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Photoelectrochemical enzymatic biosensors

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ABSTRACT

Enzymatic biosensors have been valuable bioanalytical devices for analysis of diverse targets in disease diagnosis, biological and biomedical research, etc. Photoelectrochemical (PEC) bioanalysis is a recently emerged method that promptly becoming a subject of new research interests due to its attractive potential for future bioanalysis with high sensitivity and specificity. PEC enzymatic biosensors integrate the inherent sensitivities of PEC bioanalysis and the selectivity of enzymes and thus share their both advantages. Currently, PEC enzymatic biosensors have become a hot topic of significant research and the recent impetus has grown rapidly as demonstrated by increased research papers. Given the pace of advances in this area, this review will make a thorough discussion and survey on the fundamentals, sensing strategies, applications and the state of the art in PEC enzymatic biosensors, followed by future prospects based on our own opinions. We hope this work could provide an accessible introduction to PEC enzymatic biosensors for any scientist.

1. Introduction

In 1956, the invention of oxygen electrode by Leland C. Clark, Jr. has ushered in a new era in biosensor field and also established himself as the father of the biosensor concept (Clark and Lyons, 1962; Clark, 1956). In Clark oxygen electrode, the glucose was entrapped by a dialysis membrane, and the decreased concentration of measured oxygen was proportional to glucose concentration. This work was so successful that it catalyzed numerous variations using similar design and many other (oxidase) enzymes. Since this pioneering work, the biosensor field has grown enormously and, especially, the enzymatic biosensors have been one of the most frequently applied in the biomedical field (Turner et al., 1987; Wilson and Hu, 2000). Currently, enzymatic biosensors have been valuable bioanalytical devices for qualitative and quantitative analysis of diverse targets in disease diagnosis, biological and biomedical research, food safety and environmental monitoring. In a typical enzymatic biosensor, the enzyme is the most essential component since it offers the selectivity, and enzyme electrodes thus combine the high specificity of the enzymes with the particular advantages of electrochemical detection, such as high sensitivity, low cost and simple instrumentation (Ronkainen et al., 2010; Thevenot et al., 1999). Historically, the enzyme electrodes can be divided into three generations, i.e., the first generation of oxygen-based, the second generation of mediator-based, and the third generation of direct electrochemistry-based. Conventional electrochemical enzymatic biosensors have been discussed in several excellent reviews, and interested readers are referred to these well-documented literatures (Kimmel et al., 2012; Privett et al., 2008, 2010; Zhu et al., 2015).

With the ever-increasing needs for ultrasensitive bioanalysis, the analytical chemists have always been pursuing advanced bioanalytical modalities compatible with future requirements (Chen et al., 2016a; Li and Shi, 2014). Among various techniques, photoelectrochemical (PEC) bioanalysis is a recently appeared method that promptly becoming a subject of new research interests due to its attractive potential for future bioanalysis with high sensitivity and specificity (Freeman et al., 2013; Gill et al., 2008; Yue et al., 2013). Essentially, the PEC bioanalysis is the advanced generation of the traditional electrochemical bioanalysis. So it naturally inherits the merits of electrochemical bioanalysis, but possesses higher sensitivity due to its unique PEC set-up consisting of total separated energy forms of light and electricity as excitation source and detection signal, respectively (Lisdat et al., 2013; Zhao et al., 2015b). Besides, due to the use of an electronic readout, the instruments of PEC bioanalysis are also generally simpler, cheaper, and easier to miniaturize than those of optical modalities. Specifically, the PEC instrument system generally includes an excitation source (irradiation light, a monochromator, and a chopper), a cell, and an electrochemical workstation with a three-electrode system (working, reference and counter electrodes). Especially, the working electrode comprises of various semiconductor materials that connected to a metal contact and then to the external electronics and eventually to a metal counter electrode. Concurrently, the semiconductor-based working electrode is also connected to the

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counter electrode via the solution that integrates the circuit. Based on such a system, the studied biochemical information (e.g. the analyte concentration) associated with a specific biorecognition event could be elegantly converted by the semiconductors to the output electrical signals. About the semiconductors, common organic/inorganic ones, e.g. tris(bipyridyl) ruthenium complex $[Ru(bpy)_3]^{2+}$(bpy=2,20-bipyridine) and CdS quantum dots (QDs), are frequently used in reported works. With the development of this technique, inorganic semiconductor nanomaterials and nanostructures such as TiO_2/ZnO/Cu_2O nanoparticles (NPs)/nanowires (NWs)/nanorods (NRs)/nanocones (NCs)/nanosheets (NSs)/nanoclusters (NCls)/nanoporous films, and organic semiconductor species such as porphyrin and its derivatives, phthalocyanine and its derivatives, azo dyes, chlorophyll, bacteriorhodopsin, and polymers such as phenylenevinylene (PPV), poly(thiophene), and their derivatives, as well as diverse semiconductor-based hybrids such as CdS/TiO_2, Au NPs/TiO_2, porphyrin/ZnO, CdS/grapheme have also been gradually exploited for exquisite PEC bioanalysis. As to the biological recognition elements, in addition to the usual ones (i.e., DNA, antibody and enzyme), some other bioactive materials (e.g., anticalins, affibodies and nanobodies) and recognition elements (e.g., whole cells, phages and molecularly-imprinted polymers) will further offer unique platforms for future developments of novel PEC bioanalysis. Due to its obvious advantages, PEC bioanalysis has been dynamically developing and extensively studied for innovative biomolecular probing, especially in the areas of DNA analysis (Zhao et al., 2014b), immunoassay as well as enzymatic biosensing. In fact, limitations and hurdles are also associated with this technique, for example, the common photoactive species are subject to low photo-to-current conversion efficiencies and susceptibilities to photobleaching. Besides, the established signaling mechanisms are still highly limited and it remains a challenge to implement these protocols in real-world applications. For more information on PEC bioanalysis, readers could use many recent reviews and the references therein to pursue their interest in this field (Devadoss et al., 2015; Freeman et al., 2013; Gill et al., 2008; Lisdat et al., 2013; Tang et al., 2015; Yue et al., 2013; Zhang et al., 2013; Zhao et al., 2014b, 2015b, 2016a, 2016b; Zhou et al., 2015a).

PEC enzymatic biosensors, a new subclass of enzymatic biosensors, integrate the inherent sensitivities of PEC bioanalysis and the selectivity of enzymes and thus share their both advantages. Ever since the very beginning of PEC bioanalysis, the study on enzymatic biosensors has been a focus of significant research due to its relevant importance. In typical PEC enzymatic biosensors, the PEC enzymatic systems, upon irradiation, could convert the specific biocatalytic events into electrical signals via the interactions between the semiconductor species and the biocatalyzed reaction chain. Currently, along with PEC DNA biosensors, PEC enzymatic biosensors has also become a hot topic in this area and the recent impetus has grown rapidly for the advanced PEC enzymatic biosensors, as demonstrated by increased research papers (Guo et al., 1995; Han et al., 2013; Huang et al., 2005; Lee et al., 2016; Liu et al., 2007, 2014b; Ren et al., 2009; Stoll et al., 2006; Swainsbury et al., 2014; Umar et al., 2009; Zayats et al., 2003; Zhang et al., 2015; Zhou et al., 2005; Zhu et al., 2007). Previously, we have illustrated the state of the art in the broad field of PEC bioanalysis, within which the recent progresses in the subfield of enzymatic biosensing were discussed briefly (Zhao et al., 2015b). Beyond that, no effort has yet been made for addressing specifically on the PEC enzymatic biosensors. On the other hand, the summary about the PEC DNA biosensors has been presented in an in-depth work and has been well-received previously (Zhao et al., 2014b). Given the pace of advances in PEC enzymatic biosensors, this review, using the selected typical examples, will make a thorough discussion and survey on the fundamentals, sensing strategies, applications and the state of the art in PEC enzymatic biosensors, followed by future prospects in this area based on our own opinions. This work will serves as a useful source to inform the interested audience of the developments in PEC enzymatic biosensors.

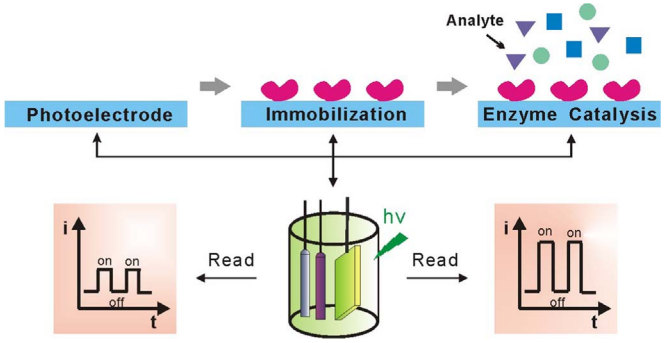

Fig. 1. General PEC enzymatic biosensor design. The major process involves the enzyme immobilization on the photoelectrode substrate as the recognition elements for the subsequent biocatalytic transformation.

2. PEC enzymatic biosensors

PEC enzymatic biosensors principally involves tracking the electrical signal prior to and after the enzymatic transformation. In a typical configuration, as shown in Fig. 1, the biorecognition elements of enzymes are initially confined onto the electrode surfaces, and the subsequent biocatalytic events would then be recorded by the electrical signals. This section will first introduce the fundamentals of enzymes and common enzymatic reactions (Section 2.1), and then summarize the enzyme immobilization (Section 2.2), followed by the discussion of PEC enzymatic biosensors according to the respective configurations, i.e. first generation (Section 2.3), second generation (Section 2.4), third generation (Section 2.5) and other (Section 2.6) type. Incidentally, the detailed summary about the PEC transducers have been well-documented and will not be discussed here, interested readers are referred to comprehensive reviews in this topic (Devadoss et al., 2015; Tang et al., 2015; Zhang et al., 2013; Zhao et al., 2015a; Zhou et al., 2015a).

2.1. Common enzymes

Enzymes, generally globular proteins composed of linear chains of amino acids that fold into a 3D structure, are highly efficient macromolecular biological catalysts that could accelerate, or catalyze chemical reactions. Different from most other catalysts, the sequences of the amino acids specify the unique 3D structures of enzymes and in turn endow them much high ***catalytic activities*** and ***specificities***, making them selective for one type of ***substrates*** molecules. In enzymes, the ***active sites***, usually a groove or pocket of the enzymes where substrate molecules bind and undergo a chemical reaction, consist of one or more ***binding sites*** where residues orient the substrates via forming temporary bonds and the neighboring ***catalytic sites*** where residues catalyze specific reactions of substrates and convert them into ***products*** in normal catalytic cycles. The enzyme–substrate interactions can be characterized by ***Michaelis–Menten kinetics***. The active sites can catalyze the specific chemical reactions repeatedly as their residues are not altered at the end of the reaction, while the remaining enzyme structure serve to maintain the precise orientation and dynamics of the active sites. Enzyme structures may also contain ***allosteric sites*** where the binding of a small molecule could induce a conformational change that increases or decreases activity. Comparing with those enzymes free of additional components to show full activity, some other enzymes necessitate non-protein ***cofactors*** to be bound for activity. Cofactors can be either inorganic (e.g., metal ions Mg^{2+}, Cu^{+}, Mn^{2+}, or iron-sulfur clusters), or organic compounds (e.g., flavin and heme) that could sometimes further divided into ***coenzymes*** and ***prosthetic groups***. Coenzymes, small organic molecules (e.g., dihydronicotinamide adenine dinucleotide (NADH), NADPH, biotin, lipoamide, flavin adenine dinucleotide (FAD), and adenosine triphosphate (ATP)) that can be loosely or