Talanta 102 (2012) 156–163

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Label-free aptasensor for thrombin determination based on the nanostructured phenazine mediator

Gennady A. Evtugyn [a,*], Veronika B. Kostyleva [a,b], Anna V. Porfireva [a], Maria A. Savelieva [a], Vladimir G. Evtugyn [c], Rusal R. Sitdikov [d], Ivan I. Stoikov [d], Igor S. Antipin [d], Tibor Hianik [b]

[a] Analytical Chemistry Department of Kazan Federal University, 18 Kremlevskaya Street, Kazan 420008, Russian Federation
[b] Department of Nuclear Physics and Biophysics, Comenius University, Mlynska dolina F1, 842 48 Bratislava, Slovakia
[c] Electron Microscopy Laboratory of Biology Faculty of Kazan Federal University, 18 Kremlevskaya Street, Kazan 420008, Russian Federation
[d] Organic Chemistry Department of Kazan Federal University, 18 Kremlevskaya Street, Kazan 420008, Russian Federation

ARTICLE INFO

Available online 8 July 2012

Keywords:
Aptasensor
Thrombin
DNA aptamer
Thiacalixarene
Neutral Red

ABSTRACT

New aptasensors based on DNA aptamer and polycarboxylated thiacalix[4]arenes in *cone*, 1,3-*alternate* and *partial cone* configurations bearing Neutral Red (NR) at substituents at the lower rim have been developed and applied for thrombin detection. The assembly of the biorecognition layer was optimized by AFM and EIS study to reach the maximal coverage and regular composition of the surface layer. The interaction of the NR groups with thrombin suppressed the electron hopping between oxidized and reduced mediator groups. This regularly decreased the NR peak current and increased the resistance of the charge transfer. The aptasensor makes it possible to detect from 1 nM to 1 μM of thrombin with the detection limit of 0.05–0.5 nM. No effect of the 1000 excess of bovine serum albumin on the signal was observed. The influence of thiacalix[4]arene configuration on the sensitivity of aptasensor signal toward thrombin is discussed.

1. Introduction

DNA and RNA aptamers are synthetic oligonucleotides binding low-molecular compounds, proteins and other biomolecules with high specificity and efficiency compared to those of antigen–antibody interactions [1]. The interest toward aptamers expressed in the past decades is related both to the relatively simple strategy of their selection based on a combinatorial chemistry approach and the high potential of their use in the assembly of biorecognition devices, i.e., biosensors, logic gates, electrophoresis, affine chromatography systems, etc. [2]. In comparison with antibodies, the aptamers exhibit long-term stability, a simpler operation mode and a better reproducibility of binding abilities during production and purification. The modification of the aptamers with optical or redox active labels or with functional groups required for immobilization is usually easier than that of antibodies as well [3]. The range of the affinity constant of aptamers toward their substrates varies from micro- to nanomolar values. This offers new opportunities for the highly sensitive detection of various species based on their binding with aptamers in the assembly of appropriate biosensors.

Various strategies are employed for the generation of the biosensor signal related to the aptamer—analyte interaction. Most often, the following approaches are applied: (1) the use of redox labels, i.e., ferrocenes [4–6] , phenothiazine dyes [7–10], thionine [11] which shifts their potential or oxidation current due to a recognition event; (2) conformational changes of the aptamer structure caused by the analyte binding [12–14] and (3) accumulation of the redox labels within the surface layer similar to that of sandwich immunoassay [15–18]. The use of redox labels provides the high sensitivity of the signal measurement. Nevertheless, the further enhancement of the aptasensor assembly is required to simplify the measurement protocol, reduce its cost and labor content.

Nanosized supports for aptamers and redox labels are of special interest in this area. The application of colloidal gold [11,15,17–21] or, carbon nanotubes, [9,22–24] not only amplifies the signal due to electron mediation but also increases the surface loading and accessibility of the aptamer binding sites for the analytes. This results in a faster electron exchange and a lower working potential as well as in a lesser interference with electroactive matrix components.

Thrombin is a multifunctional serine protease that plays an important role in the procoagulant and anticoagulant functions [24,25]. Thrombin is also involved in the other activities like inflammation and wound healing. The first DNA aptamer selective

* Corresponding author.
E-mail address: Gennady.Evtugyn@ksu.ru (G.A. Evtugyn).

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http://dx.doi.org/10.1016/j.talanta.2012.07.007