Ferrocenecarboxaldehyde labeled DNA probe for the study on DNA damage and protection

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Abstract A ferrocenecarboxaldehyde (FCA) labeled DNA probe is used for the first time in the study of DNA damage and protection. The electrochemically active reagent FCA was labeled successfully on to a denatured calf-thymus DNA by 1-ethyl-3- (3-dimethyl-aminopropyl) carbodiimide (EDC). This FCA labeled DNA probe was used to hybridize with the sample DNA sequence accumulated on the surface of a graphite electrode. Only the complementary sequence (cDNA) can form a double-stranded DNA (dsDNA) with the FCA labeled DNA probe. The FCA labeled probe can detect the damage and protection of DNA. The method proposed is sensitive, simple and cheap. It enlarges the application of electrochemical DNA probes.

2 Experimental

2.1 Apparatus

CHI 630 Electrochemical Analyzer (CHI Instruments Inc., USA), JB-1 stirring machine (Branson), TDL-16B centrifuge (Anting Science Instrument Inc., Shanghai). Three-electrode system: the working electrode was a graphite electrode with a diameter of 4 mm, the reference electrode was an Ag/AgCl electrode, and the counter electrode was a platinum electrode. All measurements are carried out in a 10 mL cell.

2.2 Reagents

Calf-thymus DNA (Beijing BaiTai Biochemical Technology Company, Beijing), Sperm DNA and yeast RNA (Shanghai Institute of Biochemistry, Chinese Academy of Sciences), (λDNA and pBR322 DNA were purchased from Hua Mei Biotechnology Company (China). Denatured single-stranded DNA (ssDNA) is produced by heating native double-stranded DNA in a water bath at 100°C for ca. 5 min followed by rapid cooling in an ice bath. Ferrocenecarboxaldehyde (from Tohoku University, Japan), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and tris-(hydroxymethyl)-aminomethane are purchased from Sigma. N-methylimidazole and sodium dodecylsulfate (SDS) are obtained from Jiangsu Guangyao Chemicals. 2 × SSC buffer: 0.3 mol/L NaCl+0.03 mol/L sodium citrate (pH 7.0), TE buffer: 10 mmol/L Tris-HCl+1 mmol/L EDTA (pH 8.0). Other reagents are commercially available and are all of analytical grade. Solutions are prepared with double-distilled water.

2.3 Methods

2.3.1 Preparation of an electrochemically labeled DNA probe. 1.0 mL 0.1 mg/mL denatured calf-thymus DNA was added to 1.0 mL 0.1 mol/L imidazole solution (pH 6.8). After a few minutes, a droplet of ethylenediamine was added and shaken. Finally, 1.0 mL 2.5 × 10−4 mol/L ferrocenecarboxaldehyde and 40 µL 0.1 mol/L EDC [12] are added into the solution. The solution was shaken at room temperature (25 ± 0.5°C) for 30 min, then stirred overnight. Ethanol and 3 mol/L acetate buffer (pH 5.2) at the ratio of 20:1 (v/v) 6 mL were added and put in a refrigerator (<−15°C) for 20 min. After being centrifuged, the precipitate was washed with ethanol (70%, 40 µL × 3) to remove unreacted labeling reagent [13]. This FCA labeled DNA probe is dissolved in 100 µL TE buffer (0.01 mol/L, pH 8.0). It is stored in a refrigerator (<−15°C) prior to hybridization (Reaction scheme see Fig.1).

2.3.2 Accumulation of sample ssDNA onto graphite electrode. J.Wang [14] et al. reported a quick way to accumulate DNA on the electrode within several minutes. This diminished the time of our usual accumulation of ssDNA on the electrode [10]. In this paper, we used electrochemical accumulation of the sample ssDNA onto the graphite electrode. A freshly smoothed graphite electrode was first pretreated by applying a potential

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of +1.7 V (vs Ag/AgCl) for 1 min in an acetate buffer solution (0.2 mol/L, pH 5.0). Then it was immersed in TE buffer (pH 8.0) containing the sample ssDNA, meanwhile applying a potential of +0.5 V (vs Ag/AgCl) for 2 min. The sample ssDNA was subsequently accumulated onto the electrode surface. After that the electrode was washed with distilled water and rinsed in 0.01 mol/L TE buffer (pH 8.0).

2.3.3 Formation of hydroxyl radicals and their damage to sample ssDNA. The electrodes accumulated with sample DNA were immersed into the solution containing EDTA (5 mmol/L) 40 μL, FeSO₄ (0.5 mmol/L) 40 μL, phosphate buffer (0.15 mol/L, pH 7.4) 1.9 mL, H₂O₂ (0.088 mmol/L) 20 μL. Then they were kept in an incubator at 37 °C for 60 min. The electrodes were washed with distilled water three times prior to hybridization.

2.3.4 Protection by hydroxyl radical scavengers to DNA damage. Other electrodes accumulated with sample DNA were immersed into the solution containing EDTA (5 mmol/L) 40 μL, FeSO₄ (0.5 mmol/L) 40 μL, phosphate buffer (0.15 mol/L, pH 7.4) 1.7 mL, 200 μL hydroxyl radical scavengers such as thiourea, sodium benzoic acid and isopropanol (0.01 mol/L) were added to the solution, respectively. Finally, 20 μL H₂O₂ (0.088 mmol/L) was added to the mixture and the whole solution was kept in an incubator at 37 °C for 60 min. The electrodes were washed with distilled water three times prior to hybridization.

2.3.5 Hybridization. The electrodes accumulated with sample ssDNA (undamaged, damaged by hydroxyl radicals and protected by hydroxyl radical scavengers) were immersed into the hybridization buffer (300 mmol/L NaCl+30 mmol/L sodium citrate) containing the FCA labeled DNA probe. The solution is incubated in a water bath at 42 °C for 1 h with shaking. After hybridization the dsDNA/FCA electrode was washed with 0.4 mol/L NaOH and 0.25% SDS solution three times to remove the absorbed FCA labeled probe.

2.3.6 Electrochemical detection. Differential pulse voltammetry (DPV) was carried out in a 10 mL electrochemical cell with the dsDNA/FCA electrode as working electrode, an Ag/AgCl as the reference electrode, and a platinum wire as counter electrode. Voltammetry was performed at 100 mV/s in 0.01 mol/L blank TE buffer. The scan range was from 0.0 V to +0.5 V (vs. Ag/AgCl).

3 Results and discussion

3.1 Electrochemical detection of sample ssDNA damage from hydroxyl radicals by Fenton reaction

The Fenton reaction is the common way to form hydroxyl radicals. The whole reaction is as follows:

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\text{Fe(EDTA)}^{2-} + \text{H}_2\text{O}_2 \rightarrow \text{Fe(EDTA)}^- + \text{OH}^- + \cdot\text{OH}
\]

In this paper, we used the Fenton reaction in a phosphate buffer system with EDTA to form hydroxyl radicals, the damage caused to sample ssDNA could be detected by the FCA labeled probe. The damage by hydroxyl radicals to sample ssDNA can cause breakage of DNA strands (i.e. cleavage of the DNA sugar-phosphate backbone) and some base breakage [15]. When the partly damaged sample ssDNA-electrode is hybridized with FCA labeled probe, the anodic peak current of FCA on the surface of the electrode is decreased visibly. Thus, the anodic peak current of FCA using DPV can detect the degree of sample ssDNA damage. Figure 2 shows the electrochemical detection of sample ssDNA damage. Figure 2a shows the electrochemical behavior of the undamaged sample ssDNA electrode and Fig.2b is that of the damaged sample ssDNA electrode. The anodic peak current of FCA on the damaged sample ssDNA electrode can be seen to decrease visibly, confirming damage of the sample ssDNA.

3.2 Effect of Fe (II) concentration on sample ssDNA damage

If in the Fenton reaction system, Fe (II) was replaced by Fe (III) or Cu(II) or Mg(II) at the same concentration, no damage of sample ssDNA was observed. This suggests that Fe (II) was essential for the formation of hydroxyl radicals. This is consistent with [16].

Fe (II) concentration affected the damage of hydroxyl radicals to sample ssDNA. DNA damage increased gradually withincreasing Fe(II) concentration from 0.1 mmol/L to 0.5 mmol/L. At Fe(II) concentrations of 0.5 mmol/L, the anodic peak current of FCA decreased almost to zero, indicating the sample complementary ssDNA was totally damaged and that could no longer hybridize with the FCA labeled probe. Therefore, an Fe(II) concentration of 0.5 m mol/L was chosen.

Fig.1 Preparation of the ferrocenecarboxaldehyde labeled DNA probe

Fig. 2 Electrochemical detection of sample ssDNA damage by DPV; a. Undamaged sample ssDNA electrode, b. Damaged sample ssDNA electrode