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Optimization of cytochrome c detection by acoustic and electrochemical methods based on aptamer sensors

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ABSTRACT

We report the effect of various factors such as oligonucleotide sequence, buffer composition, ionic strength for optimal determination of cytochrome c (cyt c) by DNA aptamer sensors using thickness shear mode acoustics (TSM) and electrochemical methods. Up to now, several DNA aptamers specific to cyt c have been selected and used in various sensing approaches including optical, electrochemical and mass sensitive transducers. We have analyzed the response of three different aptamers immobilized via biotin-neutravidin method on a gold support by TSM technique. Using this approach we have shown that only 76-length base sequence (apt 76) exhibited specific binding to cyt c with detection limit of 0.50 ± 0.05 nM. This aptamer was then studied under different ionic conditions showing an optimal response for HEPES buffer. Apt 76 based sensor has been also examined by electrochemical methods. However due to the electroactive nature of cyt c, the response of this aptamer was less favorable in comparison with TSM. The apt 76 based sensor was tested also in spiked samples of human plasma by TSM achieving a recovery of $92 \pm 6.6\%$ for 1 nM cyt c.

1. Introduction

Cytochrome c (cyt c) is a small peripheral membrane heme protein that takes part in essential biological functions as electron carrier and metal-ion liaison. Cyt c acts sometimes as a launching pad in living processes such as ATP synthesis, protein dephosphorylation, cellular respiration or metabolism and others. It operates as a precursor of programmed apoptotic death of cells when released into the cytosol after mitochondrial membrane permeability is disturbed [1]. Under physiological conditions, cyt c is positively charged owing to its lysine and arginine amino acid residues. Cyt c also exhibits high electrochemical activity and it is one of the most investigated redox proteins. Since the first quasi-reversible electrochemistry of cyt c at a gold electrode reported in 1977 [2,3], other compounds have been used to enhance the electron transfer of cyt c at surfaces using gold nanoparticles-chitosan-carbon nanotubes composite films [4], sulfonated polyaniline nano-networks [5], or gold nanoparticles-DNA networks [6]. The incorporation of DNA strands to cyt c has been demonstrated to promote the electronic transfer principally due to electrostatic interactions [7–9]. The interaction of cyt c using self-assembled monolayers of alkyl thiols with different hydrocarbon chains was studied by Surface Plasmon Resonance (SPR) [10], Quartz Crystal Microbalance (QCM) and Atomic Force Microscopy (AFM) [11]. Thanks to its redox properties, cyt c has been applied as mediator in detection of hydrogen peroxide [12], superoxide radicals [13,14], metallic ions and other compounds serving as catalytic agent and redox indicator in electrochemical reactions [15,16].

On the other side, detection of cyt c by diverse platforms and approaches has been also reported due to its importance as promising cancer marker. Once released into cytosol, cyt c cooperates with the activation and initiation of the apoptotic protease cascade. The normal level of cyt c in human serum was reported to be approximately <25 ng/mL (<2 nM) [17,18]. However, increased concentration of cyt c has been observed in serum from patients with hematologic malignancies. Upon onset of cancer chemotherapy, the serum level of cyt c in the majority of the patients raised as a result of increased cell death [19]. Therefore, higher concentra-

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