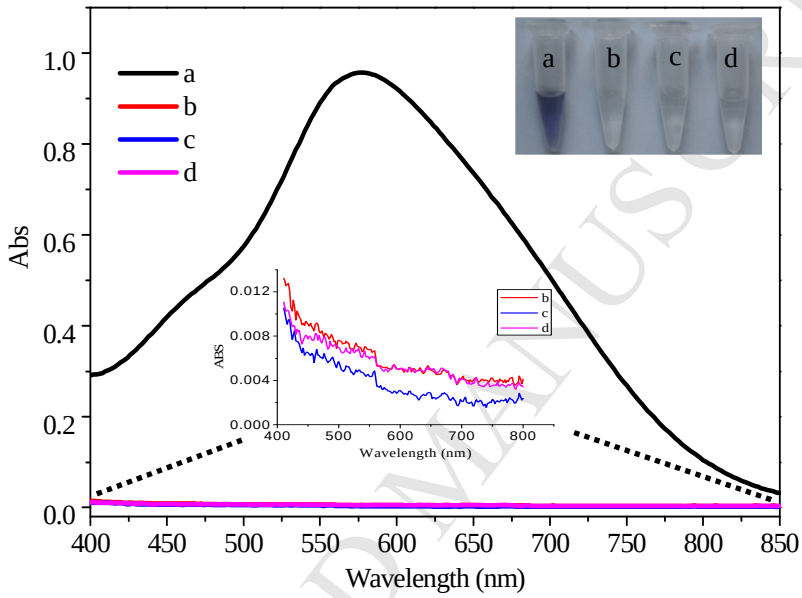


Figure 1.

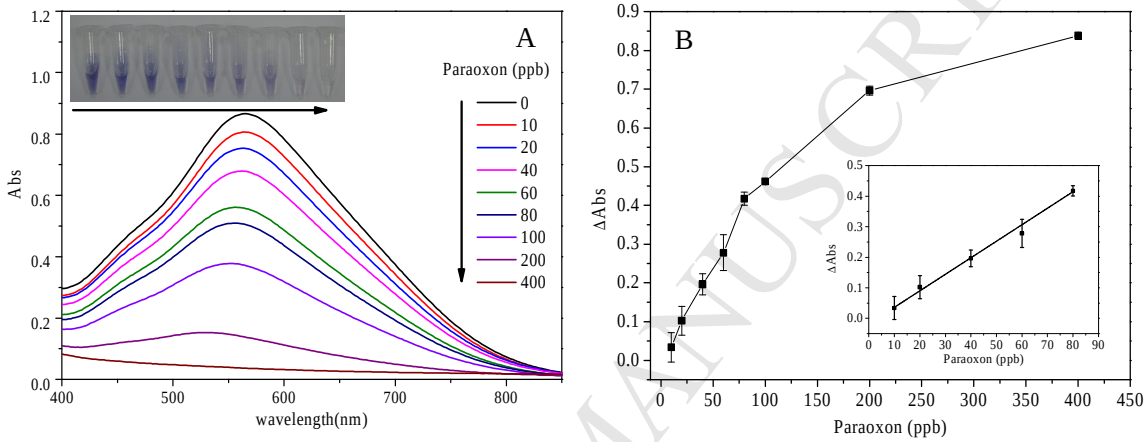


Figure 2.

Highlight

►The iodine-starch color reaction was applied for colorimetric assay of paraoxon.

► The assay of paraoxon can be performed in homogenous solution without the need of reagent synthesis and enzyme immobilization.

►A colorimetric assay for paraoxon was achieved with a limit of detection 4.7 ppb.

►This biosensor has the potential of on-site assay of OPs residues in environmental samples.

Colorimetric biosensor for the assay of paraoxon in environmental water samples based on the iodine-starch color reaction

Liangqia Guo*, Zhihong Li, Huan Chen, Yarong Wu, Ling Chen, Zhiping Song, Tianran Lin

Ministry of Education Key Laboratory of Analysis and Detection for Food Safety, Fujian Provincial Key Laboratory of Analysis and Detection Technology for Food Safety, College of Chemistry, Fuzhou University, Fuzhou 350116, China

Email:lqguo@fzu.edu.cn

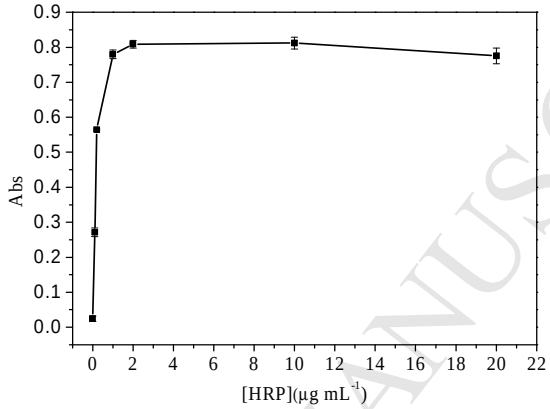


Figure S1. Effect of HRP concentration on the absorbance of iodine-starch complexes

Reaction condition: 50 μL KI (100 mmol L^{-1}) + 50 μL H_2O + 50 μL PBS (pH 7.4, 10 mmol L^{-1}) + 200 μL H_2O_2 (60 μmol L^{-1}) + 50 μL HRP + 50 μL starch (1%)

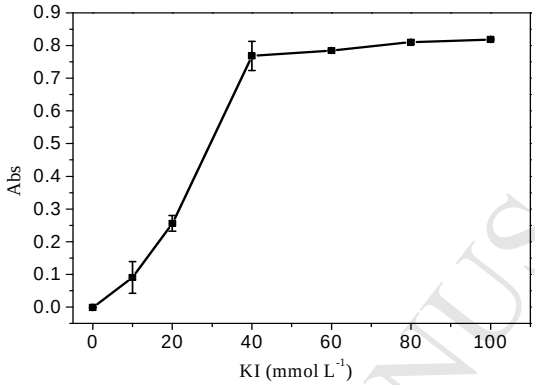


Figure S2. Effect of KI concentration on the absorbance of iodine-starch complexes

Reaction condition: 50 μL KI + 50 μL H_2O + 50 μL PBS (pH 7.4, 10 mmol L^{-1}) + 200 μL H_2O_2 (60 μmol L^{-1}) + 50 μL HRP (2 μg mL^{-1}) + 50 μL starch (1%)

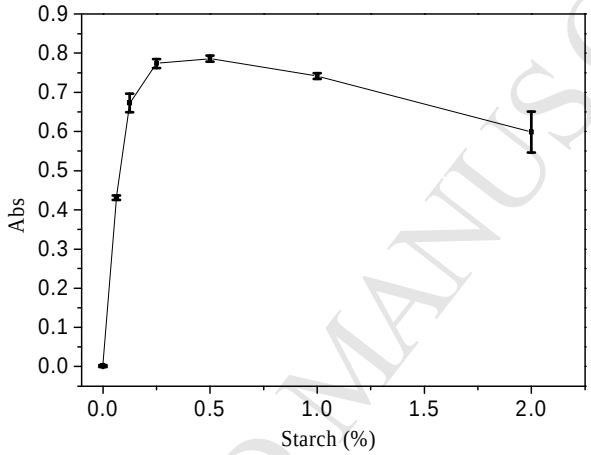


Figure S3. Effect of starch concentration on the absorbance of iodine-starch complexes

Reaction condition: 50 μL KI (40 mmol L^{-1}) + 50 μL H_2O+ 50 μL PBS (pH 7.4, 10 mmol L^{-1}) + 200 μL H_2O_2 (60 μmol L^{-1}) + 50 μL HRP (2 μg mL^{-1}) + 50 μL starch

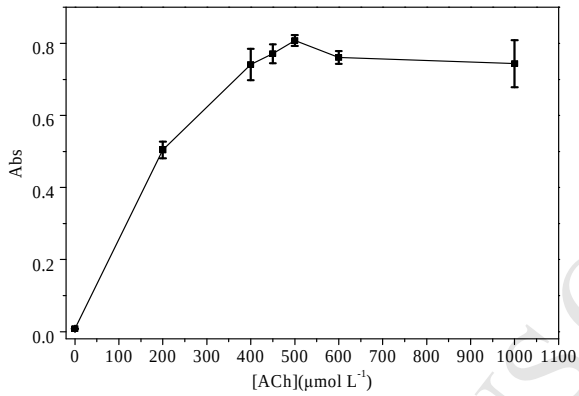


Figure S4. Effect of ACh concentration on the absorbance of iodine-starch complexes

Reaction condition: 50 μL AChE (0.05 U mL^{-1}), 50 μL PBS (pH 7.4, 10 mmol L^{-1}) and 50 μL H_2O were mixed and incubated for 30 min, then 100 μL ACh with different concentration and 50 μL ChO (2 U mL^{-1}) were added and further incubated for 30 min, followed by adding 50 μL KI (40 mmol L^{-1}), 50 μL HRP (2 μg mL^{-1}) and 50 μL starch (0.5%)

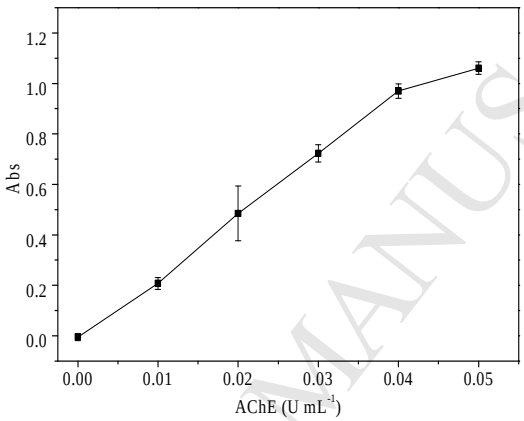


Figure S5. Effect of AChE concentration on the absorbance of iodine-starch complexes

Reaction condition: 50 μL AChE with different concentration, 50 μL PBS (pH 7.4, 10 mmol L^{-1}) and 50 μL H_2O were mixed and incubated for 30 min, then 100 μL ACh (500 μmol L^{-1}) and 50 μL ChO (2 U mL^{-1}) were added and further incubated for 30 min, followed by adding 50 μL KI (40 mmol L^{-1}), 50 μL HRP (2 μg mL^{-1}) and 50 μL starch (0.5%)

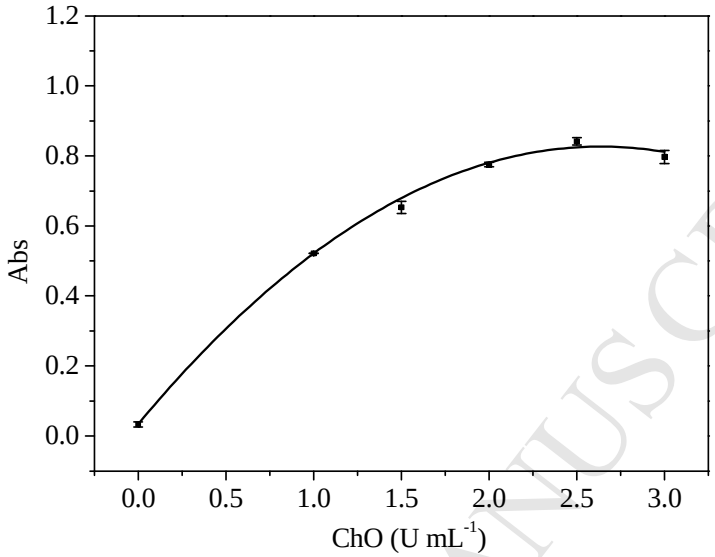


Figure S6. Effect of ChO concentration on the absorbance of iodine-starch complexes

Reaction condition: 50 μL AChE (0.03 U mL^{-1}), 50 μL PBS (pH 7.4, 10 mmol L^{-1}) and 50 μL H_2O were mixed and incubated for 30 min, then 100 μL ACh (500 μmol L^{-1}) and 50 μL ChO with different concentration were added and further incubated for 30 min, followed by adding 50 μL KI (40 mmol L^{-1}), 50 μL HRP (2 μg mL^{-1}) and 50 μL starch (0.5%)

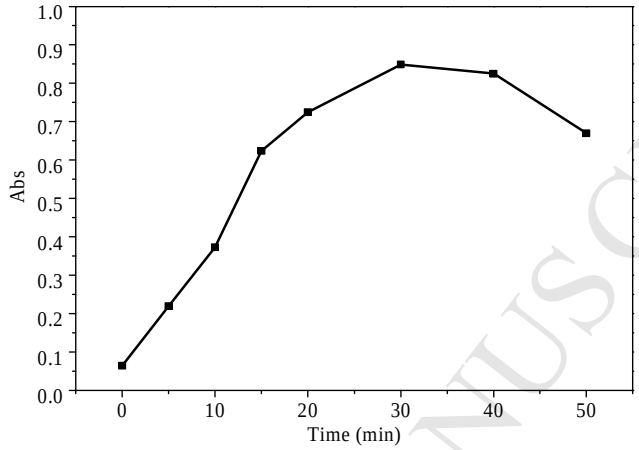


Figure S7. Effect of incubation time of enzyme catalytic reaction on the absorbance of iodine-starch complexes

Reaction condition: 50 μL AChE (0.03 U mL^{-1}), 50 μL PBS (pH 7.4, 10 mmol L^{-1}) and 50 μL H_2O were mixed and incubated for 30 min, then 100 μL ACh (500 μmol L^{-1}) and 50 μL ChO (2 U mL^{-1}) were added and further incubated for a certain time, followed by adding 50 μL KI (40 mmol L^{-1}), 50 μL HRP (2 μg mL^{-1}) and 50 μL starch (0.5%)

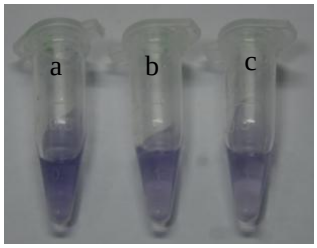


Figure S8. Photos of mixture solution for the assay of paraoxon in vegetable irrigation water sample spiked with 10, 40, 80 ppb of paraoxon, respectively.