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Electrochemical aptasensors for the assessment of food quality and safety

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ABSTRACT

Development of highly sensitive analytical procedures for food contaminants is one of the critical points in addressing new challenges related to food safety worldwide. Electrochemical aptamer-based sensors have been intensively investigated as potential analytical tools providing the desired portability, fast response, sensitivity and specificity in addition to lower cost and simplicity versus classical methods. The paper summarizes the aptasensors reported in the literature in the last 3 years, that have been used in applications related to food safety. New trends pertaining to increasing the sensitivity of detection by using nanomaterials and engineering of new aptamers are briefly discussed. With the recent development of new aptamers and following the lead of aptasensors devoted to biomedical field, the next years will witness an avalanche of new exciting electrochemical aptasensors for food safety.

Contents

1. Introduction

Food safety is a major concern worldwide and a priority of governmental programs in many countries. A globalised economy, together with changes in food consumption patterns (e.g increased

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preference for raw and undercooked foods) as well as the appearance of new processing technologies have led to the emergence of novel challenges related to food safety. Among others, the development of better analytical procedures is critical for ensuring fast reaction in situations of food contamination by toxic compounds. In this context, biosensors have been intensively investigated [1] as potential analytical tools providing the desired portability, fast response, sensitivity and specificity in addition to lower cost and simplicity versus classical methods (mostly chromatography-based, ELISA, PCR or cell culture in case of pathogenic bacteria). Electrochemical aptasensors appear particularly promising as they combine sensitivity and advantages of electrochemical detection proven with the commercial glucose sensors with the numerous advantages offered by aptamers for the specific recognition of target analyte. Aptamers are small single stranded DNA or RNA sequences which inherently adopt stable three dimensional sequence-dependent structures and bind a target ligand with high affinity [2]. Aptamers have been selected for a wide range of molecules from small ones such as ions, organic dyes, drug residues, mycotoxins to proteins and up to bacteria or viruses. Their production by systematic evolution of ligands by exponential enrichment (SELEX [3,4],) is reproducible, well established, they can be labeled easily without affecting the affinity for their ligands and are more stable than antibodies and many enzymes-all the attributes required for a successful commercial application. The effervescence of aptamer-focussed research is illustrated by numerous reviews summarizing data on development of aptamers and applications in food analysis [5–8], electrochemical aptasensors [9–12] and combinations of aptamers with different nanomaterials for biosensing applications [13,14]. In this paper we will focus on aptasensors described in the last 3 years that have been applied for the analysis of food samples.

2. Discussions

2.1. Construction of aptasensors and sensing strategies

Electrochemical aptasensors are developed by immobilizing an aptamer on a conducting substrate, Au and carbon-based electrodes being preferred in applications related to food safety. Among various electrochemical methods Electrochemical Impedance Spectroscopy-EIS, voltammetry (Cyclic Voltammetry-CV, Differential Pulse Voltammetry-DPV, Square Wave Voltammetry-SWV, Linear Sweep Voltammetry-LSV), Field Effect Transistor and potentiometry have been used in conjunction with aptasensors as reviewed elsewhere [9–12].

Either the aptamers have been directly immobilized on these electrodes (e.g chemisorption of thiolated aptamers on Au electrodes) or, most of the time, the surface was first functionalized, to allow the robust attachment of appropriate amounts of aptamer and improve electron-transfer properties, or even to immobilize electroactive probes that could be used as reporters of binding events. Surface functionalisation strategies range from simple electrochemical deposition of diazonium salts or Au nanoparticles (AuNPs) on carbon-based electrodes, to modification with conducting polymers and up to sequential deposition of several nanocomposites. Nanomaterials and nanocomposites have been used to increase the electroactive area, increase the loading with aptamer and provide a tridimensional support, facilitating aptamer immobilisation and minimizing steric hindrances [13,14].

Aptamer immobilisation on the transducer surface is a determining step for the performances of obtained aptasensor. Most common approaches for ensuring adequate stability, surface coverage by aptamer and maintaining the same binding affinity as displayed in solution include: chemisorption of thiolated aptamers on Au electrodes; attachment of biotinylated aptamer to avidin-modified sensor surfaces; click chemistry immobilisation of azide-ended aptamer to alkyne-modified surfaces; covalent immobilisation of amine-ended aptamers by amine coupling to carboxyl groups on functionalized surfaces, covalent immobilisation of amine-ended aptamer to functionalized surfaces containing amine groups via glutaraldehyde etc. Spacers are sometimes used to allow sufficient flexibility conformational freedom to the aptamer.

The assay format and aptasensing strategy are largely determined by the size of the target ligand [11]. Aptamers selected for small molecules have lower affinity to their targets, with dissociation constants Kd in the nM-μM range as compared to aptamers for large ligands, which typically have Kds in the pM-nM range [11]. Similarly to immunoassays, direct, competitive (displacement) and sandwich assays have been proposed with electrochemical aptasensors (Fig. 1). In sandwich assays (Fig. 1a) the target molecule is captured between two aptamers or between one aptamer and an antibody that bind to different regions of the molecule. The capture aptamer is anchored to sensor surface, while the other biorecognition element is used for detection and is labeled with an enzyme (E), nanoparticles, or various catalysts [11]. Sandwich-type assays are mostly used for large molecules such as proteins, while displacement assays (Fig. 1b) or direct detection based on conformational changes of the aptamer (Fig. 1c), induced by binding to the target molecule were preferred for smaller analytes. Both label-free aptasensors and sensors using either labeled aptamers or detection probes have been developed so far.

Translation of the binding event into an electrochemical signal is achieved by many ingenious ways illustrated in several reviews [6,9,10,12]. These include:

(i) use of electrochemically active species that bind to DNA (e.g Methylene Blue which binds to guanine bases on DNA and it is known as a DNA intercalator or $Ru(NH_3)_6^{3+}$ that binds electrostatically to the phosphate backbone in DNA);

(ii) aptamers or complementary strands labeled with electroactive species like Methylene Blue or ferrocene, for which analyte binding results in modification of label's proximity to the electrode or steric hindrances, making their electrochemical signal to change accordingly. Upon conformational changes of the aptamer, the label can get further away from the electrode and its electrochemical signal decreases ("signal off"), or on the contrary, the probe is getting closer to the electrode surface and its electrochemical signal increases ("signal on").

(iii) redox species freely diffusing in solution for which the binding event will cause to either block or ease their electron transfer to the electrode. A widely preffered, label–free detection strategy is Faradaic EIS relying on $Fe(CN)_6^{3-/4-}$ as reporter of the aptamer-analyte binding event [15–18]. In addition to impedimetric sensors, DPV detection was also explored in conjunction with $[Fe(CN)_6]^{3-/4-}$ to achieve sensitive detection in electrochemical aptasensors for food safety [19,20].

(iv) oftentimes in electrochemical aptasensor assays, enzyme labels are used in amplification systems, similarly to ELISA tests. In this case, the amount of analyte involved in the binding event is correlated with the amount/activity of enzymatic label. The electrochemical signal used for quantitative measurements originates from the oxidation/reduction of a common substrate or product of the enzymatic reaction catalysed by alkaline phosphatase (ALP) or horseradish peroxidase (HRP). ALP catalyses the dephosphorylation of 1-naphtylphosphate to 1-naphtol, which is further electrochemically oxidised [21]. Typical amplification systems based on HRP require the addition of electroactive substrates such as 3,3',5,5'-tetramethylbenzidine (TMB) [22] and hydroquinone(HQ) [23]. These are oxidised by HRP in the presence of H_2O_2 to TMB_{ox} or benzoquinone (BQ), respectively. Detection is achieved by electrochemical reduction of TMB_{ox}