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Research review paper

Voltammetric aptasensors for protein disease biomarkers detection: A review

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ABSTRACT

An electrochemical aptasensor is a compact analytical device where the bioreceptor (aptamer) is coupled to a transducer surface to convert a biological interaction into a measurable signal (current) that can be easily processed, recorded and displayed. Since the discovery of the Systematic Evolution of Ligands by Enrichment (SELEX) methodology, the selection of aptamers and their application as bioreceptors has become a promising tool in the design of electrochemical aptasensors. Aptamers present several advantages that highlight their usefulness as bioreceptors such as chemical stability, cost effectiveness and ease of modification towards detection and immobilization at different transducer surfaces. In this review, a special emphasis is given to the potential use of electrochemical aptasensors for the detection of protein disease biomarkers using voltammetry techniques. Methods for the immobilization of aptamers onto electrode surfaces are discussed, as well as different electrochemical strategies that can be used for the design of aptasensors.

Contents

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1. Introduction

In biomedical applications, the development of methodologies to detect and quantify proteins, specifically protein biomarkers, has increasingly become essential for areas such as clinical analysis, detection and treatment of certain diseases that can be correlated with changes in concentration of a protein biomarker in biological fluids. Determining the amounts of specific proteins in a given sample is particularly interesting and challenging. These proteins are effective diagnostic and prognostic tools for many diseases depending on the capability of easily quantifying their low concentrations in biological samples (Csordas et al., 2010; Hanash, 2011). Several strategies have been pursued to develop accurate and simple diagnosis methodologies, using different recognition elements (e.g. antibodies, aptamers) and different sensing principles and techniques (e.g. optical, electrochemical). Up to now, a variety of aptamers exhibiting high selectivity and affinity towards relevant protein disease biomarkers have been reported, thus allowing the fabrication of new, simple and sensitive diagnostic methods to determine such proteins in standard solutions and in complex samples such as blood and serum. The use of electrochemical aptasensors is particularly interesting given their simplicity, sensitivity, specificity and suitability for the detection of low levels of protein disease biomarkers using several available techniques including cyclic voltammetry (CV), square-wave voltammetry (SWV) and differential pulse voltammetry (DPV). Commonly, thrombin is used as a model protein in the design of aptasensors. However, other disease-related proteins such as platelet-derived growth factor (PDGF), osteopontin (OPN), vascular endothelial growth factor (VEGF) and Mucin 1 (MUC1) have been broadly described. These proteins can be associated to several diseases and have been found in many cancers, but particularly in breast cancer. Globally, cancer is a relevant health risk given the amount of persons annually diagnosed with this disease, thus progress leading to enhanced survival is a global priority. For instance, breast cancer is the most prevalent cancer in women worldwide and its cancer-related mortality is closely associated with the metastatic potential of the primary tumor (Xu et al., 2015), accounting for 25% (1.67 million) of the all cancer cases and 15% (522,000) of the cancer deaths. Despite the efforts to reduce the number of cases, breast cancer metastasis and breast cancer–related mortality, the prognosis remains poor (Xu et al., 2015), thus reinforcing the relevance of developing new diagnostic methods with improved features, including high specificity and sensitivity, low cost, suitability for prognosis and disease monitoring. Therefore, in this review, a special emphasis is given to the potential use of electrochemical aptasensors for the detection of protein disease biomarkers (e.g. breast cancer biomarkers) using voltammetry techniques.

2. Aptamer-based biosensors: aptasensors

According to the International Union of Pure and Applied Chemistry (IUPAC) "a biosensor is a self-contained integrated device which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor) which is in direct spatial contact with a transducer element" (Thévenot et al., 1999, 2001). Another definition commonly used for biosensors is "analytical devices that are based on a bioreceptor and that are capable of sensing biologically-relevant analytes with either electrical or optical readout" (Cheng et al., 2009). As illustrated in Fig. 1, the main components of biosensors are the bioreceptors or biorecognition elements; transducers or the detection devices; and displays or electronic parts comprised by a signal amplifier and the data processor (Cheng et al., 2009; Strehlitz et al., 2008; Velusamy et al., 2010). The basic principle of the biosensor detection is the specific binding of the target of interest to the bioreceptor immobilized on a suitable support matrix, usually an electrode, to produce either discrete or continuous signals, which are proportional to a property of a single target or a related group of analytes. The signals are then transformed into a digital format that can be recognized by the end users (Strehlitz et al., 2008). The bioreceptor will be responsible for the selectivity/specificity of the sensor response to a given target or group of targets of interest, thus minimizing the interference from other substances in complex mixtures (Viswanathan and Radecki, 2008). On the other hand, the transducer determines the sensitivity of the biosensor and is responsible for converting the biological signal into a measurable signal (Monošík et al., 2012; Sassolas et al., 2009). The selectivity and sensitivity of the bioreceptor and transducer make the biosensors an attractive analytical tool in several areas and applicable to a large variety of samples including body fluids, food, cell cultures and environmental samples (Cheng et al., 2009; Grieshaber et al., 2008; Sassolas et al., 2009).

Biosensors that use DNA or RNA aptamers as bioreceptors have been named "aptasensors" (Cheng et al., 2009; Hianik and Wang, 2009; Radi, 2011; Sassolas et al., 2009). The aptasensors emerged in the early 1990s with the discovery and introduction of aptamers as sensing probes (Ellington and Szostak, 1990; Tuerk and Gold, 1990 cited in Cheng et al., 2009). Indeed, these were then considered an important alternative to the classical analytical methods for protein detection. Aptamers are short nucleic acids (≈12–80 nucleotides long) of RNA or single-stranded DNA that possess unique binding characteristics to their targets, such as high sensitivity/affinity, specificity and ability to fold into numerous tertiary conformations (e.g. hairpin, G-quartet, stem-bulge, pseudoknot, T-junction) (Cho et al., 2009; de-los-Santos-Álvarez et al., 2008; Famulok and Mayer, 2011; Lakhin et al., 2013; Meyer et al., 2011; Radom et al., 2013). Several aptamers have been selected against a large variety of targets including proteins, ions, toxins, drug molecules, cells, and tissues (Lee et al., 2008; Shamah et al., 2008; Stoltenburg et al., 2007), through an in vitro selection procedure known as SELEX (Systematic Evolution of Ligands by Exponential Enrichment) (Fig. 2).

The SELEX methodology starts with a chemically synthesized random oligonucleotides library (up to 10^{15} different sequences). As illustrated in Fig. 2, the selection process may be divided into 3 steps, namely binding, separation/partitioning and amplification, which are iteratively repeated to obtain nucleotides with improved binding ability towards the desired target. After a number of cycles (generally 5–

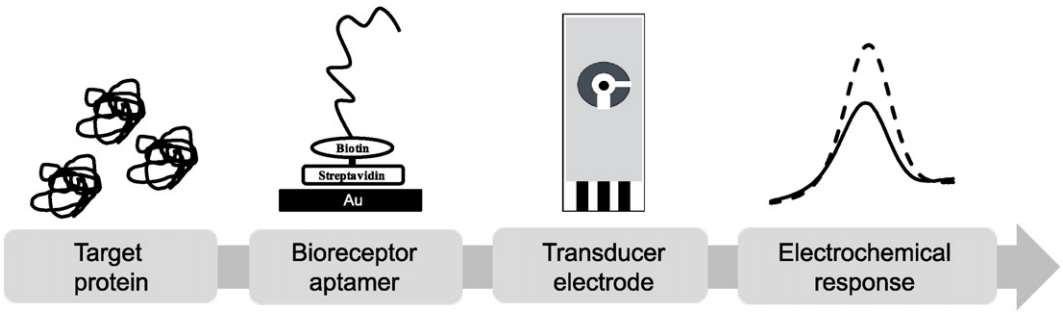

Fig. 1. Schematic illustration of a biosensor. The bioreceptor is immobilized on the surface of the transducer (signal converter). The binding event (i.e. binding of the bioreceptor to the target molecule) is then transformed into a measurable signal by the transducer, which leads to a measurement unit within the electronic device.