Talanta 130 (2014) 370–376

Contents lists available at ScienceDirect

Talanta

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

Electrogenerated chemiluminescence aptasensor for ultrasensitive detection of thrombin incorporating an auxiliary probe

Zhejian Li, Lijuan Sun, Ying Zhao, Libin Yang, Honglan Qi, Qiang Gao, Chengxiao Zhang*

Key Laboratory of Analytical Chemistry for Life Science of Shaanxi Province, School of Chemistry and Chemical Engineering, Shaanxi Normal University, Xi'an 710062, China

ARTICLE INFO

Article history:
Received 4 April 2014
Received in revised form
3 July 2014
Accepted 10 July 2014
Available online 18 July 2014

Keywords:
Aptasensor
Electrogenerated chemiluminescence
Thrombin
Aptamer
Ruthenium(II) complex
Auxiliary probe

ABSTRACT

A novel electrogenerated chemiluminescence (ECL) aptasensor for ultrasensitive detection of thrombin incorporating an auxiliary probe was designed by employing specific anti-thrombin aptamer as a capture probe and a ruthenium(II) complex-tagged reporter probe as an ECL probe and an auxiliary probe to assist the ECL probe close to the surface of the electrode. The ECL aptasensor was fabricated by self-assembling a thiolated capture probe on the surface of gold electrode and then hybridizing the ECL probe with the capture probe, and further self-assembling the auxiliary probe. When analyte thrombin was bound with the capture probe, the part of the dehybridized ECL probe was hybridized with the neighboring auxiliary probe, led to the tagged ruthenium(II) complex close to the electrode surface, resulted in great increase in the ECL intensity. The results showed that the increased ECL intensity was directly related to the logarithm of thrombin concentrations in the range from 5.0×10^{-15} M to 5.0×10^{-12} M with a detection limit of 2.0×10^{-15} M. This work demonstrates that employing an auxiliary probe which exists nearby the capture probe can enhance the sensitivity of the ECL aptasensor. This promising strategy will be extended to the design of other biosensors for detection of other proteins and genes.

1. Introduction

The detection and quantification of extremely low concentrations of specific proteins play pivotal roles in basic research and clinical applications. Aptamers are artificial oligonucleotides (DNA or RNA) that can bind to a wide variety of entities (e.g., metal ions, small organic molecules, proteins, and cells) with high affinity, equal to or often superior to that of antibodies [1–4]. In recent years, aptamers taken as molecular recognition elements have been received considerable attention in protein analysis due to their advantages such as simple synthesis, easy labeling, good stability, and wide applicability [5]. Aptasensors (aptamer-based biosensors) which fuse the exquisite sensitivity and specificity of the probes with the suitable transducers, are simple and inexpensive analytical devices that may be able to provide escalating quantities of protein information [6–9]. A variety of aptasensors including optical [10], piezoelectric [11], voltammetric [12–13], electrochemical impedimetric [14,15], and electrogenerated chemiluminescence (ECL) [16–18] aptasensor have been reported for the detection of small molecules and proteins. Among them, ECL aptasensor is a promising one owing to the combination of advantages of both electrochemical aptasensor and chemiluminescent aptasensor, such as high sensitivity and easy controllability [19–21]. However, these reported aptasensors have common detection limits in the nM range. Therefore, design of ultrasensitive aptasensors for the detection of protein biomarkers at an ultralow level in the early stage of diseases is required.

Many reports have been devoted to improve the sensitivity of ECL aptasensors for the determination of proteins. These include employment of nanomaterials, such as electrode modified materials [22], probe carriers [23–25], and signal materials [26] to enhance and to amplify signal; investigation of transduction strategies, such as target-induced strand displacement [27–29], target-binding induced conformational changes of surface confined aptamers in which the distance of ECL signal is altered [30–32]; exploration of the smart assist approaching, such as supersandwhich approachs [33], rolling circle amplification (RCA) [34], strand displacement amplification (SDA) [35] and enzyme label [36]. These signal amplification methods bear the advantage of high sensitivity, yet most of the previously reported ECL aptasensors involve complicated structural design of the capture probes, complex pretreatment of electrodes, and frequent interference from the detection environment [37]. Mao's group first reported an electrochemical aptasensor with ferrocene-tagged aptamer-complementary DNA oligonucleotides as probe [38]. Upon target (thrombin) binding, the aptamers confined onto electrode surface

* Corresponding author. Tel.: +86 29 8153 0833; fax: +86 29 8153 0727.
E-mail address: cxzhang@snnu.edu.cn (C. Zhang).

http://dx.doi.org/10.1016/j.talanta.2014.07.029
0039-9140/

dissociate from their respective cDNA(aptamer-complementary DNA) oligonucleotides into the solution and the single-stranded cDNA could thus tend to form a hairpin structure through the hybridization of the complementary sequences at both its ends. Such a conformational change of the cDNA resulting from the target binding-induced dissociation of the aptamers essentially leads to the change in the voltammetric signal of the redox moiety labeled onto the cDNA and thus constitutes the mechanism for the electrochemical aptasensors for specific target sensing. Zhang's group demonstrated a new approach to improve the sensitivity of the electrochemical aptasensor fabricated by self-assembling a thiol functionalized capture probe containing aptamer and an auxiliary probe on the surface of gold electrode and then complementarily hybridizing a methyl blue-tagged reporter probe with the capture probe [13]. The auxiliary adjunct probe can increase the chance of the dissociative reporter probe to collide with the electrode surface and facilitating the electron transfer. The biosensor with an adjunct probe exhibits improved sensitivity and a large dynamic range for DNA and the thrombin assay. To the best of our knowledge, an ECL aptasensor was designed by employing an auxiliary probe to assist the ECL probe close to the surface of the electrode that has not been reported.

The aim of the present work is to explore the development of a highly sensitive ECL aptasensor for the detection of proteins by simply introducing an auxiliary probe. As a model system, thrombin was chosen as a model analyte since it is a common protein that catalyzes many coagulation related reactions responsible for blood clotting [39]. The fabrication of the ECL aptasensor and ECL detection of thrombin is showed in Scheme 1. The ECL aptasensor was fabricated by self-assembling a thiolated capture probe on the surface of gold electrode and then hybridizing the ECL probe with the capture probe, and further self-assembling the auxiliary probe. When the analyte thrombin was bound with the capture probe, the part of the dehybridized ECL probe was hybridized with the neighboring auxiliary probe, which led to the tagged ruthenium(II) complex becoming close to the electrode surface, and resulted in a great increase in the ECL intensity. In this paper, the function of the designed auxiliary probe is discussed and the fabrication and analytical performance of the ECL aptasensor are presented.

2. Experimental

2.1. Reagents and apparatus

All oligonucleotides used were purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). Their sequences are listed as follows.

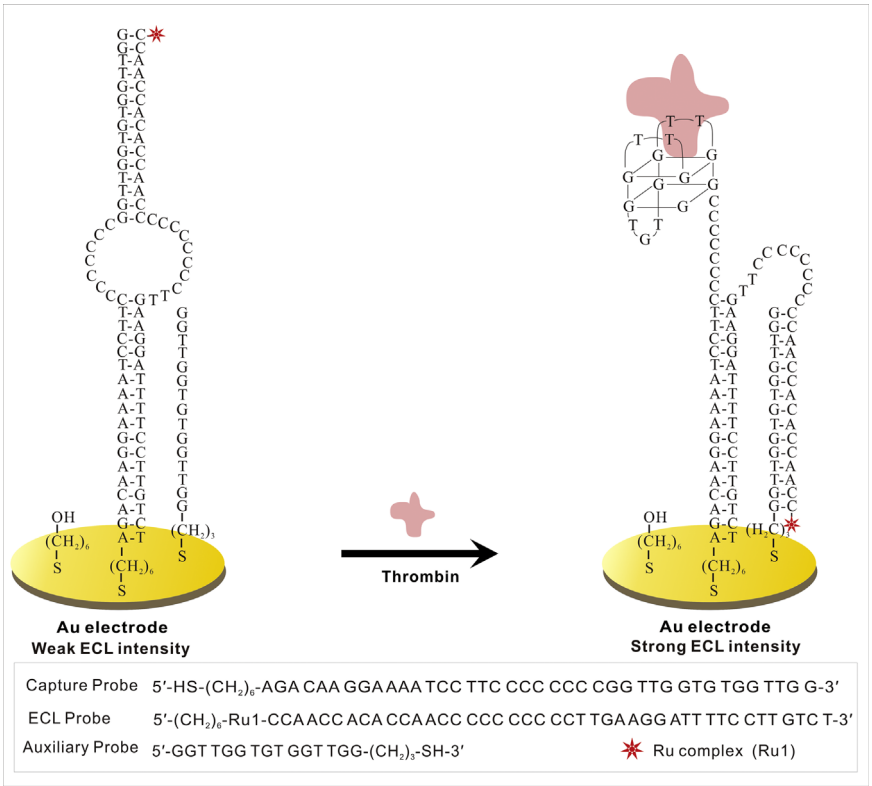
Capture probe, 5′-HS-$(CH_2)_6$-AGA CAA GGA AAA TCC TTC CCC CCC CGG TTG GTG TGG TTG G-3′ (Italic bases are the sequence of anti-thrombin aptamer).

Auxiliary probe, 5′-GGT TGG TGT GGT TGG-$(CH_2)_3$-SH-3′ (It is partly matched with the Italic part of the capture probe).

One base mismatched auxiliary probe, 5′-GGT TGG ***A***GT GGT TGG-$(CH_2)_3$-SH-3′

Reporter probe, 5′-NH_2-$(CH_2)_6$-*CCA ACC ACA CCA ACC* CCC CCC CCT TGA AGG ATT TTC CTT GTC T-3′ (Italic bases can be hybridized with the Italic part of the capture probe. The reporter probe was tagged with ruthenium(II) complex as ECL probe.).

$Ru(bpy)_2(mcbpy\text{-}O\text{-}Su\text{-}ester)(PF_6)_2$ (abbreviated as Ru1, ruthenium (II) complex), human alpha-thrombin, bovine plasma albumin (BSA), hemoglobin (Hb) and immunoglobulin G (IgG, Goat Anti-rabbit IgG) were purchased from Sigma-Aldrich, Inc. (U.S.A.) and used as received without further purification. 6-mercaptohexanol (MCH), Tri-*n*-propylamine (TPrA) and N,N-dimethylformamide (DMF) were obtained from Xi'an Chemical Reagent Factory (China). All chemical reagents were analytical grade and used without further purification. Millipore Milli-Q water (18.2 MΩ cm) was used throughout. 10 mM phosphate buffers saline (PBS, pH 7.4, 0.10 M NaCl + 10 mM NaH_2PO_4/Na_2HPO_4) was used as incubation buffer and washing buffer.



Scheme 1. Scheme for ECL detection of thrombin.