Biosensors and Bioelectronics ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Engineering the bioelectrochemical interface using functional nanomaterials and microchip technique toward sensitive and portable electrochemical biosensors

Xiaofang Jia, Shaojun Dong, Erkang Wang*

State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, China

ARTICLE INFO

Article history:
Received 30 March 2015
Received in revised form
13 May 2015
Accepted 14 May 2015

Keywords:
Electrochemical
Biosensors
Nanomaterials
Microfluidic chip
Aptamer
Cytosensor
Enzyme
Biofuel cell

ABSTRACT

Electrochemical biosensors have played active roles at the forefront of bioanalysis because they have the potential to achieve sensitive, specific and low-cost detection of biomolecules and many others. Engineering the electrochemical sensing interface with functional nanomaterials leads to novel electrochemical biosensors with improved performances in terms of sensitivity, selectivity, stability and simplicity. Functional nanomaterials possess good conductivity, catalytic activity, biocompatibility and high surface area. Coupled with bio-recognition elements, these features can amplify signal transduction and biorecognition events, resulting in highly sensitive biosensing. Additionally, microfluidic electrochemical biosensors have attracted considerable attention on account of their miniature, portable and low-cost systems as well as high fabrication throughput and ease of scaleup. For example, electrochemical enzymetic biosensors and aptamer biosensors (aptasensors) based on the integrated microchip can be used for portable point-of-care diagnostics and environmental monitoring. This review is a summary of our recent progress in the field of electrochemical biosensors, including aptasensors, cytosensors, enzymatic biosensors and self-powered biosensors based on biofuel cells. We presented the advantages that functional nanomaterials and microfluidic chip technology bring to the electrochemical biosensors, together with future prospects and possible challenges.

1. Introduction

Electrochemical biosensor is a device transducing biological sensing element-target recognition events into detectable electrochemical signals (Turner et al., 1987). Biological sensing elements (*e.g.*, enzymes, aptamers or antibodies) are immobilized/integrated at the electrochemical interface. Upon target binding, the electrochemical signal of redox reporter is changed, which is directly related to the concentration of target species. Electrochemical biosensors have been attracting much attention owing to their simple configurations, low cost, multiplexed detection capabilities, high sensitivity and selectivity, as well as ease of miniaturization for portable point-of-care diagnostics and environmental monitoring (Kimmel et al., 2012; Privett et al., 2010).

Engineering the bioelectrochemical sensing interface is crucial for improving the sensitivity and stablility of electrochemical biosensors. Nanomaterials display many unique properties that are dependent on their sizes and shapes, such as, size-dependent optical properties of metal nanoparticles (NPs) (Mayer and Hafner, 2011), electrical conductivity of carbon nanomaterials (Guo and Dong, 2011), electrocatalytic properties of metal NPs and nanocarbons (Banks and Compton, 2005), and high surface area. Coupling functional nanomaterials, especially the carbon and metal nanomaterials, with bioelectrochemical sensing interface, injects fresh energy to the development of electrochemical biosensors (Guo and Dong, 2009; Guo and Wang, 2011). The advantages that nanomaterials bring to electrochemical biosensors are presented as follows but not limited to: enlarging the electrochemically active areas, accelerating electron transfer between electrodes and detection species, and behaving as biocompatible scaffolds for biomolecule immobilization. These remarkable features lead to novel electrochemical biosensors with better performances such as improving the sensitivity and stability.

With the rapid development of chemical/biosensor networks (Byrne and Diamond, 2006) and growing concern about healthcare, food safety and environment pollution, fast access to bio (chemical) information is highly in demand. Therefore, it is of paramount importance to develop low-cost point-of-care diagnostics and environmental monitoring devices. Fueled by the

* Corresponding author.
E-mail addresses: dongsj@ciac.ac.cn (S. Dong), ekwang@ciac.ac.cn (E. Wang).

http://dx.doi.org/10.1016/j.bios.2015.05.037
0956-5663/

urgent need, researchers turn their attention to microfluidic electrochemical biosensors due to their miniature, portable and low-cost systems as well as high throughput and automation.

In this review, we summarized our recent advances in engineering the bio-nano sensing interface for sensitive and portable electrochemical biosensors. Our work covered four kinds of electrochemical biosensors, involving aptamer biosensors (aptasensors), cytosensors, enzymatic biosensors and self-powered biosensors based on biofuel cells (BFCs). We presented the advantages that nanomaterials and microfluidic chip technology bring to the electrochemical biosensors.

2. Electrochemical aptasensors

Since firstly discovered by three groups independently in 1990 (Ellington and Szostak, 1990; Robertson and Joyce, 1990; Tuerk and Gold, 1990), aptamers have aroused enormous interest in the scientific community. Aptamers are artificial single-stranded DNA, RNA or peptide sequences. Upon folding into unique secondary and tertiary structures, aptamers are capable of adaptively binding to certain targets with high specificity and affinity (Mascini et al., 2012). They are isolated from a library pool with $\sim 10^{16}$ random nucleic acid sequences through *in vitro* selection process known as systematic evolution of ligands by exponential enrichment (SELEX). The targets of aptamers are quitely wide from small molecules to proteins and even whole cells, with binding affinities comparable to or even stronger than that of antibodies (K_ds from picomolar to nanomolar level) (Famulok and Mayer, 2011; Iliuk et al., 2011). Besides the merits mentioned above, aptamers are produced *in vitro*, making it easier to tailor them with functional groups for diverse applications. Also, aptamers are more stable than antibodies and can endure harsh conditions such as high temperature or extreme pH. These features make aptamers more competitive as recognition elements compared with antibodies for a variety of applications in the fields of biosensing, biomedicine and cell biology.

Up to date, a great deal of electrochemical aptasensors have been developed, most of which required the step of labeling aptamers or other nucleic acids with the redox reporters (Li et al., 2010; Liu et al., 2011; Lubin and Plaxco, 2010). The labeling process suffers from cost- and labor-intensive, and to some extent reducing the binding affinity of the aptamers to their targets. In response, our group is devoted to the exploration of "label-free" strategies without the modification of "special electrochemical probes".

2.1. Electrochemical impedance technique

Electrochemical impedance spectroscopy (EIS) has been recognized as a sensitive tool for probing the interfacial properties of surface-modified electrodes (A.X. Li et al., 2007). When the targets bind with aptamers at the electrode surfaces, the capacitance and interfacial electron transfer resistance of the electrodes are changed. This provides a convenient method to construct highly sensitive and cost-effective label-free aptasensors.

Early in 2007, we developed an EIS aptasensor for the detection of adenosine on the basis of duplex-to-complex design (B. Li et al., 2007). As depicted in Fig. 1A, the adenosine-binding aptamer (ABA) strand was partially hybridized with 5'-thiolated part complementary strand (PCS). The formed self-assembly layers with negative charge repulsed the negatively charged probe $[Fe(CN)_6]^{4-/3-}$ anions, and hindered interfacial electron-transfer kinetics of the redox probes with high charge transfer resistance (R_{ct}). Upon addition of adenosine, the amount of the aptamers on the electrode surface was reduced, accompanied with the decreased R_{ct}. The EIS method provided a sensitive detection for adenosine with the detection limit of $\sim 10^{-7}$ M. Additionally, the designed aptasensor can be easily regenerated by hybridizing ABA with free PCS on the electrode. Subsequently, the α-thrombin binding aptamer (TBA) was grafted onto the ABA strand, achieving the parallel detection of ATP and α-thrombin (Du et al., 2008). Addition of α-thrombin amplified the sensitivity of ATP molecule, and the sensing surface was directly regenerated by treating with a large amount of ATP.

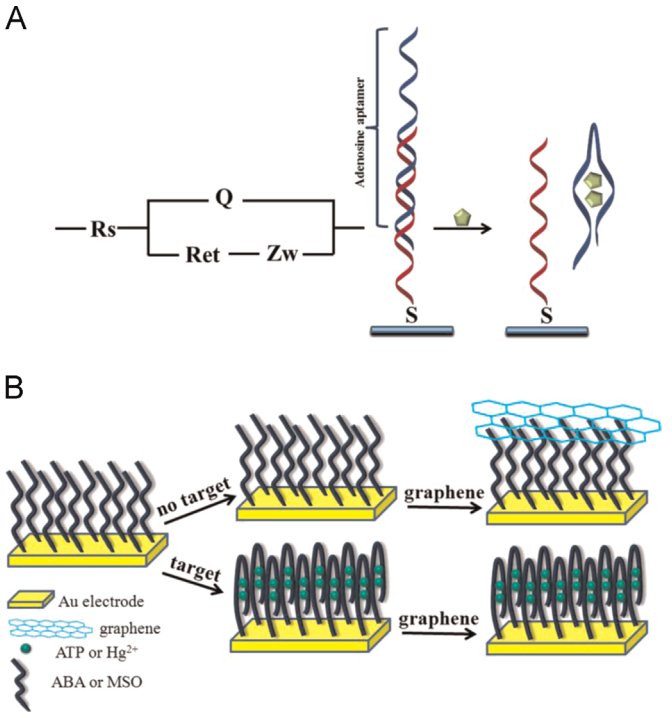

Fig. 1. The schematic representation of EIS aptasensors. (A) Circle model for the EIS and the schematic of EIS aptasensor for adenosine based on the duplex-to-complex design. (B) The sensing strategy for EIS detection of ATP. Without target, graphene was absorbed on ABA modified Au electrode, resulting in a decreased R_{ct}. In the presence of target, the formed duplex could not adsorb graphene, leading to the restoration of the high R_{ct}. The developed strategy was also applicable for the detection of Hg^{2+}. Adapted from B. Li et al. (2007) and Wang et al. (2012) with permission.

For the EIS aptasensors for protein, the signal heavily relied on the size or charge of protein, limiting the detection sensitivity (Xu et al., 2005). To solve this problem, we fabricated a sandwich sensing platform in which the thrombin molecules were captured by the thiolated aptamers immobilized on the Au electrode, and then the aptamer functionalized AuNPs were used to amplify the impedimetric signals (Li et al., 2008). Through the amplified sensing strategy, a very low detection limit of 0.02 nM was realized for the detection of thrombin.

Graphene is a two-dimensional carbon material with single-atom-thick. It possesses the advantages of large surface area and excellent electron conductivity (Fang and Wang, 2013; Guo and Dong, 2011), which provides new opportunity for developing more sensitive EIS aptasensors. As shown in Fig. 1B, through strong π–π stacking interaction between nucleotide and graphene, graphene was absorbed on the ssDNA ABA modified Au electrode, resulting in largely decreased R_{ct} (Wang et al., 2012). Upon addition of ATP, ABA underwent a conformational alteration to form duplex strands. It led to the desorption of graphene and the restoration of high R_{ct}. On the basis of this, a sensitive EIS aptasensor was fabricated for the detection of ATP with the detection limit down to 15 nM. The proposed detection strategy was versatile, which was demonstrated by the detection of Hg^{2+} using the Hg^{2+}-specific