An Electrochemical Study Based on Thymine-Hg-Thymine DNA Base Pairs Mediated Charge Transfer Process

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Experimental

Materials:

6-mercaptohexanol (MCH, Aladdin Co., 98%), MB (Aladdin Co.), hexaammineruthenium (III) chloride, dithiothreitol (DTT Aladdin Chem. Co., Shanghai) were used as received. All the chemicals used in this work were at least analytical grade. The buffer used were made of sodium phosphate (Na_2HPO_4 and NaH_2PO_4, $[PO_4^{3-}]$ = 50 mM, PBS, pH 7.4) were dissolved in PBS. Water used in this work was prepared using a Milli-Q pure water system (18 MΩ·cm resistivity).

The DNA sequences were purchased from Sangon Biotech Co. (Shanghai). The target sequences containing a 6-mercaptohexyl linker and a MB molecule covalently attached to DNA through a flexible C_6 alkyl linker. There are 25 bases in each of these probes. The mismatched DNA duplexes contained mismatched part of $(TT)_n$ with different *n* values of 1, 3, 6, 12 and the mismatched part were in the middle of the DNA sequence.

matched dsDNA

5'-SH-C6-ATATTAATAAATTTATAAACGGCCG-C6-MB-3'

5'-CGGCCGTTTATAAATTTATTAATAT-3'

dsDNA with $(TT)_1$ sequence

5'-SH-C6-ATATTAATAAATTTATAAACGGCCG-C6-MB-3'

5'-CGGCCGTTTATATATTTATTAATAT-3'

dsDNA with $(TT)_3$ sequence

5'-SH-C6-ATATTAATAAATTTATAAACGGCCG-C6-MB-3'

5'-CGGCCGTTTATTTTTTTATTAATAT-3'

dsDNA with $(TT)_6$ sequence

5'-SH-C6-ATATTAATATTTTTTTAAACGGCCG-C6-MB-3'

5'-CGGCCGTTTATTTTTTTATTAATAT-3'

dsDNA with $(TT)_{12}$ sequence

5'-SH-C6-ATATTATTTTTTTTTTTTACGGCCG-C6-MB-3'

5'-CGGCCGTTTTTTTTTTTTTTTAATAT-3'

DNA duplexes were hybridized in 2×SSC (0.3mol/L NaCl, 3.0×10^{-5}mol/L sodium citrate) solution for 4 hours at room temperature (20℃), then deprotected using DTT, purified by dialysis (M_{cutoff} of dialysis membrane, 1000) in deoxygenated water for 48 hours and change the water every 12 hours.

The matched/mismatched DNA Self-assembled monolayers on Au surface

A fresh Au(111) facet was prepared by crystallization of a molten ball formed at the end of a Au wire (99.999%) in a hydrogen-oxygen flame. The fresh Au (111) was immersed in the duplex DNA solution (purified by dialysis) immediately and then put them in an oxygen-free environment to allow the adsorption to occur for 48 hours to form DNA monolayer. Afterwards, the Au (111) electrode was rinsed with deionized water, and immersed in the aqueous solution containing 1mM MCH for 3 hours to block the facet.

Gelelectrophoresis

10μL of samples were mixed with 1 μL 10× loading buffer, respectively. The mixtures were subjected to electrophoresis on 1% agarose gel containing ethidium bromide (1 μg/mL) in 1 × TAE buffer (40 mM Tris, 20 mM glacial acetic acid, 1 mM EDTA, pH 8.0) at a constant potential of 110 V for 30 min. The separated bands were photographed by Bio-Rad gel imaging system (USA).

AFM imaging

These samples were thoroughly rinsed with deionized water and mounted on a MultiMode 8 atomic force microscopy (AFM) with a Nanoscope V controller (Bruker, USA). For imaging with commercially available sharpened Si_3N_4 probes (radius of curvature approximately 2 nm, Bruker). All images were collected in ScanAsyst Mode (PeakForce Tapping).

Electrochemistry

The Au disk electrodes (d = 2 mm, Shanghai Chenhua Instruments Co.) were polished successively with 1μm and 0.05 μm α-Al_2O_3 for 15 min and then washed with deionized water, ultrasonicated in ethanol and water for 10 min, cleaned in a Piranha solution (mixture of H_2SO_4 and 30 % H_2O_2 at a weight ratio of 7:3. Warning: Piranha solution is a strong oxidant and must be handled with extreme caution) for 30 min, and then thoroughly rinsed with deionized water. Finally, the Au electrode was subjected to electrochemical pretreatment by consecutive potential cycling in a 0.5 M H_2SO_4 solution within a potential window between −0.20 and +1.50 V (vs. Ag/AgCl) at 100 mV/s using an CH760 C electrochemical analyzer (Shanghai Chenhua Instruments Co.). $Ag/AgCl_{3MKCl}$ and Pt wire (d = 0.5 mm) were used as reference and counter electrode, respectively. The cycling was continued until reproducible voltammograms were obtained. After cleaning, Au disk electrodes were immediately transferred to DNA solution to allow DNA adsorption for 48 hours in an oxygen-free environment. And then the DNA modified electrode was rinsed and blocked in 1 mM MCH for 3 hours. Cyclic voltammetry (CV) were used in CT kinetic study.

Measurement of surface density of DNA monolayer at Au electrode surface

The supporting electrolyte (10 mM Tris buffer, pH 7.4) was deoxygenated via purging with argon for 15 min before measurements, and continued purging with argon for the duration of the experiments. In a typical experiment as was used previously,[26] chronocoulometry (pulse period, 4 s; pulse width, 500 mV) of DNA modified Au electrode was performed in absence of 50 μM $[Ru(NH_3)_6]^{3+}$. And then the DNA monolayer was allowed to equilibrate in 50 μM $[Ru(NH_3)_6]^{3+}$ solution for 30 s with stirring followed by chronocoulometry measurements in the same $[Ru(NH_3)_6]^{3+}$ solution. The surface density of coverage of the redox marker $[Ru(NH_3)_6]^{3+}$ was calculated as the difference in the chronocoulometric intercepts of the chronocoulometry plots (charge *vs.* square root of time) in the absence and presence of $[Ru(NH_3)_6]^{3+}$.