BIOCHIP JOURNAL, Vol. 2, No. 3, 175-182, September 2008

Mini Review

Electrochemical Aptamer-based Biosensors

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Accepted 15 July 2008

Abstract

Aptamers are newly emerged sensing elements that are competitive with or sometimes even better than antibodies due to their various advantages: a compact size, cost effectiveness, chemical stability, in vitro synthesis, easy modification, labeling and so on. Therefore, there have recently been intensive advances in aptamer-based biosensors including electrochemical aptasensors that are more proper to the miniaturization and integration of biosensors for high-throughput analysis and point-of-care (POC). In this review, we have summarized new state of the art strategies for an aptamer-based electrochemical sensing platform and manners of signal amplification in the categories of simple label-free electrochemical detection, electrochemical signal amplification using enzymes or nanoparticles, target-induced conformational changes, and displacement formats.

Keywords: Aptamer, Biosensor, Aptasensor, Electrochemical, Signal amplification

Introduction

Aptamers are oligonucleotides that can bind to a wide range of target molecules, such as proteins, peptides, nucleic acids, lipids or low-molecular organic or inorganic compounds with high affinity and specificity[1-3]. Aptamers could be developed using in vitro selection, known as SELEX (Systematic Evolution of Ligands by EXponential enrichment), which makes it possible to isolate functional oligonucleotides against a specific target from a random ssDNA or RNA library (usually 10^{15})[4,5]. The dissociation constant of aptamers to their targets is typically from the micromolar to picomolar range, which is comparable or better to the affinity of antibodies to their antigens[6]. Aptamers have also showed high specificity, which makes it possible to discriminate specific target molecules from their derivatives, as demonstrated in previous reports for a theophyllin binding aptamer against caffeine (difference of only a methyl group)[6], by an L-arginine binding aptamer against D-arginine[7] or by an oxytetracycline binding aptamer against tetracycline (difference of only an OH-group)[8]. Aptamers have many advantages compared with antibodies. (a) There is no need for *in vivo* immunization to obtain an aptamer, which can be chemically synthesized *in vitro*. This is a very big advantage because it is not easy to produce antibodies against some targets, i.e. small toxic compounds. In contrast, aptamers are not limited to their targets. This makes it possible for the production of purified aptamers with low cost and without batch-to-batch variation. (b) Aptamers are easy to handle due to their thermo-stability. And (c), they are easy to be modified, linked with labeling molecules like dyes, or immobilized on the surface of beads or an electrode for different applications[9]. Aptamers also have some defects when they are used in an *in vivo* system. DNA, and especially RNA, is very sensitive to nucleases, thus some methods to transform aptamers into nuclease-resistant moieties by modification of the ribose ring at the 2'-position or by a specific modification of the pyrimidine nucleotide have been reported[10,11]. Since 1990, and the first report on aptamers, a large number of aptamers has been selected for various targets such as proteins[12-14], amino acids[7,15], antibiotics[8,16,17], and other small organic and inorganic molecules[18-20].

Based on these advantages, recently aptamers have been widely used in various fields of herapeutics[21-23], medical diagnostics[24], biosensors[25-27], drug delivery systems[23,28], and separation techniques[29,30]. In particular, studies on the application of aptamers for biosensors or medical diagnostics have intensively increased due to the multiple advantages of aptamers as sensing elements such as the easy reactivation of immobilized aptamers and the possibility of using variant detection formats[31-33]. Indeed, numerous studies have reported on aptamer-based biosensors with different signal transducer manners such as an optical method[34-37], mass-dependant measurement[38-40], colorimetric detection[41-45], and an electrochemical system[31]. Electrochemical detection is an attractive sensing platform because it is simple, rapid, cost-effective, and has easy miniaturization, which is necessary for a high-throughput system or point-of-care applications. Therefore, recent variant electrochemical de-

tection methods have reported for the development of aptamer-based biosensor systems. The interaction between target molecules and aptamers immobilized on a conductive surface make a change in the current and resistance of a solution-electrode interface, so specific binding of targets to aptamers could be evaluated by impedance measurement or potentiometric analysis. Herein, the various strategies for an electrochemical signal producing design and signal amplification manners have been incorporated to develop novel and sensitive electrochemical aptasensors, i.e., enzyme or nanoparticle-based signal amplification, signal-on measurement by the conformational change of aptamers and a target-induced displacement method. This minireview summarizes these strategies and methodologies of electrochemical aptasensor systems.

Electrochemical Aptasensors

1. Simple Label-Free Electrochemical Detection

Many label-free electrochemical aptasensors have been developed based on a simple interaction or sandwich assay between target molecules and aptamers. Faradaic impedance microscopy is an effective method to analyze the biorecognition events of a target to an aptamer by measuring the changes of electron-transfer resistances between a redox molecule and electrode[46]. The effect of binding events on electron-transfer resistance depends on the character of the target molecules (Figure 1). If the target is positively charged, the resistance will be decreased due to a screening of the negative charge of aptamers, while a negatively charged or bulky protein target causes an increasing resistance by the formation of an insulating layer[47-52]. Potentiometric measurement is also widely used label-free electrochemical detection method. In this analysis, the binding event of targets to aptamers is analyzed by the measurement of current changes which depend on the conditions of the electrode surface. Generally, the binding of a target reduces the current because the electron flow between a redox molecule and the electrode is interfered by the aptamer-target complex (Figure 1)[53-55].

2. Enzyme-based Signal Amplification

Enzymes, especially redox enzymes, can be labeled to aptamers or antibodies, and these produce conductive compounds by biocatalytic activity; subsequently, an electrochemical signal can be amplified. Studies on the applications of an alkaline phosphatase and horse radish phosphatase (HRP) for signal amplification have been reported. The primary aptamer against thrombin or IgE was immobilized on a gold electrode, and a target protein was bound to a primary aptamer. According to a sandwich assay, a secondary aptamer conjugated with an alkaline phosphatase was bound to another site of the target proteins; subsequently, the alkaline phosphatase generated *p*-aminophenol as an electrochemically detectable product by the hydrolysis of *p*-aminophenyl phosphate (Figure 2a)[56-58]. Instead of an alkaline phosphatase, a radish phosphatase (catalyze the reduction of H_2O_2) or a pyrroloquinoline quinone dependent glucose dehydrogenase (GDH) can be employed for electrochemical signal amplification. The glucose is oxidized in the presence of 1-methoxy-5-methylphenazinium methylsulfate (mPMS) as a diffusional mediator, enabling electrochemical detection by the biocatalytic activity of the glucose dehydrogenase[59]. In another type of enzyme application for the signal enhancement of an aptasensor, an aptamer was designed to carry out rolling circle amplification (RCA)[60]. The aptamer-primer sequence initiates an RCA reaction that produced hundreds of copies of a repeated sequence, on which an ALP was coupled (Figure 2b). As a result, the sensitivity was significantly improved. A related application substituted the enzymatic reaction with platinum nanoparticles as a catalytic probe[61]. In this study, Pt NPs, functionalized with a secondary aptamer, catalyzed the electrochemical reduction of H_2O_2 (Figure 2c). As a result, the sensitivity for thrombin detection was increased to 1 nM. This is 80-fold higher than that of HRP and GDH applications. The

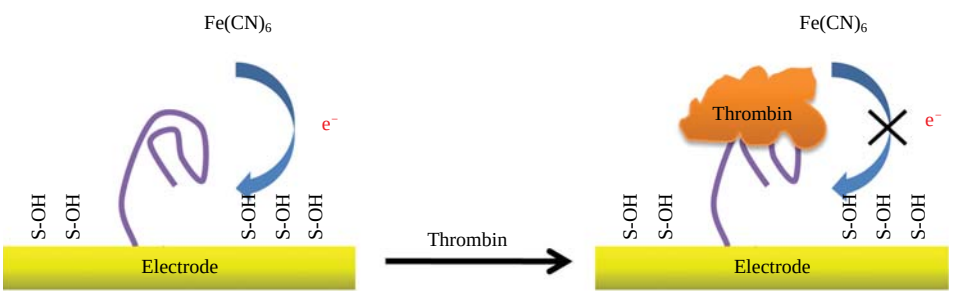

Figure 1. Simple label-free electrochemical analysis. The electron-transfer resistance or current is changed by the binding of targets to an aptamer immobilized on an electrode.

enhancement of sensitivity by Pt NPs may be attributed to the need to activate the enzymatic reaction using a diffusional electron mediator that is weakly coupled with the electrode surface as a result of the aptamer layer and protein nonspecific adsorptions.

3. Nanoparticle-based Signal Amplification

The application of nanoparticles in an electrochemical aptasensor also introduces novel methods to develop highly sensitive sensing techniques. An impedimetric aptasensor was developed based on thrombin binding aptamer functionalized gold nanoparticles (AuNPs), which produces an amplified impedance signal[62]. According to a sandwich assay, a primary thrombin binding aptamer (TBA) was first immobilized on an electrode, followed by a thrombin being bound to the primary TBA. Finally a secondary TBA functionalized on AuNPs was bound to the TBA-thrombin complex (Figure 3a). Here, the AuNPs are no longer good conductors but are negatively charged complexes, which interfere with an electron transfer on the surface. The AuNPs binding to the thrombin leads to a significant increase of electron-transfer resistance, which amplifies the signal effectively. Nanoparticles, especially gold nanoparticles (AuNPs), have also been used as the carriers of electrochemical signal producing materials[63]. In this format, AuNPs were functionalized with TBA containing a poly-ad-

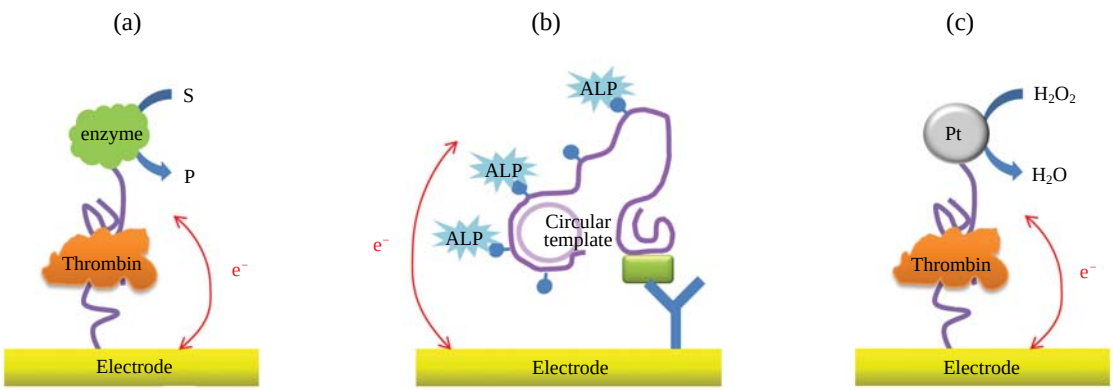

Figure 2. Schemes of enzyme-based signal amplifications: (a) A redox enzyme linked with aptamers produces a large amount of conductive compounds, consequently the electrochemical signal is amplified. (b) An aptamer-primer sequence mediating an in-situ RNA reaction leads to a significant enhancement of sensitivity. (c) Pt NPs-labeled aptamers act as electrocatalysts such as a redox enzyme.

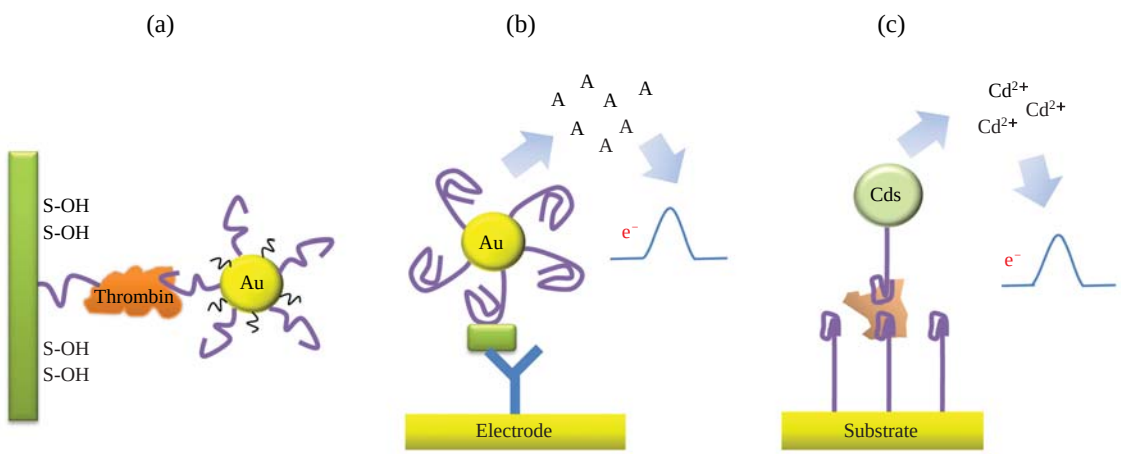

Figure 3. Schemes of nanoparticle-based signal amplifications: (a) The electron-transfer is affected by AuNPs functionalized with aptamers. (b) After hydrolysis of adenosine-rich aptamers immobilized on AuNPs, a large number of adenosines generate an amplified electrochemical signal. (c) After the formation of a complex with targets and QDs functionalized aptamers, the electrochemical signal is measured based on metal ions released from the QDs.

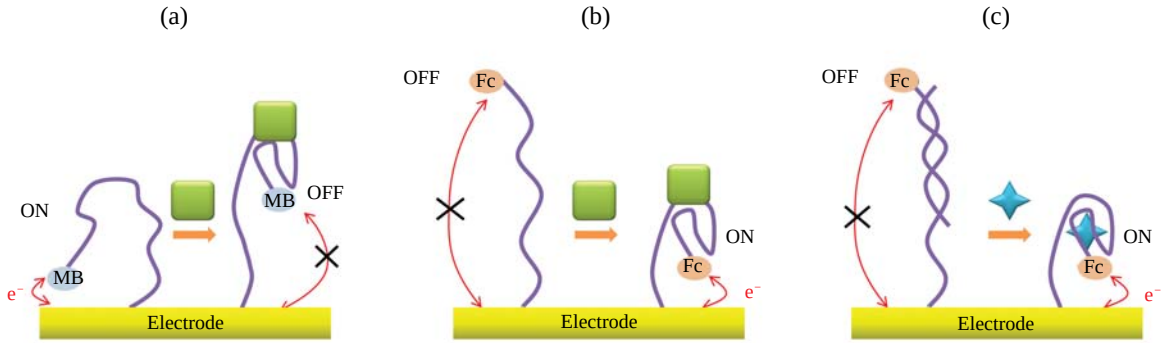

Figure 4. Schemes of conformational change-based electrochemical aptasensors: (a) After the binding of targets, the aptamer folded into a rigid structure and methylene blue (MB) was closed to the electrode. (b) The binding of targets to an aptamer makes the ferrocene close to electrode surface and results in producing positive signals. (c) In the presence of targets, a complementary DNA is released from a rigid DNA duplex; subsequently the ferrocene linked with the aptamer was closed to the electrode and turned on an electron-transfer.

enine sequence. After the forming of a sandwich structure of anti-thrombin antibodies/thrombin/TBA, single-adenines were generated by acid or nuclease degradation and detected using a pyrolytic graphite electrode directly (Figure 3b). Because one group of AuNPs carried a large number of TBAs containing a poly-adenine sequence, the sensitivity of thrombin detection was intensively improved. Similarly, semiconductor quantum dots (QDs) were employed in an electrochemical aptasonsor system for signal amplification[64]. In this study, the QDs were labeled to a secondary aptamer, and a sandwich assay was performed under the same procedure with previous studies. Finally, the binding event was evaluated using a solid-contact Cd^{2+} selective microelectrode (ISE) after dissolution of the CdS (Figure 3c).

4. Binding-Induced Conformational Changes

Nucleic acids aptamers fold into a flexible but well-defined three-dimensional structure upon binding to their target molecules. This property of aptamers enables the development of novel and unique aptamer-based sensing platforms. Thus, several electrochemical aptasensors based on the conformational change of aptamers have been continuously reported. Recently, Heeger, Plaxco and others developed a few novel electrochemical aptamer-based (E-AB) sensors for thrombin, cocaine and potassium. These E-AB sensors are based on the binding-induced conformational changes of redox-tagged and surface-confined aptamers, which have proven to be highly sensitive and selective. One example is a redox-active methylene blue (MB) labeled thrombin binding aptamer immobilized on an electrode (Figure 4a). The flexible conformation of the aptamer labeled with MB enabled the electrical contacting of the MB with the electrode, and a voltammetric response of the methylene blue was observed. The forming of a G-quadruplex structure upon binding the thrombin shielded the MB from electron-transfer communication with the electrode[65,66]. But this sensing format has a disadvantage because of a negative readout signal. To solve this defect, several signal-on aptasensors were developed. One approach is to use a bifunctionalized thrombin binding aptamer (TBA) labeled with a terminal electroactive ferrocene as a redox group and a thiol group at the second terminus of the aptamer[67-69]. The long, flexible aptamer strand prevented electrical contact of the ferrocene with the electrode. The formation of a TBA-thrombin complex made a G-quadruplex aptamer configuration rigid and resulted in the orientation of the ferrocene towards the electrode (Figure 4b). This led to the generation of a positive signal in the presence of thrombin. In another approach, a DNA duplex structure consisting of a ferrocene-labeled aptamer and its complementary DNA was used[70-72]. In the absence of target molecules (thrombin, PDGF, and cocaine), the hybridized aptamer sustained a partially unfolded state. While in the presence of targets, the aptamer folded to bind to the targets. Consequently, an electroacitve ferrocene was closed to the electrode, and the signal was increased (Figure 4c). A further method for an electrochemical aptasensor has used a redox-active reporter that intercalates into double-stranded DNA rather than being covalently tethered to the aptamer[73,74]. The hairpin structure of aptamers was immobilized on a gold electrode, and methylene blue was intercalated in the duplex stem of the probe hairpin. The binding of thrombin with the aptamer opened the hairpin structure, thus releasing the intercalated redox-active MB. As a result, the binding of thrombin to the interface

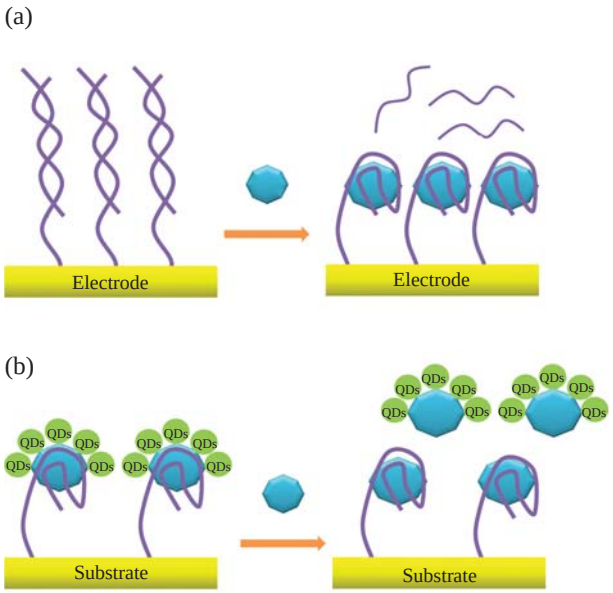

Figure 5. Schemes of target-induced displacement format: (a) A complementary DNA is separated from a DNA duplex in the presence of targets. (b) The targets competitively displace the target-QDs conjugates, which are already bound to the aptamer.

decreased the electrochemical signals.

5. Target-Induced Displacement Format

Studies on the structure of aptamer binding have indicated that small targets are often pocketed into the aptamer structure, so it is difficult to perform a sandwich assay. Because of this limitation, aptasensors for small molecules have been commonly developed with a single-site binding format. But a simple label-free electrochemical detection method is still limited for the development of a sensitive aptasensor for small molecules because the electron-transfer resistance or current changes by the binding of small molecular targets is usually low as compared to macromolecules. In an approach to overcome this difficulty, a target-induced displacement format is a very effective method. Two strategies for a displacement assay have been reported. One approach is based on the separation of the two strands of duplex nucleic acids, composed of an aptamer strand and partially complementary sequence, induced by the presence of target molecules. In the presence of a target, duplex DNA was separated and the aptamer folded binding to the target. The detachment of a complementary sequence decreases the electron-flow resistance (Figure 5a). The separation of duplex DNA was dependent on target concentration; thus, this method enables the sensitive detection of small molecules[75-79]. The other approach relies on the displacement of the aptamer-target conjugate, labeled with an electrochemical signal producing material, by the competitive binding of targets[80,81]. Target protein-QDs conjugates were already bound to an aptamer immobilized on a substrate, followed by protein-QDs conjugates being released from the aptamer by displacement in the presence of the target protein (Figure 5b). Then, an electrochemical signal from the QDs was measured to estimate the concentration of the target protein.

Conclusion and Perspectives

Not surprisingly, an interest on aptamers as sensing elements has intensively increased due to the various advantages of aptamers in biosensor and medical diagnostic studies. Recent advances have also demonstrated aptamer-based biosensors, especially electrochemical aptasensors. This review has summarized some state of the art electrochemical aptamer-based biosensors. There is no doubt that studies on aptasensors will be continuously and actively increased. It is also promising that electrochemical sensing platforms for aptasensors will emerge more intensively, as an electrochemical system is more proper to the trends of biosensors: a multiplexing for high-throughput analysis, miniaturization for a point-of-care system, and an integrated system with IT for ubiquitous networking.

In spite of these bright perspectives, aptamer-based biosensors also have some huddles to overcome. Aptasensors are still immature compared to immuno-assays and have rarely been tested in real samples. The selection of aptamers with high affinity for small molecules is still a challenging work to improve the sensitivity of the detection of organic or inorganic small molecules. While there is still a long way to go, we expect that aptamer-based biosensors, especially electrochemical aptasensors, will become one of the most effective analysis tools.

Acknowledgements

This work was supported by a Grant from the Seoul R & BD Program.

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