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Biosensors and Bioelectronics

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Recent advances on developing 3rd generation enzyme electrode for biosensor applications



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ARTICLE INFO

Article history: Received 16 September 2015 Received in revised form 17 December 2015 Accepted 18 December 2015 Available online 19 December 2015

Keywords:
Direct electrochemistry
Enzyme electrode
3rd generation biosensors
Protein film voltammetry
Cyclic voltammetry
Electron transfer rate constant

ABSTRACT

The electrochemical biosensor with enzyme as biorecognition element is traditionally pursued as an attractive research topic owing to their high commercial perspective in healthcare and environmental sectors. The research interest on the subject is sharply increased since the beginning of 21st century primarily, due to the concomitant increase in knowledge in the field of material science. The remarkable effects of many advance materials such as, conductive polymers and nanomaterials, were acknowledged in the developing efficient 3rd generation enzyme bioelectrodes which offer superior selectivity, sensitivity, reagent less detection, and label free fabrication of biosensors. The present review article compiles the major knowledge surfaced on the subject since its inception incorporating the key review and experimental papers published during the last decade which extensively cover the development on the redox enzyme based 3rd generation electrochemical biosensors. The tenet involved in the function of these direct electrochemistry based enzyme electrodes, their characterizations and various strategies reported so far for their development such as, nanofabrication, polymer based and reconstitution approaches are elucidated. In addition, the possible challenges and the future prospects in the development of efficient biosensors following this direct electrochemistry based principle are discussed. A comparative account on the design strategies and critical performance factors involved in the 3rd generation biosensors among some selected prominent works published on the subject during last decade have also been included in a tabular form for ready reference to the readers.

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Contents

1.	Introduction	386
2.	Principles on 3rd generation biosensor	387
	Characterization of the 3rd generation biosensor	
4.	Fabrication strategies and applications of the 3rd generation biosensor	389
	4.1. Nanofabrication of electrode	389
	4.2. Polymer based approach	391
	4.3. Self-assembled approach.	391
	4.4. Reconstitution based approach	392
5.	Problems and challenges for developing 3rd generation biosensors.	393
6.	Conclusion and future perspectives for developing 3rd generation biosensors	396
	knowledgments	
	erences	

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1. Introduction

Fast, specific and sensitive transduction of biochemical signals for quantitative or semi-quantitative detection of analytes of interest are the vital functional factors for a biosensor to be used for practical applications. The amperometric transducer-based biosensors are widely acclaimed not only for their inherent potential to exhibit these functional properties but also for bearing the scope of scaling down their size with tailored low production cost, easy fabrication, and simple operation with low or no sample loss (Wilson, 2005). However, the operational principle and the design approach being utilized to develop amperometric biosensors may largely influence the aforesaid functional entities and annexed characteristics. Facile electron transfer between the biocatalytic reaction and electrode is necessary to improve the functional property of the bioelectronic device. The amperometric transducer based biosensors function by the production of a current when a potential is applied on the working electrode in an electrochemical setup in response to the analyte of interest.

Enzyme-based amperometric biosensor comprises immobilized/ confined enzyme(s) (mostly oxidoreductase) as the chemically selective layer over a highly conductive support material/matrix acting as electrode to transduce biochemical signal to electrical one under the influence of a suitable applied potential (Hirst and Stevens, 1985; Willner et al., 2006). If the signal/response accrued based on the electro-activity, primarily of co-substrate, product or co-product of the enzyme catalyzed reaction the category of the biosensor is termed as 1st generation, due to its primitive in nature. The first of its kind is the Clark oxygen electrode based glucose biosensor (Clark and Lyons, 1962). There are many drawbacks of the 1st generation biosensors such as, technical difficulty of maintaining air-tight sample chamber (if oxygen is used as redox indicator), and need of high redox potential for the redox indicator (e.g. $\sim +600$ mV versus SCE at Pt electrodes to oxidize H₂O₂) sometimes affects the specificity of the constructed biosensor. Coupling of electrons between the redox active centers of the enzyme and the electrode via some specialized small electroactive molecules to generate the response constitutes the 2nd generation biosensors. These specialized molecules are referred to as 'electron transfer mediators' (ETM), which shuttles electrons between the redox center of the enzyme and the electrode, at comparatively low over potential. The ETM also surpasses the role of molecular oxygen to take-up the electron from the reactive center of many redox enzymes catalyzing the aerobic oxidations of substrates (Gilardi et al., 1994). However, the leaching susceptibility of the soluble mediator to the sample solution, diffusion barrier of the mediator between enzymeelectrode interface, are some of the drawbacks affecting stability and reproducibility of the 2nd generation biosensors that prompted to explore 3rd generation biosensors. The biosensors of the 3rd generation category involve direct electrical communication between the redox centre of the enzyme and the electrode to generate the response (Ghindilis et al., 1997). These biosensors are characterized by highselectivity and sensitivity, as they can operate in a potential window closer to the redox potential of the enzyme and the electron exchange between the redox centre of the enzyme and the electrode takes place without any diffusion barrier due to proximity of these two terminals (Gorton et al., 1999). This principle of direct electrochemistry has been known for over 30 years (Berezin et al., 1978; Tarasevich, 1979) and is also useful in identifying various distinctive properties of enzymes. One prominent application is the determination of redox potentials especially, where these relate to thermodynamically inaccessible or kinetically reactive species for which potentiometric methods are not suitable (Armstrong et al., 1988). The attractive feature of DET based biosensors is the possibility to regulate the desired properties through protein modification or interfacial engineering, which are pioneered and developed by Willner's group (Willner et al., 1996, 2006; Willner and Katz, 2000; Zayats et al., 2005) etc, Dong's group (Chen et al., 2007; Chi et al., 1994; Dong and Guo, 1995; Dong and Chi, 1992; Jiang et al., 2006; Jin et al., 2003) and Gooding's group (Ciampi and Gooding, 2010; Gooding et al., 2003; Liu et al., 2006a; Liu and Gooding, 2006) etc. Examples of different enzymes involved in DET include cytochrome c, glucose oxidase (GOD), azurin, multicopper oxidases (e.g. laccase, ascorbate oxidase, ceruloplasmin, bilirubin oxidase (BOD)) and several peroxidases (microperoxidase, horseradish peroxidase (HRP) etc). The conventional configurations of three generations of the biosensor are illustrated in Fig. 1.

The present review article summarizes the principle, characterization, and recent advancement in the fabrication strategies of the 3rd generation enzyme electrode for amperometric biosensors application. Effort has been made to incorporate major review papers that largely cover the development on the subject since its inception. The challenges and future perspectives for developing 3rd generation biosensors are also highlighted here.

2. Principles on 3rd generation biosensor

The direct electron transfer (DET) between the redox centre of enzyme and the electrode is the central requirement to the 3rd generation biosensors. Hence, the contact of the redox enzyme with the conductive electrode is essential to facilitate electron exchange. The feasibility of electron exchange between the redox centers of proteins and the electrodes may be explained by the electron-transfer (ET) theory of Marcus (Marcus and Sutin, 1985). The ET rate constant ($K_{\rm ET}$) between a donor and acceptor pair is given by Eq. (1), where, d and d° are the distance separating the electron and donor, and the van der Waals distance, respectively, β is the electron-coupling constant and ΔG° and λ are the free energy change and the reorganization energy accompanying the electron-transfer process, respectively.

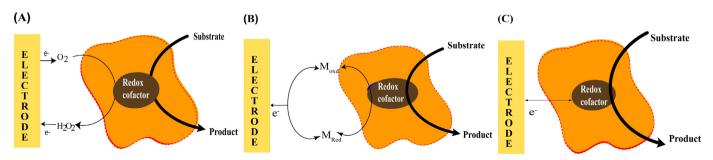


Fig. 1. Response mechanisms of different generations of amperometric enzyme biosensors, A: 1st generation biosensors where primarily, co-substrate/co-product is used as redox indicator, B: 2nd generation biosensors where artificial redox mediator is used to relay the electrons, and C: 3rd generation biosensors where direct electron transfer between enzyme and the electrode is established to generate the response.

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