Electrochemical Aptasensor Based on a Macrocyclic Ligand Bearing Neutral Red

Gennady Evtugyn,*[a] *Veronika Kostyleva*,[a, c] *Rusal Sitdikov*,[b] *Anna Porfireva*,[a] *Maria Savelieva*,[a] *Ivan Stoikov*,[b] *Igor Antipin*,[b] *Tibor Hianik*[c]

[a] Analytical Chemistry Department, Kazan Federal University, 18 Kremlevskaya Street, Kazan, 420008, Russian Federation
[b] Organic Chemistry Department, Kazan Federal University, 18 Kremlevskaya Street, Kazan, 420008, Russian Federation
[c] Department of Nuclear Physics and Biophysics, Comenius University, Mlynska dolina F1, 842 48 Bratislava, Slovakia
*e-mail: Gennady.Evtugyn@ksu.ru

Received: August 10, 2011
Accepted: September 17, 2011

Abstract
An amperometric aptasensor has been developed by immobilization of DNA aptamer with Neutral Red (NR) on polycarboxylated thiacalix[4]arene onto the electropolymerized NR layer at a glassy carbon electrode. The NR reduction current recorded after 10 min incubation decayed with increased thrombin concentration due to limitation of the electron exchange in the surface layer. The aptasensor makes it possible to determine 0.1–50 nM of thrombin (limit of detection 0.05 nM). The aptasensor can be used for the direct determination of thrombin in blood serum and does not exert any alteration of the response in the presence of 100 fold excess of serum proteins.

Keywords: Aptasensor, Thrombin, DNA Aptamer, Thiacalixarene, Neutral Red

DOI: 10.1002/elan.201100435

Highlights

- Novel aptasensor based on the Neutral Red (NR) and aptamer toward thrombin co-immobilized on polycarboxylated thiacalix[4]arene has been proposed for sensitive thrombin determination.
- After the contact of the aptasensor with thrombin, the current of the NR reduction decayed due to steric limitation of the electron exchange in the surface layer.
- To accelerate the aptasensor recovery, stabilize the signal and reduce measurement time, the electrode was first covered with a polymeric NR film and aptasensor treated with ferricyanide ions
- The aptasensor makes it possible to detect 0.1–50 nM of thrombin (limit of detection 0.05 nM) with a minimal influence of serum proteins

1 Introduction

DNA and RNA aptamers [1,2] are of growing interest due to high selectivity and efficiency of biorecognition of proteins [3–5], toxins [6,7] and some other compounds important for clinical diagnostics and environmental pollution control [8,9]. In comparison with the traditional antibodies, aptamers show long-term stability, easier operation mode, multiple possibilities for modification and relatively low cost of mass production.

Many of the strategies of aptamer application in biosensor assembly are based on electrochemical transducers [10,11] which record changes in the signals related to the redox labels introduced into the aptamer structure or to diffusionally free indicators. Methylene Blue [12–14], thionine [15], Methylene Green [16], and ferrocene [17,18] have been successfully used for amperometric signal detection. In many cases, electrochemically active labels are combined with nanosized materials which both increase the specific concentration of aptamers onto the surface and electrically wire the labels to the electrode. Until now, electroconductive nanoparticles were predominantly used for assembling such a biorecognition layer, e.g., colloidal gold [19–22], single- [23,24] and multiwalled [25,26] carbon nanotubes. To some extent, they also participate in the signal generation and improve the operational performance of the aptasensors due to a faster electron exchange and a lower working potential.

In many cases, the aptamer–analyte interaction is quantified by changes in the voltammetric signal generated by redox active labels. The formation of the complex especially with bulky molecules limits the electron transfer and decreases the signal in comparison with blank experiment. However, the use of electroconductive materials as aptamer support decreases the effect of steric factors on the signal due to their involvement in the electron exchange which becomes less sensitive to the analyte molecules. For this reason, alternative approaches to the aptamer immobilization on the support with reduced adsorptive and electrochemical activity have been elaborated. With thrombin determination, silica nanocapsules bearing

