Analytica Chimica Acta 812 (2014) 176–183

Contents lists available at ScienceDirect

Analytica Chimica Acta

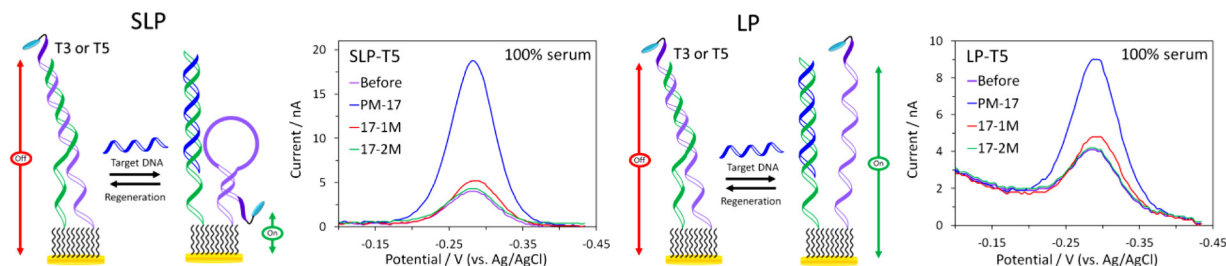
journal homepage: www.elsevier.com/locate/aca

Effect of redox label tether length and flexibility on sensor performance of displacement-based electrochemical DNA sensors

Zhi-gang Yu[a,b,*], Anita J. Zaitouna[b], Rebecca Y. Lai[b,*]

[a] *Key Laboratory of Green Chemical Engineering and Technology of College of Heilongjiang Province, College of Chemical and Environmental Engineering, Harbin University of Science and Technology, Harbin 150040, PR China*
[b] *University of Nebraska-Lincoln, 651 Hamilton Hall, Lincoln, NE 68588-0304, USA*

GRAPHICAL ABSTRACT



HIGHLIGHTS

- Four "signal-on" displacement-based E-DNA sensors were investigated.
- Stem-loop probe sensors have better signaling capability than linear probe sensors.
- Sensors that used a signaling probe with a T3 tether have a lower detection limit.
- Sensors that used a signaling probe with a T5 tether are more specific.
- Sensors that used a signaling probe with a T5 tether are more selective.

ARTICLE INFO

Article history:
Received 28 October 2013
Received in revised form
19 December 2013
Accepted 28 December 2013
Available online 9 January 2014

Keywords:
Electrochemical DNA sensor
Methylene blue
Linear probe, Stem-loop probe
Mismatch discrimination
Serum

ABSTRACT

This article summarizes the sensor performance of four electrochemical DNA sensors that exploit the recently developed displacement-replacement sensing motif. In the absence of the target, the capture probe is partially hybridized to the signaling probe at the distal end, positioning the redox label, methylene blue (MB), away from the electrode. In the presence of the target, the MB-modified signaling probe is released; one type of probe is capable of assuming a stem-loop probe (SLP) conformation, whereas the other type adopts a linear probe (LP) conformation. Independent of the sensor architecture, all four sensors showed "signal-on" sensor behavior. Unlike the previous report, here we focused on elucidating the effect of the redox label tether length and flexibility on sensor sensitivity, specificity, selectivity, and reusability. For both SLP and LP sensors, the limit of detection was 10 pM for sensors fabricated using a signaling probe with three extra thymine (T3) bases linked to the MB label. A limit of detection of 100 pM was determined for sensors fabricated using a signaling probe with five extra thymine (T5) bases. The linear dynamic range was between 10 pM and 100 nM for the T3 sensors, and between 100 pM and 100 nM for the T5 sensors. When compared to the LP sensors, the SLP sensors showed higher signal enhancement in the presence of the full-complement target. More importantly, the SLP-T5 sensor was

* Corresponding authors at: University of Nebraska-Lincoln, 651 Hamilton Hall, Department of Chemistry, Lincoln, NE 68588-0304, USA.
Tel.: +1 402 472 5340; fax: +1 402 472 9402.
E-mail addresses: yzgyh@126.com (Z.-g. Yu), rlai2@unl.edu (R.Y. Lai).

0003-2670/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aca.2013.12.040

found to be highly specific; it is capable of discriminating between the full complement and single-base mismatch targets even when employed in undiluted blood serum. Overall, these results highlight the advantages of using oligo-T(s) as a tunable linker to control flexibility of the tethered redox label, so as to achieve the desired sensor response.

© 2014 Elsevier B.V. All rights reserved.

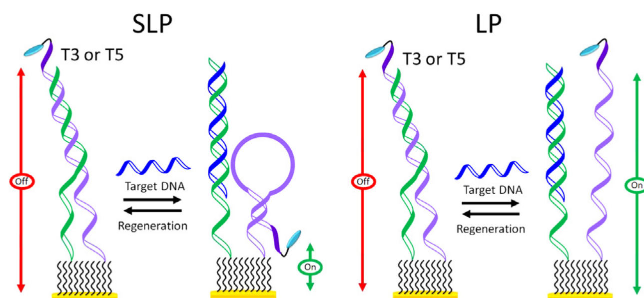
1. Introduction

Research has been conducted in the field of biosensors, specifically for detection of DNA and RNA, over the past few decades [1–3]. A wide range of optical and electrochemical detection strategies have since been reported [4–6]. While many of the sensing approaches feature impressive sensitivity, most are multi step processes and require the addition of complex reagents, rendering them less compatible with on-site and real-time sample analysis. Sensors that are sensitive, operationally convenient yet require low mass/power/volume are thus in high demand. Electrochemical DNA and RNA sensing approaches have gained substantial interest, among them, the electrochemical DNA (E-DNA) sensing platform has received the major share of attention since its development in 2003[7–9]. This first generation folding-based E-DNA sensor utilizes a stem-loop DNA probe (SLP), which signaling mechanism is based mostly on target-induced charge in the probe conformation [10,11]. In 2007, a linear probe (LP)-based version of this sensor was reported. Unlike the SLP E-DNA sensor, the LP sensor's signaling mechanism is based entirely on the change in the probe dynamics or flexibility upon target binding [12]. Independent of the signaling mechanism, both SLP and LP E-DNA sensors can be classified as "reagentless" sensors since the signal change is induced by target binding only; no exogenous reagents are required. They have also been proven to be sensitive, specific, and most importantly, selective enough to be employed directly in whole blood and other realistically complex biological samples [13].

Despite the merits associated with this electrochemical sensing platform, both SLP and LP sensors are innately "signal-off" sensors, which suffer from limited signaling capacity, in which only a maximum of 100% signal suppression can be attained under any experimental conditions [14,15]. "Signal-off" sensors are more susceptible to "false positive" results; the reduction in signal could be due to events other than target binding. Monolayer stability is, in particular, critical in maintaining good sensor performance for this class of "signal-off" sensors. Monolayer degradation often leads to a decrease in the signal even in the absence of the target. Thus, in recent years researchers have shifted their focus to the development of "signal-on" E-DNA sensors [16–21].

Most reported sensors only use one type of surface-immobilized probe; we recently designed two "signal-on" E-DNA sensors that features two surface-attached DNA probes (Scheme 1) [22,23]. For both sensors, an unlabeled capture probe and a MB-modified signaling probe are co-immobilized onto the interrogating electrode. The two probes are designed to be partially complementary to each other, enabling the formation of a short DNA duplex at the distal end of the probes. In the absence of the target DNA, the short duplex prevents the MB label on the signaling probe from accessing the electrode surface for efficient electron transfer; formation of the capture probe/target duplex, however, disrupts the 10-base inter-probe duplex, liberating the signaling probe. For the SLP sensor, the released signaling probe is capable of assuming a stem-loop conformation. The change in probe conformation and mobility confines the MB label close to the electrode, resulting in an increase in redox current. For the LP sensor, the liberated probe remains in the linear conformation, but the enhanced probe flexibility leads to the increase in redox signal. Similar to the "signal-off" E-DNA sensors, these two sensors have been demonstrated to be sensitive, specific and to work well under a wide range of experimental conditions [22,23].

Previous research has shown that the location of the redox label can have a large impact on sensor performance, which includes the signal change obtained in the presence of the target and binding kinetics [24]. For the newly developed dual-probe E-DNA sensors, this effect has not been investigated. Here we systematically characterized both SLP and LP "signal-on" E-DNA sensors that are fabricated using close-to-identical 25-mer signaling probe sequences (Scheme 1). Alternating current voltammetry (ACV) was used to investigate the sensors' hybridization efficiency and binding kinetics. Cyclic voltammetry (CV) was also used to characterize the sensors. It is worth noting that the four probes used in this study contain either a 3-thymine (T3) or 5-thymine (T5) linker between the last base of the probe and the methylene blue (MB) redox label. The use of T(s) to increase the tether length has several advantages. First, tether length and flexibility can be easily adjusted a by simply adding extra bases at the end of the probe sequence. The synthesis of these probes is also less challenging since the added T(s) is part of the DNA sequence [20]. The key difference between the current work and the previous works is the use of a longer tether between the last base on the probe strand and the redox label [23]. The tuning of the tether length by incorporating extra T(s) in the tether to alter sensor performance is universal and can potentially be used in the design of other E-DNA and electrochemical aptamer-based (E-AB) sensors [25].



Scheme 1. Design and signaling mechanism of the stem-loop (**SLP-T3**/**SLP-T5**) (A) and linear probe-based (**LP-T3**/**LP-T5**) (B) E-DNA sensors.

2. Experimental

2.1. Materials

Four thiolated and MB-modified DNA probes (**SLP-T3**, **SLP-T5**, **LP-T3**, **LP-T5**) were used as surface-immobilized signaling probes. A 25-base thiolated DNA probe was used as the target capturing probe (**T8-P**). All five probes were purchased from Biosearch Technologies Inc. (Novato, CA) (Fig. S1 in Supplementary data). Five DNA targets purchased from Integrated DNA Technologies (Coralville, IA) were used as received. The sequence information of the probes and targets are shown in the following. The single underlined portion of signaling probes forms a 10-base duplex with the italicized portion of the unlabeled capture probe (**T8-P**), whereas the double underlined portion of **T8-P** hybridizes to the target DNA. The mismatches in the targets are highlighted in bold.[0,1-2]DNA probes**SLP-T3**:5′

Download English Version:

https://daneshyari.com/en/article/1164149

Download Persian Version:

https://daneshyari.com/article/1164149

Daneshyari.com