



# An i-DNA based electrochemical sensor for proton detection

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## ARTICLE INFO

### Article history:

Received 19 March 2010

Received in revised form 6 June 2010

Accepted 10 June 2010

Available online 23 June 2010

### Keywords:

i-DNA

i-Motif

Conformation change

Electrochemical proton sensor

## ABSTRACT

An i-DNA based electrochemical proton sensor which is fabricated by attaching the ferrocene-labeled i-DNA (Fc-i-DNA) onto a gold electrode is reported. This type of i-DNA is a cytidine-rich single-stranded oligonucleotide that its conformation can be switched between the random coil conformation and the folded i-motif structure at different pH values. The Fc-i-DNA is thiol terminated and can be bound to the gold electrode surface by Au–S interaction. With the variation of solution pH, the distance between ferrocene moiety and electrode surface is changed, leading to different redox currents. The pH can then be determined by measurement of the corresponding currents. In the range of pH 5.6–7.1, it is shown a linear relationship between the currents and pH values. The proton sensor also exhibits quick response, easy fabrication, and good selectivity.

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## 1. Introduction

DNA has been demonstrated recently to be an attractive unit component for the design of nanostructures, nanodevices and biosensors, because of its unique properties such as pairing specificity, good conformational flexibility and designable sequences. Most of its applications are based upon its classical double helix structure or three-dimensional structure, for example, in developments of G-quadruplexes [1,2], molecular beacons [3,4], and aptamers [5,6]. Comparing with conventional labeling probe technique, the DNA based biosensor has some unique potential applications in biochemical engineering, clinic diagnostics, and single cell detection.

Some artificially designed DNAs, which are obtained via SELEX (systemic evolution of ligands by exponential enrichment) technique [7,8], can be folded into well-ordered, three-dimensional structures that either recognize target molecules (aptamer), or catalyze specific chemical reactions (DNAzyme). In comparison to antibody and enzyme, aptamer and DNAzyme have a number of advantages, including relative ease of production, designable binding affinity, and resistance against denaturizing. These advantages make them very promising in analytical and diagnostic applications [9–11]. For example, aptamer and DNAzyme have been lately used to design and fabrication of biosensors, such as for the detection of thrombin [12–15], cocaine [16,17], and metal ions [18,19].

i-DNA is another type of functionalized oligonucleotide with interesting characteristics. It is a cytidine-rich single-strand oligonucleotide which can form a quadruplex structure (called i-

motif) under slight acidic condition when cytosine residues are hemi-protonated [20,21]. It means that the conformations of i-DNA may be switched at different pH values, between the closed i-motif structure and the extended random coil structure. In the i-motif structure, two parallel stranded duplexes are associated, with their cytosine-protonated cytosine (C–C<sup>+</sup>) pairs face to face and fully intercalated. Due to such a unique characteristic, the i-DNA has been attracted much attention lately, and been widely used in nanostructures or nanodevices [22]. Liedl et al. have fabricated i-DNA based switches both in solution or on a surface, which was driven by a chemical pH oscillator [23,24]. Shu et al. have made an i-DNA based molecular motor, to translate biochemical reactions into mechanical work [25]. In addition, the same i-DNA (which also used in this work, but with Fc at one end) has also been investigated by fluorescent [26,27] and colorimetric methods [28,29]. All of these reported devices are based on the conformational transition of i-DNA induced by pH variation.

In this work, an i-DNA based electrochemical pH sensor has been developed and characterized electrochemically based on the conformational changes of i-DNA induced by pH variation. The experimental results show that it has advantages of quick response, easy fabrication and good selectivity. Comparing with fluorescent and colorimetric methods reported, this type of electrochemical sensor needs simpler equipment and costs less.

## 2. Experimental

### 2.1. Chemicals

Ethyl disulfide and ferrocene-labeled i-DNA was synthesized by FRIZ Biochem. (Munich, Germany). The sequence is given below: (3') C<sub>2</sub>H<sub>5</sub>–S–S–(CH<sub>2</sub>)<sub>3</sub>–(CCCTAA)<sub>4</sub>–TTT–(CH<sub>2</sub>)<sub>6</sub>–Fc (5').

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Tris(2-carboxyethyl) phosphine hydrochloride (TCEP), 6-mercaptohexanol (MCH) and ferrocenecarboxylic acid (97%, FcCOOH) were purchased from Sigma–Aldrich (Shanghai, China). Boric acid ( $\text{H}_3\text{BO}_3$ ), phosphoric acid ( $\text{H}_3\text{PO}_4$ ), acetic acid (HAc), sodium hydroxide (NaOH), disodium hydrogen phosphate dodecahydrate ( $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ ), potassium phosphate monobasic ( $\text{KH}_2\text{PO}_4$ ), lithium chloride (LiCl), sodium chloride (NaCl), potassium chloride (KCl), magnesium chloride ( $\text{MgCl}_2$ ), and calcium chloride ( $\text{CaCl}_2$ ) were analytical grade. All aqueous solutions were prepared with double-distilled water.

The buffers were prepared as follows: Britton–Robinson buffer (B–R buffer) was adjusted to the appropriate pH with 0.2 M NaOH. And phosphate buffered solution (PBS) was prepared by mixing 0.067 M  $\text{Na}_2\text{HPO}_4$  with 0.067 M  $\text{KH}_2\text{PO}_4$  in different ratios. The pH values could be measured by a PHSJ-3F pH meter (Shanghai, China).

## 2.2. Electrochemical measurements

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed with a CHI 660C or CHI 900 electrochemical workstation (CH Instruments Inc., Shanghai, China). A conventional three-electrode system was used with a bare gold disk electrode (2 mm in diameter, CH Instruments Inc.) or a modified gold electrode as a working electrode, a saturated calomel electrode (SCE) and a platinum foil as respective reference and counter electrodes. Unless otherwise stated, all experiments were carried out at room temperature ( $22 \pm 2^\circ\text{C}$ ).

## 2.3. Fabrication of the Fc-i-DNA modified gold electrode

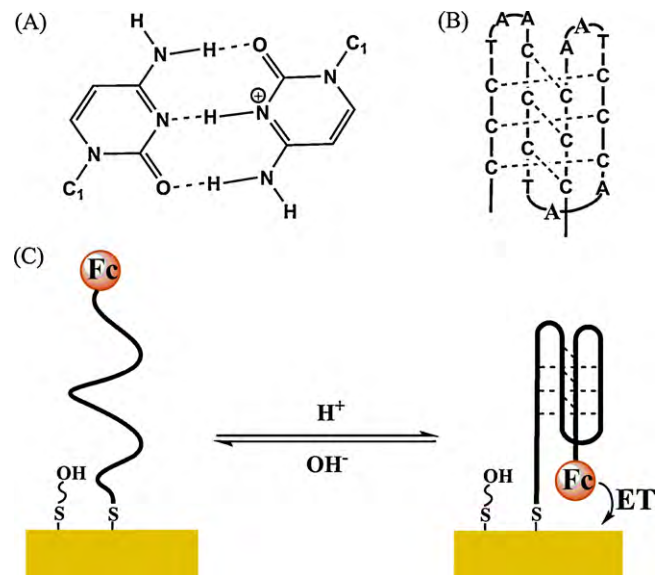
The method of immobilization of Fc-i-DNA onto a gold electrode is similar with the procedures reported previously [30,31]. First, the electrode is exposure to the hot piranha solution (a mixture of 30%  $\text{H}_2\text{O}_2$  and concentrated  $\text{H}_2\text{SO}_4$ , 1:3 in volumes) for 0.5 h. Then, it is polished with alumina (1 and 0.05  $\mu\text{m}$ ), sonicated in ethanol and water each for 5 min, and electrochemically cleaned in 0.5 M  $\text{H}_2\text{SO}_4$  to remove any remaining impurities. After that, the electrode is immersed in 1.0 M  $\text{KH}_2\text{PO}_4$  solution (pH 3.8) containing 1  $\mu\text{M}$  Fc-i-DNA and 1 mM TCEP, and kept at room temperature for 12 h. The modification is conducted under acidic condition to obtain low grafting density of DNA chains on the electrode surface, which can provide enough space for conformational change of i-DNA [32]. The addition of TCEP is to cut the disulfide bond of Fc-i-DNA and induce the formation of Au–S bond [33]. The fabricated Fc-i-DNA modified electrode is rinsed with water, and dried under nitrogen gas flow. Finally, the electrode surface is passivated with 1 mM MCH solution for 1 h, to remove the unspecifically adsorbed DNA, and the Fc-i-DNA modified gold electrode is ready for further investigation.

## 3. Results and discussion

### 3.1. The design principle and electrochemical response of the pH sensor

The triple-hydrogen bonding between cytosine and protonated cytosine is presented in Fig. 1A. Manzini et al. have found that only hemi-protonation is needed for the formation of an i-motif structure, and the sugar-phosphate backbone is held in *trans* state [20]. In the i-motif structure, two parallel duplexes associate in a head-to-tail orientation with their C–C<sup>+</sup> pairs face to face (Fig. 1B). This structure has also been elucidated and confirmed by NMR and X-ray studies [21,34].

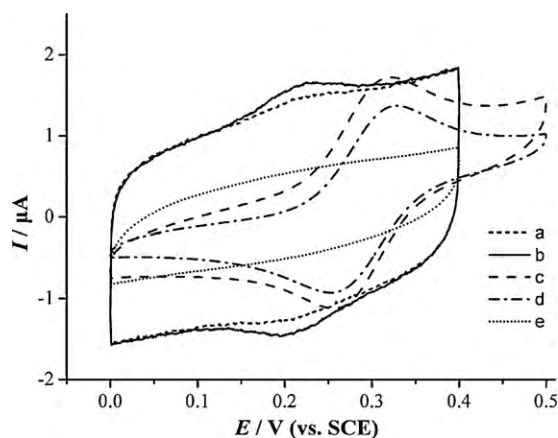
The synthesized i-DNA is labeled with a ferrocene moiety at the 5' end and a thiol-C<sub>3</sub> spacer at the 3' end. It can be bound to the gold electrode through Au–S interaction [30]. And the purpose of ferrocene-labeling is to generate the redox response when the



**Fig. 1.** The design principle of an i-DNA based pH sensor: (A) triple-hydrogen bonded C–C<sup>+</sup> pair; (B) four-stranded i-motif structure; (C) schematic representation of i-DNA based electrochemical pH sensor.

potential is applied to the electrode under certain pH value. Here ferrocene is chosen but not methylene blue (MB) as a redox label, it is mainly because the solution needs to be oxygen removed when using MB, and it is not so convenient as that using of ferrocene. After the modification of Fc-i-DNA on the electrode surface, MCH is also introduced to remove the unspecifically adsorbed DNA and keep the DNA chains upstanding on the electrode surface [30].

The Fc-i-DNA modified gold electrode is immersed into the buffers of different pH values for electrochemically characterization. It is an equilibrium process of transition of structures of i-DNA at different pH values (Fig. 1C). At basic pH, the cytosine residues of i-DNA are not protonated, and the random coil conformation of i-DNA is predominated. Due to the large distance separation in this state, there is no efficient electron transfer (ET) between the electrode surface and the ferrocene moiety. Hence, it shows small redox current (Fig. 2a). When the pH is acidic, the cytosine residues are partially protonated, and the compact i-motif structure is dominated. Under such circumstance, the ferrocene moiety has the chance to approaching the electrode surface and the ET may occur, causing the enhancement of the current (Fig. 2b). Based on



**Fig. 2.** Cyclic voltammograms of: the Fc-i-DNA modified gold electrode in PBS of pH 8.0 (a) and 4.0 (b), scan rate: 2.0 V/s; a bare gold electrode (c) and a MCH modified gold electrode (d) in PBS containing 0.2 mM FcCOOH (pH 8.0), scan rate: 0.05 V/s; and a bare gold electrode in PBS of pH 4.0 (e), scan rate: 2.0 V/s.

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