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Label-free electrochemical aptasensor for cytochrome *c* detection using pillar[5]arene bearing neutral red

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ABSTRACT

Novel electrochemical aptasensor toward cytochrome *c* (Cyt *c*) has been developed on the base of glassy carbon electrode (GCE) modified with electropolymerized neutral red (Poly-NR) and decacarboxylated pillar[5]arene (P[5]A-COOH) bearing terminal neutral red (NR) and aminated aptamer specific to Cyt *c*. Addition of Cyt *c* resulted in decrease of the cathodic peak current of NR on cyclic voltammogram due to suppression of the electron exchange between reduced and oxidized NR forms in the surface layer. The implementation of Cyt *c* in the surface layer was confirmed by scanning electron microscopy (SEM), atomic force microscopy (AFM) and electrochemical impedance spectroscopy (EIS). Depending on the content of the surface layer and assembling protocol, the limits of detection (LODs) varied from 0.02 to 1.0 nM and linear range of concentrations was within three orders of magnitude. Interfering influence of some proteins and polyethylene glycol was characterized. The aptasensors developed can find application in detection of Cyt *c* as apoptosis agent in blood serum. This has been partially validated in model blood serum mimicking the ionic composition of the plasma.

1. Introduction

There is an urgent need in the development of simple and reliable biosensors devoted to the detection of biomolecules that play significant role in medical diagnostics and therapeutic treatment [1]. The introduction of modern biosensor technologies expands the number of such biomarkers and is considered as promising alternative to expensive and sometimes time consuming laboratory tests. Further progress in this area is mainly directed to the development of new technologies for the detection of specific interactions between an analytes and biorecognition element to reach faster and more sensitive response [2]. Aptamers as new recognition elements obtained by combinatorial chemistry from random DNA library and selected against targets by affine chromatography have recently found increased attention in medical diagnostics and targeted therapy [3]. Aptamer based biosensors called as aptasensors have been reported for the detection of various species, e.g., thrombin [3,4], mycotoxins [5,6], lysozyme [7], dopamine [8], prions [9], cancer biomarkers [10,11], cells [12] and viruses [13]. The determination of other analytes as well as the prospects of application of aptasensors in various areas were recently summarized in reviews [14–16].

Cyt *c* is an electron-carrying mitochondrial protein that is used in various electrochemical sensors as redox mediator for the detection of nitric oxide [17], nitrite [18] and hydrogen peroxide [19,20]. Besides, Cyt *c* biosensors were successfully applied for the detection of superoxide radical and subsequent assessment of antioxidant capacity [21–23]. The participation of the Cyt *c* molecules in the electron transfer reactions is also considered as a model of similar redox paths in biological membranes [24]. Regarding diagnostic significance, the release of mitochondrial Cyt *c* into the cytoplasm indicates the cell apoptosis [25]. The monitoring of the Cyt *c* release was suggested to use for the screening of anti-cancer drugs [26]. Decrease in the Cyt *c* redox activity was successfully applied for the sensitive detection of anti-respiratory poisons [27]. The

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