Talanta 82 (2010) 1122–1125

Contents lists available at ScienceDirect

Talanta

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

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 ($C–C^+$) pairs face to face and fully intercalated. Due to such an 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_2H_5–S–S–$(CH_2)_3$–$(CCCTAA)_4$–TTT–$(CH_2)_6$–Fc (5′).

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doi:10.1016/j.talanta.2010.06.019

Tris(2-carboxyethyl) phosphine hydrochloride (TCEP), 6-mercaptohexanol (MCH) and ferrocenecarboxylic acid (97%, FcCOOH) were purchased from Sigma–Aldrich (Shanghai, China). Boric acid (H_3BO_3), phosphoric acid (H_3PO_4), acetic acid (HAc), sodium hydroxide (NaOH), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4{\cdot}12H_2O$), potassium phosphate monobasio (KH_2PO_4), lithium chloride (LiCl), sodium chloride (NaCl), potassium chloride (KCl), magnesium chloride ($MgCl_2$), and calcium chloride ($CaCl_2$) 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 Na_2HPO_4 with 0.067 M KH_2PO_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 ($22 \pm 2\,^{\circ}C$).

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% H_2O_2 and concentrated H_2SO_4, 1:3 in volumes) for 0.5 h. Then, it is polished with alumina (1 and 0.05 μm), sonicated in ethanol and water each for 5 min, and electrochemically cleaned in 0.5 M H_2SO_4 to remove any remaining impurities. After that, the electrode is immersed in 1.0 M KH_2PO_4 solution (pH 3.8) containing 1 μM 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^+ 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_3 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 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].

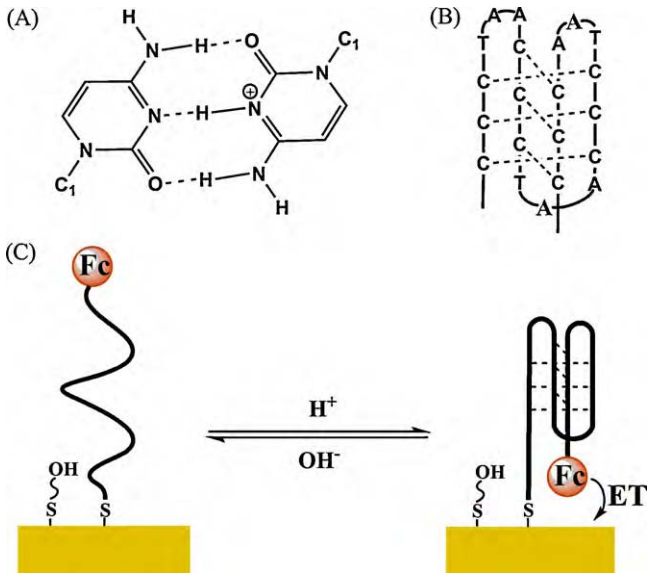

Fig. 1. The design principle of an i-DNA based pH sensor: (A) triple-hydrogen bonded C–C^+ pair; (B) four-stranded i-motif structure; (C) schematic representation of i-DNA based electrochemical pH sensor.

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

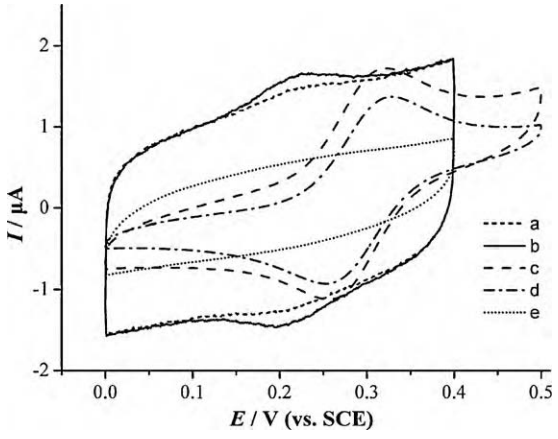

Fig. 2. Cyclic voltammograms of: the Fc-i-DNA modified gold electrode in PBS of pH 8.0 (a) and 4.0 (b), scan rate: 2.0 V/s; a bare gold electrode (c) and a MCH modified gold electrode (d) in PBS containing 0.2 mM FcCOOH (pH 8.0), scan rate: 0.05 V/s; and a bare gold electrode in PBS of pH 4.0 (e), scan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

this principle, the pH values can be determined by measurement of the currents. The transition between two different conformations is reversible, and rather fast. After immersing the electrode into different buffer solutions, the electrochemical scan is started immediately. The measured peak currents are nearly the same as the currents obtained after immersing the electrode in the same solution for longer time. Therefore, the conformational transition of i-DNA finishes very quickly, and should be in the order of several seconds. This reversible and quick conformational transition of i-DNA as shown in the CV curves is in good agreement with the phenomenon reported previously [24,26].

3.2. Voltammetric studies of the Fc-i-DNA modified electrode

The cyclic voltammetric responses of a bare gold electrode and a MCH modified electrode (obtained by immersing the gold electrode in 1 mM MCH for 1 h) in PBS containing 0.2 mM FcCOOH are shown in Fig. 2c and d. A pair of redox wave with good reversibility near 0.3 V (vs. SEC) is observed. After being modified with MCH, the electrode shows decreased peak current, due to the inhibition of electron transfer by MCH.

When the Fc-i-DNA modified gold electrode is immersed in the PBS for characterization, a pair of weak wave near 0.2 V (vs. SCE) attributed to the ferrocene moiety is observed (Fig. 2a and b), demonstrating successful immobilization of Fc-i-DNA onto the electrode surface. In contrast, the bare gold electrode does not show any redox peaks (Fig. 2e). The peak current at pH 4.0 is bigger than that at pH 8.0, because of conformational transition of i-DNA from extended state to closed state. After modification with Fc-i-DNA, the electrode shows much bigger background current. This is due to the absorption of negatively charged DNA on the electrode surface which can change the structure of electrical double layer, causing the bigger charging current. Due to the existence of big charging current, the CV response appears weak, and DPV has been employed to solve such problem.

Fig. 3 shows the DPV response of the pH sensor in different buffers. At pH higher than 7.1, Fc-i-DNA is kept in an extended conformation which inhibits efficient ET, and smaller current is observed. At lower pH, the folding of i-DNA conformation makes the ferrocene moiety closer to the electrode surface and increases the current, finally to a maximum at pH 5.6. Then the current changes little as continuing lower pH, from 5.6 to 5.0. Because the folded i-motif structure has been formed and kept stable in this condition, and the distance between the ferrocene moiety and the electrode surface does not change any more. In the range of pH 5.6–7.1, it exhibits a good linear relationship (R^2 = 0.98) between the currents and pH values (see inset in Fig. 3). This result is consistent with those reported previously by other techniques [23,25,35].

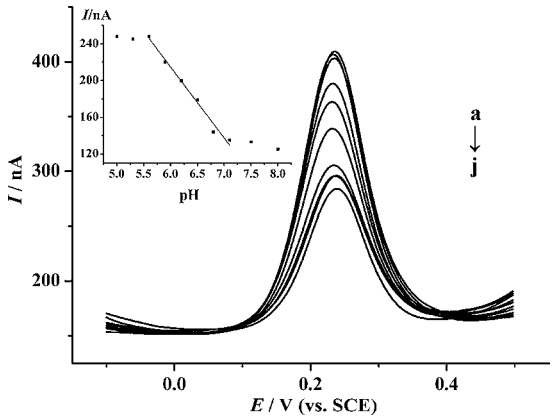

Fig. 3. Differential pulse voltammograms of the Fc-i-DNA modified gold electrode in B–R buffer at different pH, at amplitude of 0.05 V, pulse width of 0.03 s, pulse period of 0.2 s (a→j: pH 5.0, 5.3, 5.6, 5.9, 6.2, 6.5, 6.8, 7.1, 7.5 and 8.0). Inset: the corresponding calibration plot of the peak current vs. the pH values (5.6–7.1).

3.3. Stability and selectivity of the Fc-i-DNA modified electrode

The stability of the sensor is tested by measurement of the variation of DPV peak current after immersing the Fc-i-DNA modified electrode in B–R buffer (pH 5.9) for different time. The current does not change significantly in 4 h, demonstrating relatively good stability, as shown in Fig. 4A. The error bar represents the standard deviation of the data sets which obtained with three different Fc-i-DNA modified gold electrodes, and it also demonstrates that the proton sensor has a good reproducibility.

The i-motif structure is formed through the interaction of C–C^+, only when cytosine residues are hemi-protonated in slightly acidic conditions. Thus, other cations will theoretically not interfere with the proton detection, and the experiment results convince this assumption. Fig. 4B shows the interference of different metal cations for the detection of proton. After addition of 0.1 M different metal cations in B–R buffer (pH 5.9), the DPV response of Fc-i-DNA modified electrode does not change much, showing very good selectivity. Three Fc-i-DNA modified gold electrodes fabricated by the same method have been introduced to conduct the measurements, and they show good consistence and reproducibility.

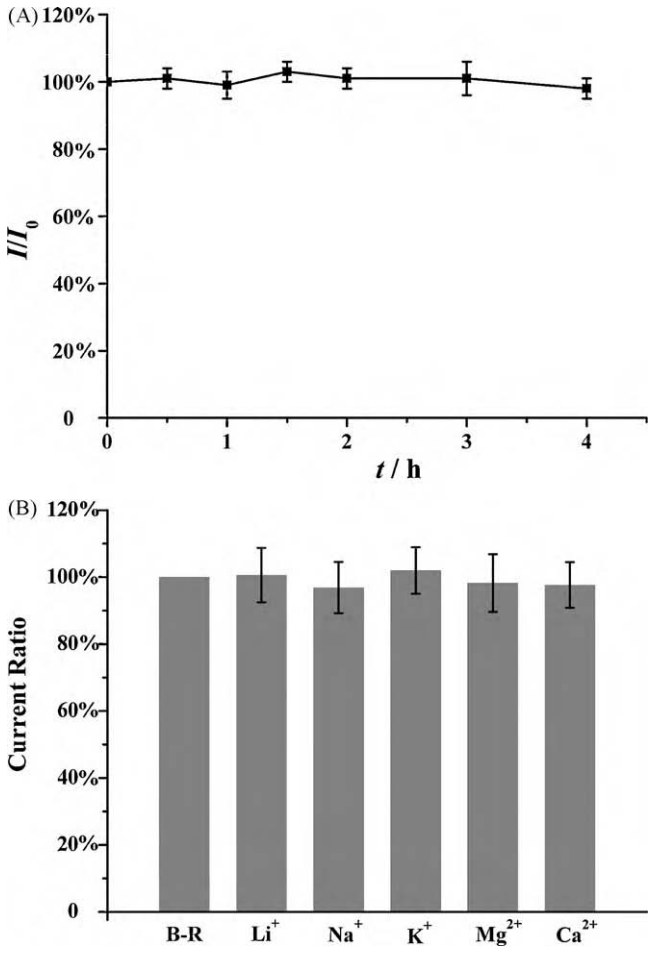

Fig. 4. (A) Stability of i-DNA based pH sensor with time (I: DPV peak currents at different time, I_0: the initial current). (B) Interference of different metal cations: Y scale is the ratio of the currents which obtained in B–R buffer (pH 5.9) with or without 0.1 M different cations added. The error bar each represents the standard deviation of measurements conducted with three Fc-i-DNA modified gold electrodes.

4. Conclusions

In summary, the i-DNA based electrochemical sensor developed in this work exhibits good performance for the measurement of H^+. The transition between compact and extended states of i-DNA leads to a substantial quick response and has selectivity. This sensor provides a possible platform for pH measurement and regulating in precise manufacturing that needs rigid pH control. In the present protocol, a routine-size electrode is employed, but limited by its relatively big dimension. To have further applications, our current effort is aimed at creating subminiature pH sensor by modifying Fc-i-DNA onto ultramicroelectrodes. The pH linear range which can be determined by this sensor is only up to 7.1, but not to 7.4, this might be improved by modification of the DNA sequence, and this type of work is also undertaken in our lab.

Acknowledgments

This work was supported by NSFC (20735001 and 20628506) and the Innovation Team Programs of the Ministry of Education of China.

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