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Electrochemical DNA sensors and aptasensors based on electropolymerized materials and polyelectrolyte complexes

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ABSTRACT

DNA sensors based on oligonucleotides and aptamers immobilized using electropolymerization and layer-by-layer assembling are reviewed. The conditions of electropolymerization and the role of electrosynthesized layers are considered for polyaniline, polypyrrole, polythiophene, polyphenazines and their derivatives with particular attention to immobilization of bioreceptors and signal detection principles. The performance of DNA sensors for hybridization detection and for the determination of low-molecular intercalators and DNA damaging factors is reviewed. Besides, the composition of polyelectrolyte complexes utilizing DNA receptors are considered depending on the analyte nature and functions of polyionic components and auxiliary reagents used for surface layer coatings.

Contents

1. Introduction

The interest to the DNA sensors has been dramatically increased in the past decades due to great significance of their application including detection of hybridization events, DNA damage and antitumor drugs analysis [1,2]. Electrochemical transducers offer broad opportunities in DNA sensor design due to simple experiment protocols, inexpensive and mostly commercially available equipment. Together with well-developed theory of electrochemical phenomena, these advantages result in intensive progress in electrochemical DNA sensors development.

The use of electrochemical transduction principles has a natural limitation in rather low electrochemical activity of DNA molecules, which can be detected only with special measurement technique like differential pulse voltammentry (DPV) and adsorptive stripping voltammetry and chronoamperometry on mercury or carbon electrodes [3]. Recently, new approaches have been suggested to overcome this limitation by special design of appropriate transducer. Electropolymerized materials and polyelectrolyte complexes implementing DNA receptor elements are of special importance due to advantages they possess, i.e. variety of electrochemical characteristics, easy implementation of the DNA probes, preservation of native structure of DNA, high reproducibility of the biosensor characteristics and compatibility with different transducer types with no respect of their dimensions and shape.

In this review, electrochemical DNA sensors based on electropolymerized materials and polyelectrolyte complexes are

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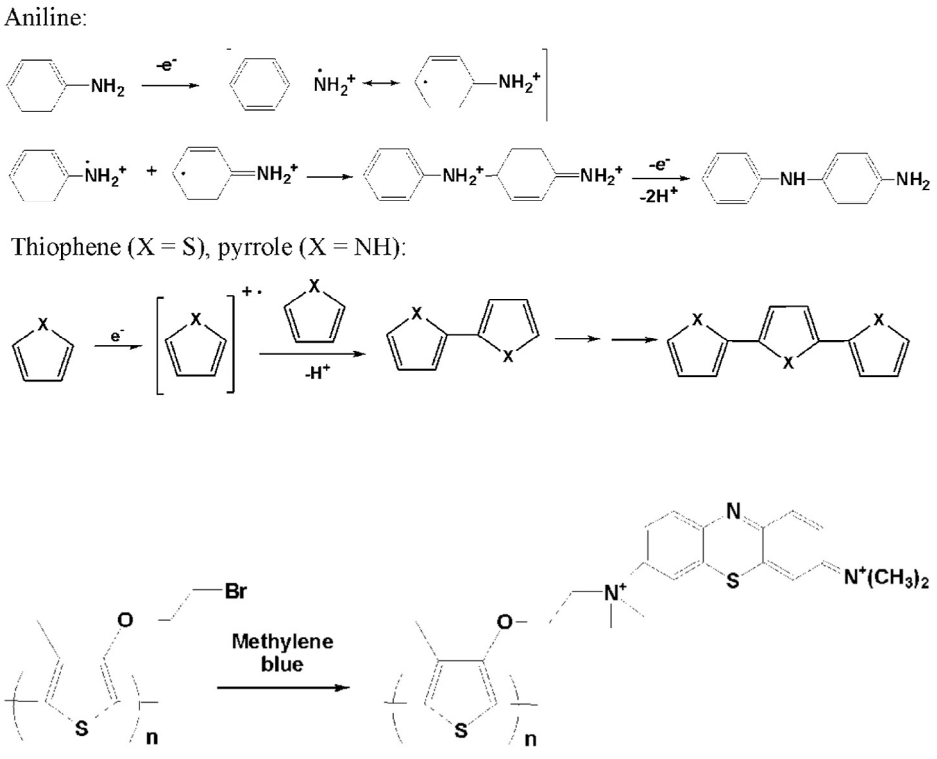
reviewed with examples of recent publications illustrating the advantages and limitations of these carriers within last decade.

2. Electropolymerized materials in DNA sensors and aptasensors

2.1. General characterization of electropolymerized materials and their inclusion in DNA sensor assembly

Electropolymerization involves the formation of oligomeric or polymeric products by electrochemical oxidation of their monomers [4]. The products are insoluble and commonly deposited on the electrode surface. Although many different polymers obtained by electrolysis have been described in the DNA sensor assembly, only few of them are characterized to establish the mechanism of polymerization and product structure. The polymerization starts with the formation of a cation radical at high anodic potential. After that, head-to-tail coupling leads to the dimer formation followed by its oxidation and addition of another monomer molecule. The appropriate schemes are presented for aniline, thiophene and pyrrole polymerization (1).



(1)

(2)

The DNA addition to the reaction mixture affects polymerization due to coordination of the cationic intermediates to negatively charged phosphate residues in DNA structure [5]. The entrapment of DNA into the growing polymer film results in formation of polyelectrolyte complexes used for enhancement of redox activity or electroconductivity area and detection of specific DNA interactions especially those with small molecules, e.g., antitumor drugs.

Alternatively, the DNA molecules can be immobilized onto the polymer layer by electrostatic interactions or covalent binding to the side groups. The electropolymerized film provides regular positioning of anchoring groups on the transducer surface. The accessibility of the oligonucleotides for bulky analytes in such coatings can be extended by introduction of appropriate spacers. The mechanisms of the DNA introduction described are compatible with simultaneous entrapment of auxiliary components, e.g., with chemical or electrochemical synthesis of metal nanoparticles, covalent binding of redox mediators etc. [6].

Electrochemical characteristics of the polymers including their ability of DNA wiring depend on the monomer nature and regularity of the polymer structure. It can be modified by implementation of appropriate functional groups in the side chain substituents. Thus, introduction of Methylene blue, a DNA intercalator, in the polythiohene structure (2) was applied for hybridization of complementary oligonucleotides which changed the redox activity of the phenothiazine dye [7].

Derived monomers applied for electropolymerization, both alone and in the mixture with unsubstituted analogs, are presented in Fig. 1 for aniline, pyrrole and thiophene.

Chemical modification of the polymerization products is performed for two purposes, i.e. DNA immobilization and signal generation/transduction [8]. For polyaniline (PANI) derivatives, anionic substituents can also participate in electrostatic interaction with oxidized form of the polymer similarly to low-molecular counter anions. The formation of such "self-doped" PANI decreases the pH dependence of its electroconductivity and redox activity. Introduction of DNA bearing large negative charge of phosphate residues plays similar role. This can be used for detection of hybridization events that increase the charge density of the double stranded (ds-) DNA helix against single DNA probe. Covalent immobilization assumes the reaction between the terminal functional groups of the substituents and those of DNA probe as shown for polythiohene derivative in Equation (3) [9]. Other examples of covalent DNA immobilization are given below in the description of appropriate biosensors.

All the electropolymerization products can be subdivided into three groups in accordance with their electrochemical properties:

(1) Electroconductive polymers that exert their own electron conductivity (PANI, polypyrrole (PPY), polythiophene and their structural