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Review

Recent approaches to improving selectivity and sensitivity of enzyme-based biosensors for organophosphorus pesticides: A review

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ABSTRACT

Pesticide determination has attracted great attention due to the fact that they exhibit high acute toxicity and can cause long-term damage to the environment and human lives even at trace levels. Although classical analytical methods (including gas chromatography, high performance liquid chromatography, capillary electrophoresis and mass spectrometry) have been effectively used for analysis of pesticides in contaminated samples, they present certain limitations such as time-consuming sample preparation, complexity, and the requirement of expensive instrumentation and highly skilled personnel. For these reasons, there is an expanding need for analytical methods able to provide simple, rapid, sensitive, selective, low cost and reliable detection of pesticides at trace levels. Over the past decades, acetylcholinesterase (AChE) biosensors have emerged as simple, rapid and ultra-sensitive tools for toxicity detection of pesticides in the environment and food. These biosensors have the potential to complement or replace the classical analytical methods by simplifying or eliminating sample preparation and making field-testing easier and faster with significant decrease in cost per analysis. With the recent engineering of more sensitive AChE enzymes, the development of more reliable immobilization matrices and the progress in the area of microelectronics, AChE biosensors could become competitive for multi-analyte screening and soon be used for the development of portable instrumentation for rapid toxicity testing of samples. The enzymes organophosphorus hydrolase (OPH) and organophosphorus acid anhydrolase (OPAA) have also shown considerable potential in OP biosensor applications and they have been used for direct detection of OPs. This review presents the recent advances in the fabrication of enzyme biosensors for organophosphorus pesticides (OPs) and their possible applications for toxicity monitoring of organophosphorus pesticide residues in real samples. The focus will be on the different strategies for the biosensor construction, the analytical performance of the biosensors and the advantages and disadvantages of these biosensor methods. The recent works done to improve the analytical performance, sensitivity and selectivity of these biosensors will also be discussed.

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1. Introduction

Pesticides (e.g. insecticides, herbicides, and fungicides) are widely used in agriculture to eliminate or control a variety of agricultural pests that can damage crops and livestock and reduce farm productivity. Although pesticides are directly applied to plants and soils, only 1% of pesticide sprayed is delivered to the intended target [1]. Accidental release of pesticides due to spills, leaking pipes, underground storage tanks, and waste dumps may lead to their persistence in the environment for a long period of time [1]. As a result, pollution due to the uncontrolled use of these pesticides has become one of the most alarming challenges.

The organophosphorus pesticides (OPs) are synthetic esters, amides, or thiol derivatives of the phosphoric, phosphonic, phosphorothioic, or phosphonothioic acids [2,3] which are used extensively to control agricultural, household and structural pests globally. OPs break down quickly when exposed to light and air, are less persistent in the environment, are not subject to bioaccumulation and biomagnification, and do not release toxic break down products [2]. These features justify their application in the agricultural and veterinary practices of the modern world [2]. Consequently, they are favored over organochlorine (OC) pesticides such as DDT. However, it is not known whether OPs ever degrade fully since they have been detected in soil and drinking water long after application [4].

OPs are among the most acutely toxic pesticides and their residues in the environment can cause long-term damage to human health even at trace levels. They belong to the toxicity class I (highly toxic) or toxicity class II (moderately toxic), according to the EPA classification [5]. The toxicity of OPs is based on the inhibition of the enzyme acetylcholinesterase (AChE, EC 3.1.1.7), which is essential for the functioning of the central nervous system of mammals and insects. This results in the accumulation of acetylcholine (Ach) neurotransmitter, which interferes with muscular responses and causes respiratory and myocardial impairment and even death [6]. Although exposure to a single compound or active ingredient may not exceed the level considered to be without acceptable risk for either humans or environmental species, concurrent exposures to numerous OP compounds could exceed a safe level because of increased AChE inhibition. The toxicity of OPs is reported to vary considerably, depending on the chemical structure of the pesticide [6].

Due to the high acute toxicity of the OPs [4,7], as well as the registered chronic effects [4], the OP residue limits in food, drinking water and environmental samples are subject of regulation and control [2,4]. As a result, their rapid detection and reliable quantification has become increasingly necessary. Numerous analytical methods including gas chromatography (GC), liquid chromatography (LC), high-performance liquid chromatography (HPLC), mass spectrometry (MS), capillary electrophoresis (CE), surface plasmon resonance (SPR), fluorimetry, ultraviolet spectroscopy and GC–MS have been developed for analysis of OPs in contaminated samples [2,5,6]. Although these methods have been used successfully for the detection of OPs, they present several disadvantages due to the fact that they are time-consuming, require sample pre-treatment, expensive instrumentation, highly skilled personnel and they can only be used in laboratories. For these reasons there is an expanding need for field deployable analytical methods able to provide simple, rapid, sensitive, selective, low cost and reliable detection of OPs at low concentrations.

Numerous biosensors have been developed over the last decades in an attempt to satisfy these needs. Among the different types of biosensors, the enzyme-based electrochemical biosensors are especially desirable for field applications since they are easy to manufacture and deploy [8]. Furthermore, it is possible to miniaturize the required instrumentation providing compact and portable analysis devices, and they can potentially be engineered to be highly selective and sensitive [9]. These biosensors are well documented and have emerged in the past few years as the most promising alternative to detect OPs [8]. The applied electrochemical transduction mode of the electrochemical biosensors is commonly potentiometric or amperometric [10]. The potentiometric determinations are based on the measurement of the emf of a galvanic element, constituted by an indicator and a reference electrode [10]. The potential of the indicator electrode depends on the analyte concentration, according to the Nernst equation, while the potential of the reference electrode remains constant. The exponential character of the relationship between the potential of the indicator electrode and the analyte concentration defines the wide dynamic concentration range determinations (3–4 decades), but also defines the low accuracy and precision of the method [10]. The amperometric determinations involve the measurement of the current response of an indicator electrode, as a function of the concentration of the electroactive species present in solution, at a constant potential [10]. The amperometric detection normally presents advantages such as high sensitivity, precision, linearity of the calibration plot and the ability to control the process through the electrode potential [10]. This review therefore focuses more on the amperometric detection method.

The most commonly reported electrochemical OP biosensors are based on indirect detection by AChE enzyme. A large number of documents including several review articles on AChE-based electrochemical biosensors for the detection of OP compounds are available in literature, with limits of detection (LOD) ranging from micro-molar to pico-molar levels depending on the source of AChE enzyme, technique, and transduction mechanism employed [10]. Various amperometric and potentiometric AChE-based biosensors have been reported. The potentiometric AChE biosensors detect the pH shift resulting from the decrease (in the presence of OP pesticides) of the acid released during the enzyme catalyzed hydrolysis of the choline esters [10]. The amperometric AChE biosensors are based on the measurement of the change in concentration of the electroactive product thiocholine, produced as a result of hydrolysis of acetylthiocholine. When OPs are present, AChE is inhibited and, therefore, less thiocholine is produced [6]. The conventional or native AChE-based biosensors have LOD comparable to the classical laboratory techniques listed previously, but they have limitations such as poor selectivity, sensitivity and long analysis time due to the incubation period. In addition to sensitivity and selectivity, another difficulty of conventional AChE biosensors is the poor resolution of pesticide mixtures. They provide information on a family of compounds and not on individual compound in a family. In this sense, the use of recombinant AChE has been reported allowing dramatic enhancement of the sensitivity of these devices [9,11]. Although these biosensors show a high sensitivity, they often lack selectivity due to the fact that they react with any cholinesterase inhibitor. To address the selectivity issue, different AChE variants having different sensitivities and selectivities for various OPs have been used in array based sensors for discrimination between the different OPs [9]. The arrays of

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