Electrochimica Acta 282 (2018) 588–594

Contents lists available at ScienceDirect

Electrochimica Acta

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

Rational engineering of synergically stabilized aptamer-cDNA duplex probes for strand displacement based electrochemical sensors

Ziping Zhang*, Liping Dong, Qing Zhu

College of Life Sciences, Yantai University, Yantai, 264005, China

ARTICLE INFO

Article history:
Received 21 February 2018
Received in revised form
13 May 2018
Accepted 17 June 2018
Available online 19 June 2018

Keywords:
Aptamer
Strand displacement
Synergic stabilization
Electrochemical sensors

ABSTRACT

Electrochemical aptamer (EA) sensors employing electrode-bound aptamer-cDNA (cDNA: complementary DNA) duplex probes are simple and versatile sensing platforms for detection of many analytes. Target recognition in these sensors is a strand displacement process where target binding to aptamer displaces the cDNA strand. Despite many displacement-type EA sensors, a common drawback exists that sensor response rate is relatively slow (typically with target binding equilibrium time ranging from 0.5 to 3 h), which is mainly caused by a suboptimal conformation of the aptamer sequence in the conventional aptamer-cDNA duplex probe containing a relatively large number of aptamer-involved base pairs. Realizing that the key to optimizing sensor sensitivity and response rate of displacement-type EA sensors lies in engineering of a class of aptamer-cDNA probes with moderate duplex stability and a minimum amount of aptamer-involved base pairs, we explore here a unique means for rational design of this type of aptamer-cDNA duplex probes by using the concepts of synergic stabilization and base-pair regulation. We have integrated a short non-aptamer base-pair sequence and a short aptamer-involved base-pair sequence to engineer a class of synergically stabilized aptamer-cDNA duplex probe. Through further regulation of the number of aptamer-involved base pairs within the duplex we can tune not only the signal increase but also the response rate of these synergically stabilized duplex probes to achieve optimal sensor performance. The rationally engineered synergically stabilized aptamer-cDNA duplex probes are sensitive and exhibit significantly enhanced response rates (with target binding equilibrium times of 1 min for ATP and 2 min for cocaine). The concepts of synergic stabilization and base-pair regulation can be generalized to any aptamer-cDNA duplex probes and will help guide further development of displacement-type EA sensors for sensitive and quick detection of a wide variety of target analytes.

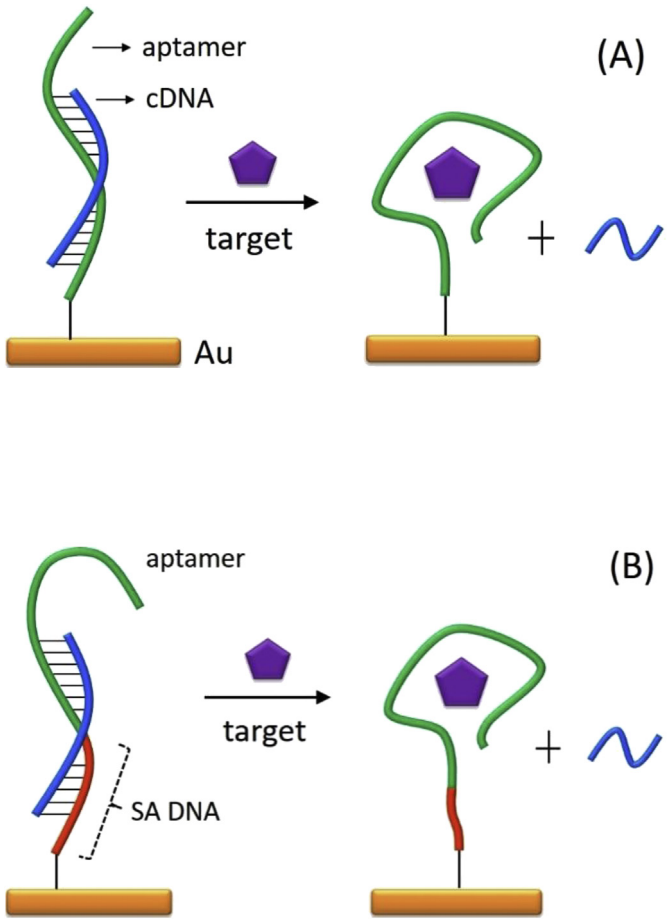
1. Introduction

Recent years have seen the development of a unique class of electrochemical aptamer (EA) sensors using a molecular recognition mechanism of target-induced strand displacement [1–3]. Examples include sensors for detecting proteins [4–6], small molecules [7–10] and metal ions [11]. Recognition elements in these sensors are electrode-immobilized aptamer-cDNA duplex probes formed by hybridization of an aptamer sequence with a cDNA strand. In the presence of target analyte, the aptamer sequence specifically interacts with its target molecule to disrupt the aptamer-cDNA duplex and the cDNA strand is displaced, which forms a displacement recognition process on the electrode surface (Scheme 1A). The main advantages of employing displacement recognition mechanism for EA sensors are that the aptamer-cDNA probe is easy to generalize for any aptamer using a simple base-pairing rule, and almost all electrochemical techniques can be well adapted for signal transduction of the displacement recognition process. Fox example, Redox reporters (methylene blue or ferrocene) were covalently labeled to either the aptamer for signal-on EA sensors or the cDNA strand for signal-off EA sensors [6,12–14]. Similarly, both the aptamer sequence and the cDNA strand were conjugated with different redox labels to construct dual-signaling or triple-signaling EA sensors [4,5,15–17]. In addition, label-free EA sensors were also developed by employing electrochemical impedance spectroscopy or chronocoulometry for signal transduction of the displacement recognitions [18,19].

Despite many examples of displacement-type EA sensors,

* Corresponding author.
E-mail address: zipingzhangytu@163.com (Z. Zhang).

https://doi.org/10.1016/j.electacta.2018.06.120
0013-4686/



Scheme 1. Schematic diagram of the target-induced strand displacement molecular recognition on electrode surfaces employing the conventional aptamer-cDNA duplex **(A)** and synergically stabilized duplex **(B)** probes (SA DNA: short additional DNA sequence). Compared to the conventional probe, the synergically stabilized probe possesses the advantage of less aptamer bases paired to the cDNA bases, which provides enhanced response rate for target binding.

almost all the previous sensors possessed a common drawback of relatively slow response rates with sensor equilibrium times ranging from 0.5 to 3 h. These slow response rates could be mainly attributed to high duplex stability and limited conformational flexibility of the aptamer sequence in the conventional aptamer-cDNA probes. For example, most of displacement-type EA sensors for ATP detecting employed a long, fully complementary duplex probe in which all aptamer bases were paired to cDNA bases to form the aptamer-involved base pairs. Duo to its high binding strength and structure rigidity of the long duplex, this class of fully complementary aptamer-cDNA probe only functioned at elevated temperature (i.e. 37 °C) and needed for a long target binding time of 3 h. In principle, reducing the number of aptamer-involved base pairs will cause decreased duplex binding strength and improved conformational flexibility of the aptamer sequence, which in turn enhances the response rate of the aptamer-cDNA probe for target binding. Consistent with this, displacement-type EA sensors using partially complementary or mismatched aptamer-cDNA probes exhibited a relatively short response time of about 0.5 h [20,21]. However, further reduction of the number of aptamer-involved base pairs for a more rapid response rate usually causes instability of the duplex structure, which leads to increased background signal and decreased sensor sensitivity [20]. Recent studies have also demonstrated that the kinetics of the displacement process was mainly dependent on the length of unpaired aptamer sequence and enhanced displacement rate could be achieved through reducing the number of aptamer-involved base pairs in the aptamer-cDNA complex [22,23]. Together, these results suggest that the key to optimizing sensitivity and response rate of displacement-type EA sensors is engineering of a class of aptamer-cDNA probes with moderate duplex stability and a minimum amount of aptamer-involved base pairs (here we term them “good probes”).

While simple reduction of the number of aptamer-involved base pairs within the conventional aptamer-cDNA probes (the duplex is made up entirely of aptamer-involved base pairs) cannot achieve the optimal probe architecture, studies about structure-switching signaling aptamers made by Nutiu and Li [24] provide a useful inspiration for engineering of this class of “good probes”. In their design of aptamer-based fluorescent reporters functioning through switching structures from aptamer-cDNA duplex to aptamer-target complex, a concept of synergic stabilization was introduced to construct the aptamer-cDNA duplex structure. More specifically, a short non-aptamer sequence of 5 bp was integrated with a short aptamer-involved base-pair sequence of 7 bp and these two unstable sequences cooperated to form a synergically stabilized, “5 + 7” type of duplex structure (here “5” and “7” represent the number of non-aptamer and aptamer-involved base pairs). Compared to the conventional aptamer-cDNA probe (containing a duplex of 12 aptamer-involved base pairs), this “5 + 7” type of synergically stabilized probe possessed comparable duplex stability but a reduced number of aptamer-involved base pairs (7 *vs.* 12), and thus exhibited a significantly enhanced response rate for fluorescent detection of ATP. Inspired by this “5 + 7” type of duplex structure, we report here the employment of the basic concept of “synergic stabilization” to engineer the “good probes” above and develop sensitive and rapidly responsive displacement-type EA sensors (Scheme 1B). We first demonstrated that the “5 + 7” type of synergically stabilized duplex probe was not generally optimal for EA sensors. We then used another concept of “base-pair regulation” to regulate the number of aptamer-involved base pairs within the synergically stabilized duplex and proposed a novel “5 + x” type of synergically stabilized duplex probes (“x” represents the various numbers of aptamer-involved base pairs for different aptamers). By combining the concepts of synergic stabilization and base-pair regulation, the “good probes” for different displacement-type EA sensors were easily engineered and these probes responded quickly to target analytes with high sensitivity.

2. Material and methods

2.1. DNA and chemical reagents

All DNA oligonucleotides listed in Table 1 are synthesized and purified by Takara Biotechnology (Dalian, China). Tris(2-carboxyethyl) phosphine hydrochloride (TCEP), 6-mercapto-1-hexanol (MCH), Adenosine-5′-triphosphate disodium salt (ATP), Guanosine-5′-triphosphate trisodium salt (GTP), Cytidine-5′-triphosphate disodium salt (CTP) and Uridine-5′-triphosphate trisodium salt (UTP) were purchased from Sigma-Aldrich. Cocaine hydrochloride, Benzoylecgonine (BZE) and cocaethylene (CE) were purchased from the State Food and Drug Administration (Beijing, China). All solutions were prepared using ultrapure water of 18 MΩ cm. The buffer containing of 200 mM NaCl and 20 mM Tris-HCl (pH = 7.8) was used for immobilization of thiolated DNA probes on the Au electrode surfaces. In the EA sensor for ATP, the buffer containing 300 mM NaCl, 5 mM $MgCl_2$ and 20 mM Tris-HCl (pH = 7.8) was used for DNA surface hybridization and