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Mini Review

Aptamers in Electrochemical Biosensors

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Biosensors are analytical devices with increasing practical significance. Various biorecognition elements are used in biosensors and new types are emerging. In the past few years, aptamers have been developed, and they have also gained popularity in analytical chemistry. Aptamers are short sequences of single-stranded chains of DNA, RNA, and peptides that exert high affinity to the target structure in a way similar to that of antibodies. Biosensors with an immobilized aptamer are called aptasensors and they represent an alternative to other biosensors based on antibodies and various bioreceptor molecules. In this review, the significance of aptamers is scrutinized, their application in the electrochemical biosensors is discussed, and significant recent examples are introduced.

Keywords: affinity interactions; amperometry; antibody; aptamer; aptasensor; bioassay; biosensor; DNA; piezoelectric sensor; potentiometry; sensor

1. INTRODUCTION

Biosensors are analytical devices in which two substantial parts are combined, resulting in unique specifications of the final instrument. The sensor is also known as a physicochemical transducer, and it is the first part of the biosensor device. The second important part of a biosensor is composed of a chemical structure of biorecognition element, a natural origin. While the sensor platform is necessary for the assay, it does not provide specificity and cannot even work alone in some cases. Just a combination of the sensor platform and the biorecognition element provides the final biosensor that can serve as a complex analytical device. Several sensor platforms and biorecognition elements exist and are described in studies in this area. Electrochemical, optical, piezoelectric, gravimetric, and thermometric biosensors can be distinguished as relevant types considering the physicochemical transducer [1-5]. Macromolecules of biological origin, such as enzymes, antibodies, receptors, genes and their fragments,

whole cells, etc. can be mentioned as the biorecognition elements used for the construction of biosensors [6-9].

Extensive research on new biorecognition elements is a part of biosensor development. Discoveries in the field of biorecognition elements can further increase the practical impact of biosensors, make them more accurate, make them easier to manufacture, and exert better stability. Aptamers are emerging material that would further improve the relevance of biosensors. These molecules exert high affinity for target structures and the biosensors are called aptasensors though other studies consider aptasensors as a sole class of analytical devices [10-12]. This review covers the recent discoveries in the use for electrochemical biosensors. Relevant and recent discoveries on aptamers and their use in electrochemical biosensors are quoted in this paper.

2. APTAMER

A single-stranded chain of DNA or RNA exerting affinity to a target structure is commonly considered an aptamer [13-15]. However, the term aptamer also covers purpose-made peptides being able to interact with their targets [16,17]. In the current literature, various chemically prepared and further modified peptides and oligonucleotides are called aptamers [18]. Aptamers can also be also a part of a nanoparticle or nanostructured material [19,20]. The history of aptamers began in the early 1990s. The use of single-stranded oligonucleotides described Ellington and Szostak for RNA in 1990 [21] and DNA in 1992 [22]. Peptide aptamers were developed in the following years, as described in the pioneering work by Colas et al. in 1996 [23].

The principle of application of aptamers in the biosensors is quite close to that of antibodies, and antibodies are the major alternative to the aptamers. Biosensors that contain an immobilized antibody are called immunosensors. Antibodies obtain selectivity to the immunosensor as they can adhere to the analyte with substantial selectivity [24,25]. Antibodies are chemically immunoglobulin proteins and they can be polyclonal when obtained from a whole animal, monoclonal when obtained from previously selected and cultured cells, and recombinant when methods of genetic engineering are chosen for production purposes [26-28]. The analytical use of antibodies is well known and many immunochemical methods and serological tests are based on manufactured antibodies [29]. Although immunochemical methods are traditional, they also have drawbacks. The molecule of an antibody is quite large depending on the exact type of immunoglobulin. The manufacturing of antibodies is quite an expensive process. Polyclonal antibodies should be prepared in laboratory animals. There are some ethical problems and the reproducibility of polyclonal antibody production. Monoclonal and recombinant antibodies are made by a biotechnological process, and immunoassays based on monoclonal and recombinant antibodies are quite reproducible. On the other hand, the price of such antibodies is quite high, and the large molecule of an antibody represents a limitation in methods based on nanomaterials. The molecular weight of an aptamer is approximately (depending on the type of the antibody and the length of aptamer) ten times lower than an immunoglobulin [30]. Aptamers would replace antibodies in immunoassays and biosensors in any aspect [31]. Molecularly imprinted polymers are another alternative to aptamers,

though molecularly imprinted polymers are more an experimental material than an material with increased practical potential [32].

Various production technologies are known for the manufacturing of aptamers and both in vitro and in vivo approaches are possible [33,34] and there are also exist aptamer-based nanomaterials [35,36]. Aptamers made from oligonucleotides are probably the most common. Depending on the type of laboratory manufacturing of the aptamer, typical commercially available structures are oligonucleotides containing between 10 and 80 nucleotides but the aptamers sized up to 120 nucleotides were also prepared as well [37]. The typical molecular weight of aptamers presented in scientific studies ranges between 5 and 15 kDa or between 15 and 50 nucleotides [38]. Peptide aptamers are typically between 5 and 20 amino acids residues [39]. Dissociation constants for complex aptamers: target structure is in a picomolar to nanomolar scale, but some aptamers exert even affinity than corresponding to the picomolar dissociation constant [40-43]. The interaction of the aptamer with the target structure is based dominantly on non-covalent interactions. Hydrogen bonding, electrostatic interactions, and interaction through van der Waals forces can be written as the common mechanism allowing the tight bond between the aptamer and the target molecule [44-46]. Aptamers have gained high popularity as a material suitable for the diagnosis of diseases, therapy and theranostic, analytical procedures, and other applications in biomedicine [47-54]. The basic specifications is written in Table 1.

Table 1. Survey of Aptamers Specifications

Specification	*Description*	*References*
The first discoveries	RNA aptamer in 1990, DNA aptamer in 1992, peptide aptamer in 1996	[21-23].
Chemical composition	oligonucleotides, peptides, related structures, and nanostructures	[13-20]
Size of oligonucleotide aptamer	The typical molecular weight of aptamers presented in scientific studies ranges between 5 and 15 kDa or 15 to 50, aptamers up to 120 nucleotides also exists	[37,38]
Size of the peptide aptamer	typically sized between 5 and 20 amino acids residues	[39]
Principle of interaction aptamer to the target	hydrogen bonding, electrostatic interactions, interaction via van der Waals forces	[44-46]
Dissociation constants for aptamer target complex	The picomolar to nanomolar scale is the common range	[40]

3. THE CONCEPT OF APTAMERS USE IN ANALYTICAL CHEMISTRY

As mentioned above, aptamers are molecules having a high affinity for the target structures. This phenomenon predetermines the applicability of aptamers as a part of assays where an affinity interaction is expected. The typical weight of aptamers of 5 to 15 kDa makes them a very light alternative to the

other molecules. Immunoglobulin G (IgG) with a weight of around 150 kDa is 10 to 30 times heavier than an aptamer. The other immunoglobulins are even heavier. The M variant of an immunoglobulin, IgM, is a pentamer of IgG with an approximate molecular weight of 970 kDa. One molecule of IgG has two antigen-binding sites called paratopes and IgM has 10 paratopes. The aptamers are still lighter than immunoglobulins even if the mass per binding site is calculated. Lectins and receptors are other affinity molecules that are applicable for an assay. Concanavalin A with molecular weight 112 to 140 kDa [55] or a *Hechtia argentea* with molecular weight 27.4 kDa [56] can be introduced as examples. Taking into account all the typical affinity molecules, aptamers are very light, which allows them to reach a high density of affinity molecules per square or volume of a biosensor or other analytical devices. Theoretically, a higher sensitivity by a device based on aptamers can be reached when compared to immunoglobulins or lectins.

Selectivity and reproducibility are other issues that should be taken into consideration when the practical impact of aptamers is studied. Although selectivity is tested in the particular experiments that are discussed in the following, a complex study on the selectivity of aptamers versus antibodies or lectins is missing. The simplicity of aptamers represents an advantage for assay development; on the other hand, the analytical robustness of the newly prepared device should be carefully examined. Although the production of antibodies is quite a standard process with minimal malfunctions in commercially available devices and kits, aptamers should reach their reputation in the future. The economy will also play a substantial role, and mass production of either of the affinity molecules can make it a highly preferred part of an essay. Aptamers are a part of various analytical devices and methods in the current time. Various spectrophotometric, colorimetric and fluorometric assays [57-63], colorimetric and fluorometric biosensors [64-69], solid phase extraction methods [70], aptamer-assisted polymerase chain reaction [71,72], microfluidic sensors with an aptamer [73], aptamer-containing affinity columns for liquid chromatography [74] and high performance liquid chromatography-mass spectrometry based on aptamer [75] can be exampled as use of aptamer for an analysis. Electrochemical devices and biosensors are another way to use aptamers for analytical purposes [76-80]. The summarizing of analytical methods based on aptamers is written in Table 2.

Table 2. Survey of analytical methods where aptamers can be used

Method	*References*
Spectrophotometric, colorimetric, and fluorometric assays	[57-63]
Colorimetric and fluorometric biosensors	[64-68]
Electrochemical devices and biosensors	[76-80]
Solid-phase extraction methods	[70]
Aptamer assisted polymerase chain reaction	[71,72]
Microfluidic sensors with an aptamer	[73]
Aptamer-containing affinity columns for liquid chromatography	[74]
Aptamer-based high-performance liquid chromatography-mass spectrometry	[75]

4. ELECTROCHEMICAL BIOSENSORS WITH APTAMERS

Electrochemical biosensors are well-known analytical devices with a long history that have started with the discoveries of the Clark electrode and improved the Clark electrode with immobilized glucose oxidase in the 1950s and 1960s [81-85]. Since then, various electrochemical platform has been proposed for biosensors, including the platforms containing catalyzing (enzymes) or affinity (antibodies, bioreceptors) attached on their surface [86-89].

Aptamer electrochemical biosensors have been developed in several studies. Liu et al. have developed an aptamer-based electrochemical biosensor based on aptamers as a tool for the detection of mercury ions [90]. The biosensor contained gold nanoparticles covered with an aptamer-based on thymine-binding mercury cation and it was based on glass carbon electrode and differential pulse voltammetry. The electrochemical assay was able to detect Hg^{2+} with a detection limit of 0.005 nmol/l and linear range 0.01 to 500 nmol/l. The aptasensor on heavy metal assay was also developed by Wang and colleagues, who prepared an electrochemical aptasensor with an aptamer attached to a composite comprising chitosan, graphene and titanium dioxide [91]. The aptasensor detected Pb^{2+} with a limit detection of 0.33 ng/l and had a linear range of 1 ng/l to 1000 ng/l. In another work, an electrochemical aptasensor was made on fabric screen-printed electrodes and the working electrode was founded on phosphorene-gold nanocomposites and an immobilized aptamer [92]. The researchers performed their devices for the norovirus-like particle assay (artificial particles with norovirus antigens) and they were able to prove the particles with a limit of 0.28 ng/ml and linear range for the analyte 1 ng / ml to 10 µg/ml. The biosensor also determined norovirus in the spiked oyster samples with quite good recovery (97.2 – 104 %). Nguyen and co-workers constructed another aptasensor on microorganisms for the detection of *Staphylococcus aureus* [93]. The electrochemical biosensor worked on the principle of chronoamperometry and showed the presence of *S. aureus* in various samples with a detection limit of 39 CFU for an *S. aureus* suspension in buffer and 414 CFU for *S. aureus* suspension in tap water. In a work by Dong and co-workers, an electrochemical aptasensor with tetrahedral DNA nanostructure was developed for the fumonisin B1 assay [94]. The biosensor was able to find fumonisin B1 with a detection limit of 0.31 fg/ml and exerted a linear dynamic range of 0.5 fg/ml to 1 ng/ml. Blidar et al. prepared another aptamer biosensor: a device for the oxytetracycline assay in milk [95]. The biosensor was based on screen-printed electrodes with a gold nanostructure and an aptamer on their surface and the biosensor was suitable for the assay based on chronoamperometry, multipulse amperometry, and chronopotentiometry. Oxytetracycline was measured with a detection limit of 8.7 nmol/l and a linear range of 50 nmol/l to 1.2 µmol/l. Electrochemical biosensors based on aptamers mentioned in the aforementioned text are summarized in Table 3.

Table 3. Electrochemical biosensors with aptamers

Description	*Analyte*	*Specifications*	*References*
Glass carbon electrode and differential pulse voltammetry,	Hg^{2+}	limit of detection 0.005 nmol/l and	[90]

aptamers selectively binding mercury cations were attached to gold nanoparticles		linear range 0.01 to 500 nmol/l	
electrochemical aptasensor with an aptamer attached on a composite comprising chitosan, graphene, and titanium dioxide	Pb^{2+}	limit detection 0.33 ng/l and it had a linear range of 1 ng/l to 1000 ng/l	[91]
Electrochemical aptasensor made on screen printed electrodes on fabric and the working electrode founded on phosphorene-gold nanocomposites and an immobilized aptamer	norovirus	limit of detection for norovirus-like particles 0.28 ng/ml and linear range 1 ng/ml up to 10 µg/ml	[92]
Chronoamperometric biosensor	*Staphylococcus aureus*	limit of detection 39 CFU for a suspension of *S. auresus* in buffer and 414 CFU for *S. aureus* suspension in tap water	[93]
Electrochemical aptasensor with tetrahedral DNA nanostructure	fumonisin B1	limit of detection 0.31 fg/ml and exerted linear dynamic range 0.5 fg/ml to 1 ng/ml	[94]
biosensor based on screen-printed electrodes with a gold nanostructure and an aptamer on their surface	oxytetracycline	limit of detection 8.7 nmol/l and a linear range of 50 nmol/l to 1.2 µmol/l	[95]

5. CONCLUSION

Aptamers have gained high popularity and applicability since their discovery approximately thirty years ago. Applications in the analysis and construction of electrochemical biosensors are not an exception. Aptamers can be used in the same way as antibodies when a biosensor is constructed and the aptamers represent an alternative to the antibodies. Analytical devices based on aptasensors can have quite decent specifications that make the aptasensors directly competitive with the immunosensors. The aptamers are also suited for mass production by chemical processes. This fact makes aptasensors more suitable for mass production and the contemporary reduction of production costs. On the other hand, every aptasensor expected to be used for a biosensor construction should be carefully checked for specificity and other issues that can reduce their applicability.

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