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Effect of Surface Pretreatment on Electron Transfer of Methylene Blue Covalently Labeled Double-Stranded DNA Self-Assembled Monolayers on Gold

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In this article we investigated the effect of surface pretreatment procedures on electron transfer of methylene blue (MB) covalently labeled double-stranded DNA (ds-DNA)/mercaptohexanol (MCH) mixed self-assembled monolayers (SAMs) on gold by cyclic voltammetry (*CV*) and chronocoulometry (*CC*). The pretreatment procedures included *M+E*, *M + C (piranha)*, *M + C (dilute aqua regia)*, *M + C + E (piranha)*, *M + C + E (dilute aqua regia)* and *RM + C + E (piranha)*. The *M* was mechanical polishing, *C* was piranha or dilute aqua regia dipping, *E* was electrochemical polishing and *RM* was roughly mechanical polishing. Results indicated electron transfer reaction of MB was mainly adsorption controlled. The electron transfer rate (k_s) values of MB labeled ds-DNA/MCH mixed SAMs on gold pretreated by *M+C(piranha)* and *M+C(dilute aqua regia)* were 0.84 ± 0.15 and 0.82 ± 0.17 s^{-1}, smaller than those (2.76 ± 0.28, 4.76 ± 2.68, 3.89 ± 2.06, 2.26 ~ 7.79 s^{-1}) by *M+E*, *M+C+E(piranha)*, *M+C+E(dilute aqua regia)* and *RM+C+E(piranha)* respectively. Thus, electrochemical polishing was an important pretreatment step, which might influence the k_s of MB. Furthermore, the k_s values of MB did not change monotonically with increasing gold surface roughness R_f, indicating that R_f was not the key factor to make the difference of k_s. We considered that the difference of elemental composition on gold surface possibly led to different k_s of MB. These conclusions provided the important reference for electrochemically studying DNA electron transfer mechanism.

Keywords: pretreatment procedures, methylene blue covalently labeled DNA, self-assembled monolayers, electron transfer rate, gold roughness

1. INTRODUCTION

Exploration of electron transfer mechanism through double stranded DNA(ds-DNA) is especially significant not only in understanding DNA damage, repair and the origin of cancer but also in designing DNA sensor with perfect performance [1,2]. Electron transfer studies through ds-DNA usually includes two systems: homogeneous system (photosensitiser labeled ds-DNA molecules were dispersed in solution and DNA charge transfer rate was detected mainly by fluorescence methods) and heterogeneous system (redox probes labeled ds-DNA molecules were immobilized on a solid surface and DNA electron transfer rate was detected by electrochemical methods such as cyclic voltammetry and electrochemical impedance spectroscopy) [3]. The heterogeneous system based on thiol and redox probes labeled ds-DNA self-assembled monolayers (SAMs) on gold is widely applied due to its simplicity and conveniency. Two main paths are proposed to transfer electrons: 1) electrons are transferred through the interior of DNA duplex and electron transfer reaction is an adsorption controlled process; 2) electrons are transferred by redox probes diffusing to electrode surface and electron transfer reaction is a diffusion controlled process [3−7]. However, the reports from literatures about DNA electron transfer mechanism are not consistent and even controversial. For example, both diffusion and adsorption controlled reaction processes are reported for electron transfer through ds-DNA SAMs on gold [4−7].

Pretreatment procedures are especially important for constructing alkanethiols SAMs on gold. For example, gold surface pretreated by dilute aqua regia might increase surface coverage of alkanethiols SAMs and decrease the amount of defects existed in SAMs, which availed to reduce electron transfer rate (k_s) of redox probes through SAMs [8−10]. However, for thiol labeled ds-DNA SAMs, the effect of gold pretreatment procedures on DNA electron transfer performance is different. For example, our previous report [11] indicated that electron transfer resistances of $Fe(CN)_6^{3-/4-}$ through thiol labeled ds-DNA SAMs on gold pretreated by the procedures including *M + E*, *M + C (piranha)*, *M + C(dilute aqua regia)*, *M + C + E (piranha)* and *RM + C + E (piranha)* were almost the same. The $Fe(CN)_6^{3-/4-}$ were dissociate redox probes, which transferred electrons through thiol labeled ds-DNA SAMs mainly by tunneling or diffusion to the collapsed sites or defects in SAMs. For the current study about thiol and redox probes labeled ds-DNA SAMs, the redox probes are away from electrode surface and the possibility of redox probes diffusing to the collapsed sites or defects in SAMs is decreased greatly, then electrons transfer mainly by tunneling or through the interior of DNA duplex. How pretreatment procedures influenced the k_s of thiol and redox probes labeled ds-DNA SAMs on gold is the problem unsolved now. Investigating the effect of pretreatment procedures on the k_s of redox probes labeled ds-DNA SAMs on gold is important for electrochemically exploring DNA electron transfer mechanism.

In this article we choose thiol and methylene blue (MB) labeled ds-DNA SAMs on gold as the research subject and investigate the effect of different pretreatment procedures (*M + E*, *M + C (piranha)*, *M + C (dilute aqua regia)*, *M + C + E (piranha)*, *M + C + E (dilute aqua regia)* and *RM + C + E (piranha)*) on k_s of MB. The electron transfer mechanism of MB labeled ds-DNA SAMs on gold is discussed combined with literature reports.

2. EXPERIMENTAL SECTION

2.1 Chemicals and apparatus

Thiol and methylene blue (MB) labeled double stranded DNA (ds-DNA) reagent was purchased from Takara biotechnology (Dalian) Co. Ltd. The base sequence was as follows:

$HS(CH_2)_6$-5'-CTC TCG TAA GCT GTG ATG GCA CTT G-3'-MB

3'-GAG AGC ATT CGA CAC TAC CGT GAA C-5'

The molecular structure of MB labeled ds-DNA was showed in Figure 1.

Mercaptohexanol ($HS(CH_2)_6OH$, abbreviated by MCH, 97%, Aldrich), Hexaammineruthenium(III) chloride ($Ru(NH_3)_6Cl_3$, 98%, Aldrich), tris(hydroxymethyl)aminomethane (tris, ≥99.9%, Sigma). Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$, ≥99.0%), sodium dihydrogen phosphate dehydrate ($NaH_2PO_4 \cdot 2H_2O$, ≥99.9%), and sodium chloride (NaCl, ≥99.8%) were purchased from Sinopharm Chemical Reagent Co. Ltd. Ultrapure water (18 MΩ·cm) was used in the whole experiment. Chronocoulometry (*CC*) and cyclic voltammetry (*CV*) experiments were performed using CHI660D electrochemical workstation (CH Instruments, USA) with a three-electrode system: working electrode (polycrystalline gold, 2 mm diameter), counter electrode (platinum sheet), and reference electrode (saturated calomel electrode, SCE). Experimental solutions were deaerated with high-purity N_2.

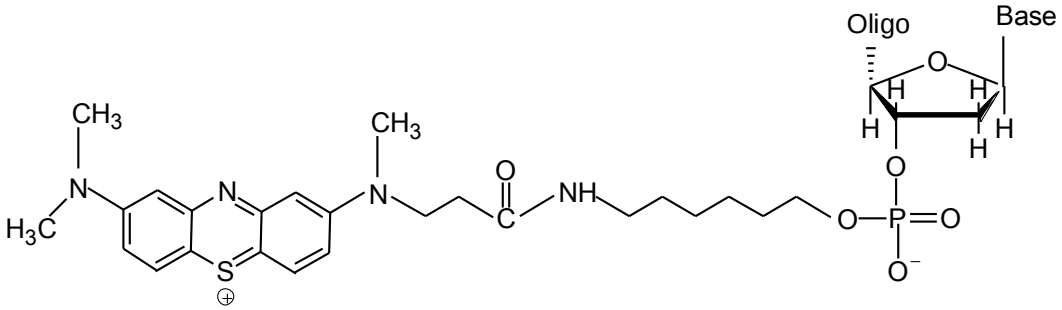


Figure 1. Molecular structure of methylene blue (MB) labeled double stranded DNA (ds-DNA).

2.2. Pretreatment of gold electrodes

The pretreatment procedures of gold electrodes were the same as those from our previous report [11], which included *M+E*, *M+C(piranha)*, *M+C(dilute aqua regia)*, *M+C+E(piranha)*, *M+C+E(dilute aqua regia)* and *RM+C+E(piranha)*. The *M* was mechanical polishing, *C* was piranha or dilute aqua regia dipping, *E* was electrochemical polishing and *RM* was roughly mechanical polishing.

M+E (Mechanical polishing and electrochemical polishing): The gold electrodes were carefully hand-polished on microcloth pads with alumina slurries (1.0, 0.3 and 0.05 μm) (i.e. *mechanical polishing*), sonicated in ultrapure water for 25 min and electrochemically polished by *CV* scans in 0.5 M H_2SO_4 solution from −0.4 to +1.5 V at 0.1 V s^{-1} until reproducible cyclic voltammograms were obtained (i.e. *electrochemical polishing*).

M+C (Mechanical polishing and chemical reagent for piranha or dilute aqua regia dipping): The gold electrodes were carefully hand-polished with alumina slurries (1.0, 0.3 and 0.05 μm), then sonicated in ultrapure water for 10 min, dipped into newly prepared piranha reagent (concentrated H_2SO_4: 30% H_2O_2 = 3:1) or dilute aqua regia (concentrated HNO_3: concentrated HCl: H_2O = 1:3:6) for 5 min. When piranha reagent was used to dip the gold electrodes, the pretreatment procedure was abbreviated as *M+C* (*piranha*); when dilute aqua regia was used to dip the gold electrodes, the pretreatment procedure was abbreviated as *M+C*(*dilute aqua regia*).

M+C+E (Mechanical polishing, chemical reagent for piranha or dilute aqua regia dipping and electrochemical polishing): The gold electrodes pretreated by *M+C* were sonicated in ultrapure water for 15 min, and then electrochemically polished in 0.5 M H_2SO_4 solution. The pretreatment procedure was abbreviated as *M+C+E* (*piranha*) or *M+C+E* (*dilute aqua regia*) respectively when piranha reagent or dilute aqua regia was used to dip the gold electrodes.

RM+C+E (Roughly mechanical polishing, chemical reagent for piranha dipping and electrochemical polishing): The pretreatment procedure was almost the same as *M+C+E* except that the gold electrodes were roughly hand-polished with alumina slurries (1.0, 0.3 and 0.05 μm) and the dipping chemical reagent was only piranha reagent. The pretreatment procedure was abbreviated as *RM+C+E* (*piranha*).

The real surface area *A* of gold electrodes was determined by integrating the charge of gold reduction peak in 0.5 M H_2SO_4 solution by *CV* scans at a scan rate of 0.1 V s^{-1} with 400 μC cm^{-2} as a monolayer of chemisorbed oxygen. The roughness factor R_f was calculated based on $R_f = A/A'$ (*A'* was the geometrical area of gold electrode and equal to 0.0314 cm^2).

2.3. Preparation of methylene blue (MB) labeled double stranded DNA (ds-DNA)/mercaptohexanol (MCH) mixed SAMs on gold

The gold electrode surface was covered with a 30 μL drop of mixed solution including 2 μM MB labeled ds-DNA, 1 M NaCl, and 5 mM sodium phosphate buffer (pH 7.0), and sealed up with a 0.5 mL centrifugal tube to protect the solution from evaporation. The assembly time was kept for 24 h at room temperature. When the assembly was finished, the gold electrode was dipped into 1 mM MCH, 10 mM sodium phosphate buffer solution (pH 7.0) for 1 h to form DNA/MCH mixed SAMs. The ds-DNA/MCH modified gold electrode was then rinsed with 50 mM NaCl and 5 mM sodium phosphate buffer solution (pH 7.0) fully for use.

2.4. Electrochemical characterization

Chronocoulometry (*CC*) and cyclic voltammetry (*CV*) were used to investigate ds-DNA surface coverage (Γ_m) and electron transfer rate (k_s) of MB through ds-DNA/MCH mixed SAMs on gold.

(1) Surface coverage (Γ_m) *of ds-DNA for ds-DNA/MCH mixed SAMs on gold from CC measurement.* Γ_m of ds-DNA was quantificationally measured by the *CC* method [12]. The experiment was performed in 10 mM tris-HCl solution (pH 7.4) or 10 mM tris-HCl solution (pH 7.4) with 50 μM

$Ru(NH_3)_6^{3+}$. The pulse width was from +100 mV to –500 mV with 500 ms pulse period. According to the integrated Cottrell equation (Equation 1):

$$Q = \frac{2nFAD_o^{1/2}C_o}{\pi^{1/2}}t^{1/2} + Q_{dl} + Q_{Ru} + Q_{MB} \quad (1)$$

Where *n* was electron transfer number of $Ru(NH_3)_6^{3+}$ reduction reaction (n = 1), *F* was Faraday constant (96485 C mol^{-1}), D_o was the diffusion coefficient of $Ru(NH_3)_6^{3+}$ (cm^2 s^{-1}), C_o was the bulk concentration of $Ru(NH_3)_6^{3+}$ (mol cm^{-3}), Q_{dl} was the double-layer charging charge (C), Q_{Ru} was the reduction charge of ds-DNA surface-adsorbed $Ru(NH_3)_6^{3+}$ (C), and Q_{MB} was the reduction charge of MB labeled ds-DNA (C). Q_{Ru} was obtained from the difference of the two extrapolated intercepts at t = 0 of the linear part of Q vs. $t^{1/2}$ plots in 10 mM tris-HCl solution (pH 7.4) or 10 mM tris-HCl solution (pH 7.4) with 50 μM $Ru(NH_3)_6^{3+}$. Γ_m of ds-DNA on gold was calculated based on Equation 2, where *z* was the charge of $Ru(NH_3)_6^{3+}$ (z = 3), *m* was the number of ds-DNA bases (m = 50).

$$\Gamma_m = \frac{zQ_{Ru}}{mnFA} \quad (2)$$

(2) Electron transfer rate (k_s) of MB labeled ds-DNA/MCH mixed SAMs on gold from CV measurement. CV experiments were performed in 50 mM NaCl and 5 mM sodium phosphate buffer solution (pH 7.0) at different scan rate *v*. Based on Laviron equation (Equation 3), when $n\Delta E_p$ was smaller than 200 mV [13]:

$$k_s = \frac{mnFv}{RT} \quad (3)$$

Where n was electron transfer amount of MB redox reaction (n = 2), ΔE_p was the difference of redox peak potentials (mV), m was a parameter related to ΔE_p value, *v* was scan rate (V s^{-1}), *R* was gas constant (8.314 $J \cdot K^{-1} \cdot mol^{-1}$), and *T* was temperature (K).

(3) Electron transfer rate (k_s) of MB in solution through MCH SAMs on gold from CV measurement. Samely, the *CV* experiments were performed in 50 mM NaCl and 5 mM sodium phosphate buffer solution (pH 7.0) at different scan rate *v*. For diffusion-controlled reaction, the k_s value was calculated based on Equation 4 and Equation 5[14]:

$$E_{pa} = E^{o'} + m\left[0.78 + \ln\frac{\sqrt{D}}{k_s} - 0.5\ln m\right] + \frac{m}{2}\ln v \quad (4)$$

$$m = \frac{RT}{(1-\alpha)nF}$$

$$E_{pc} = E^{o'} - m\left[0.78 + \ln\frac{\sqrt{D}}{k_s} - 0.5\ln m\right] - \frac{m}{2}\ln v \quad (5)$$

$$m = \frac{RT}{\alpha nF}$$

Where E_{pa} and E_{pc} were the redox peak potentials of MB (V), $E^{o'}$ was the formal potential (V), *D* wad the diffusion coefficient of MB in solution (cm^2 s^{-1}), α was transfer coefficient.

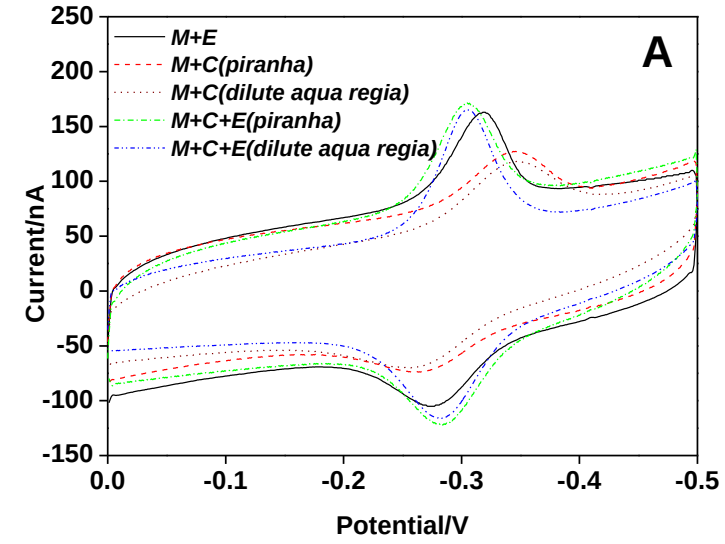
3. RESULTS AND DISCUSSION

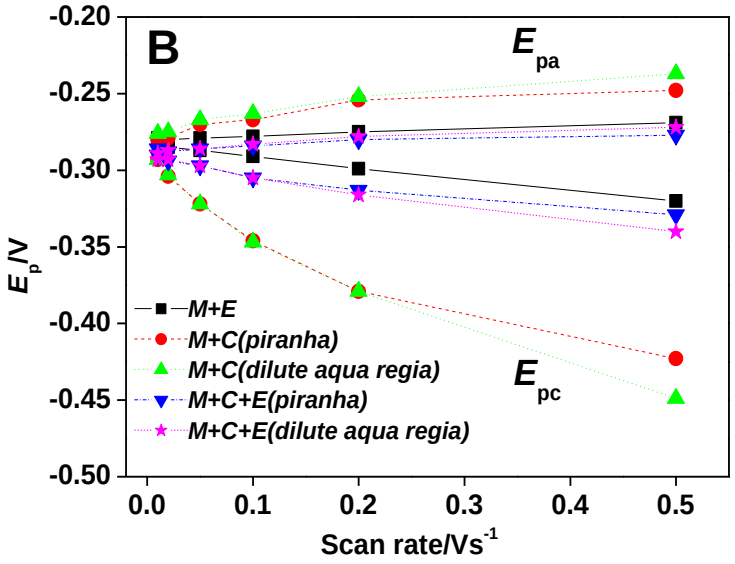
3.1 Effect of pretreatment procedures (M+E, M+C(piranha), M+C(dilute aqua regia), M+C+E(piranha), M+C+E(dilute aqua regia)) on electron transfer rate (k_s) of MB labeled ds-DNA/MCH mixed SAMs on gold

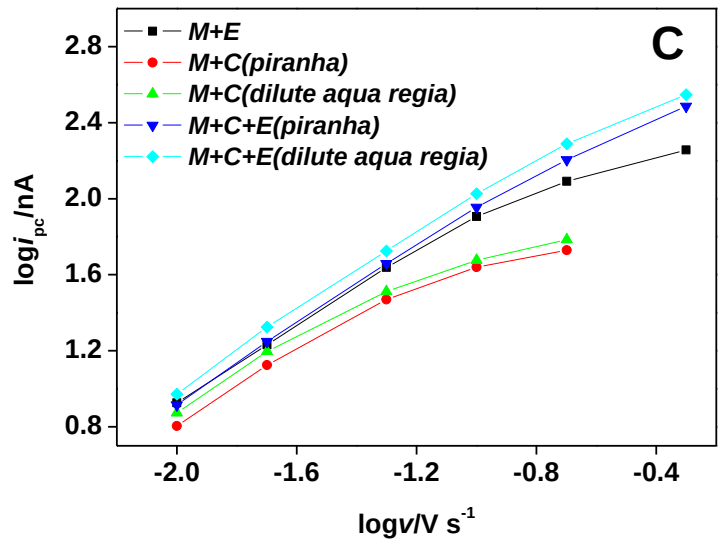
Figure 2A showed the *CV* plots of MB labeled ds-DNA/MCH mixed SAMs on pretreated gold at a scan rate v of 0.1 V s^{-1} in 50 mM NaCl and 5 mM sodium phosphate buffer solution (pH 7.0). A couple of MB redox peaks appeared and the formal potentials $E^{o'}$ were about −0.295 V for all the plots, consistent with literature report [7]. The redox peak potential difference (ΔE_p) for MB differed, which depended on gold pretreatment procedures. For *M+E*, *M+C+E(piranha)* and *M+C+E(dilute aqua regia)*, ΔE_p were 40 ± 3, 30 ± 8 and 36 ± 10 mV respectively; For *M+C(piranha)* and *M+C(dilute aqua regia)*, ΔE_p were 87 ± 12 and 83 ± 8 mV respectively. Figure 2B showed the plots of redox peak potentials (E_{pa}, oxidization peak potential; E_{pc}, reduction peak potential) with v. Also, we could observe that ΔE_p (i.e., $E_{pa}-E_{pc}$) values were bigger for MB labeled ds-DNA/MCH mixed SAMs on gold pretreated by *M+C(piranha)* and *M+C(dilute aqua regia)* than by *M+E*, *M+C+E(piranha)* and *M+C+E(dilute aqua regia)*. These results indicated that k_s of MB were smaller for gold pretreated by *M+C(piranha)* and *M+C(dilute aqua regia)*.

Figure 2C showed the plots of log cathodic peak current (i_{pc}) against log v. When v was 0.01~0.2 V s^{-1}, the linear correlation coefficient R of logi_{pc}~log$v$ plots ranged from 0.9820 to 0.9969 with the linear slope S from 0.70 to 0.99. When v was 0.01~0.1 V s^{-1}, the R ranged from 0.9926 to 0.9999 with the S from 0.84 to 1.03. Thus, MB redox reaction was mainly adsorption-controlled because the S values were close to 1.0 [15,16]. Based on Equation 3, we calculated the k_s values of MB redox reaction, which were shown in Table 1. For *M+C(piranha)* and *M+C(dilute aqua regia)*, the k_s was 0.84 ± 0.15 and 0.82 ± 0.17 s^{-1} respectively; for *M+E*, *M+C+E(piranha)* and *M+C+E(dilute aqua regia)*, the k_s was 2.76 ± 0.28, 4.76 ± 2.68 and 3.89 ± 2.06 s^{-1} respectively. k_s of MB labeled ds-DNA SAMs on gold pretreated by *M+C(piranha)* and *M+C(dilute aqua regia)* was smaller than by *M+E*, *M+C+E(piranha)* and *M+C+E(dilute aqua regia)*. Thus, electrochemical polishing was an important pretreatment step, which might influence the k_s of MB through ds-DNA SAMs on gold.

Figure 2D showed the *CC* plots of MB labeled ds-DNA/MCH mixed SAMs on pretreated gold in 50 μM $Ru(NH_3)_6^{3+}$ and 10 mM tris-HCl (pH 7.4) solution. Based on the extrapolated intercepts of Q~$t^{1/2}$ plots, DNA surface coverage $\Gamma_{m(CC)}$ were calculated to be about 8.0×10^{-12} mol cm^{-2}. Furthermore, we calculated the $\Gamma_{m(CV)}$ by Q_{MB}/nFA, where Q_{MB} was the integrated charge of MB reduction peak from *CV* plots in Figure 2A. The $\Gamma_{m(CV)}$ was about 39% of the ratio, as compared with $\Gamma_{m(CC)}$. This indicated that not all the MB molecules were electroactive, consistent with the report from Barton et al [17]. Barton et al [17,18] proposed that intercalative stacking of redox probes with DNA duplex was the precondition for DNA-mediated electron transfer. Thus, it was possible that some MB molecules could intercalate into DNA duplex and produce the electrochemical signal. On the contrary, some MB molecules remained outside DNA duplex and showed electrochemically inactive.


A
M+E
M+C(piranha)
M+C(dilute aqua regia)
M+C+E(piranha)
M+C+E(dilute aqua regia)
Current/nA
Potential/V


B
E_{pa}
E_{pc}
M+E
M+C(piranha)
M+C(dilute aqua regia)
M+C+E(piranha)
M+C+E(dilute aqua regia)
E_p/V
Scan rate/Vs^{-1}


C
M+E
M+C(piranha)
M+C(dilute aqua regia)
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M+C+E(dilute aqua regia)
logi_{pc}/nA
logv/V s^{-1}

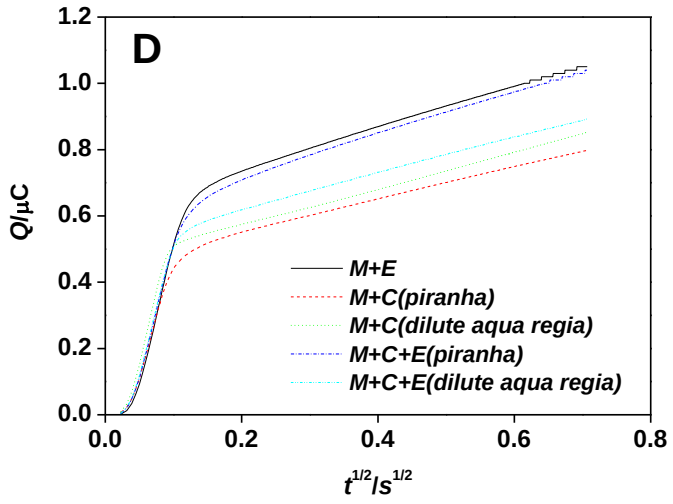


Figure 2. (A) Cyclic voltammetry (*CV*) plots of methylene blue (MB) labeled double stranded DNA (ds-DNA)/mercaptohexanol (MCH) mixed SAMs on gold pretreated by different procedures including *M+E*, *M+C(piranha)*, *M+C(dilute aqua regia)*, *M+C+E(piranha)* and *M+C+E(dilute aqua regia)*. The scan rate *v* was 0.1 V s^{-1} and the electrolytical solution was 50 mM NaCl and 5 mM sodium phosphate buffer solution (pH 7.0). (B) Plots of redox peak potential E_p (E_{pa}, oxidation peak potential; E_{pc}, reduction peak potential) for MB with scan rate *v*. (C) Plots of logi_{pc} (reduction peak current) with log *v*. (D) Chronocoulometry (*CC*) plots (background subtraction) in 50 μM $Ru(NH_3)_6^{3+}$ and 10 mM tris-HCl (pH 7.4) solution.

Table 1. Electrochemical parameters of methylene blue (MB) labeled double stranded DNA (ds-DNA)/mercaptohexanol (MCH) mixed SAMs on gold from our experimental reports

Pretreatment procedures	*Gold roughness factor* R_f	Redox peak potential difference of MB ΔE_p/mV[c]	*Surface coverage of ds-DNA from CC measurement* $\Gamma_{m(CC)}$ / 10^{-12} mol cm^{-2}	*Surface coverage ratio from CV and CC measurements* $\Gamma_{m(CV)}/\Gamma_{m(CC)}$	*Electron transfer rate of MB* k_s/s^{-1}
M+E[a]	1.27 ± 0.26	40 ± 3	8.27 ± 0.26	0.45 ± 0.08	2.76 ± 0.28
M+C(Piranha)[a]	1.19 ± 0.05	87 ± 12	8.13 ± 0.60	0.35 ± 0.01	0.84 ± 0.15
M+C(dilute aqua regia)[a]	1.00 ± 0.00	83 ± 8	9.01 ± 0.05	0.36 ± 0.05	0.82 ± 0.17
M+C+E (Piranha)[a]	1.16 ± 0.12	30 ± 8	7.86 ± 2.32	0.38 ± 0.01	4.76 ± 2.68
M+C+E (dilute aqua regia)[a]	1.04 ± 0.06	35 ± 12	8.34 ± 1.02	0.42 ± 0.08	3.89 ± 2.06
RM+C+E (Piranha)[b]	1.46	21	8.81	0.17	7.79
	1.50	41	8.30	0.52	2.60
	1.68	45	5.54	0.51	2.35
	1.83	31	7.59	0.31	4.14
	2.06	19	10.94	0.63	7.36
	2.19	41	7.23	0.30	2.60
	2.27	33	6.79	0.29	4.68
	2.61	41	7.19	0.29	2.60
	2.65	45	5.90	0.35	2.26

[a]The data were the average value with standard deviation of triplicate measurements (n = 3).

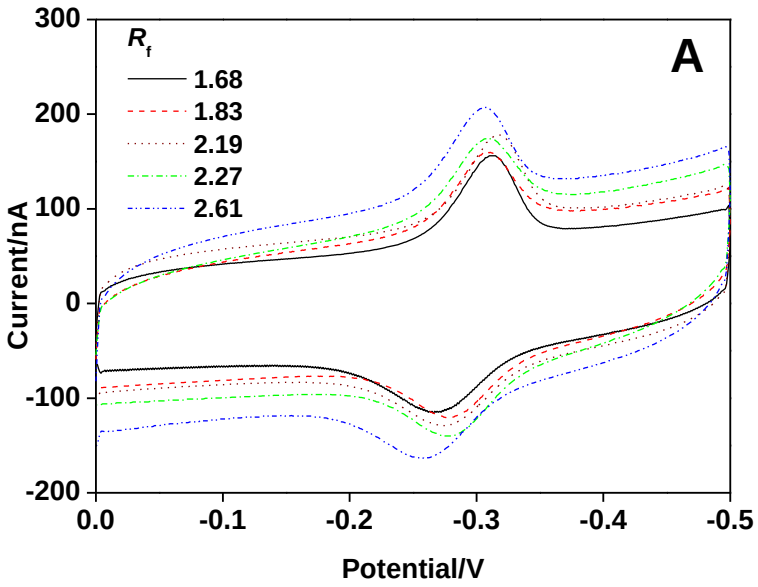
[b]Nine electrodes were pretreated by *RM+C+E (Piranha)* for investigating the electron transfer of MB labeled ds-DNA/MCH SAMs on rough gold.
[c]The ΔE_p values were calculated by *CV* measurement at a scan rate of 0.1 V s^{-1}.

3.2 Effect of pretreatment procedure (RM+C+E) on electron transfer rate (k_s) of MB labeled ds-DNA/MCH mixed SAMs on gold

We applied *RM+C+E* to pretreat the gold electrodes and investigated the effect of gold roughness factor (R_f) on k_s of MB labeled ds-DNA/MCH mixed SAMs on gold.

A couple of MB redox peaks appeared and the $E^{o'}$ were about −0.295 V for all the *CV* plots with ΔE_p values of MB ranged from 19 to 45 mV at a scan rate of 0.1 V s^{-1}(see Figure 3A and Table 1). With the increase of *v*, E_{pa} and E_{pc} shifted gradually and the ΔE_p became bigger and bigger (see Figure 3B). When *v* was 0.01~0.2 V s^{-1}(see Figure 3C), the *R* of logi_{pc}~log*v* plots was from 0.9567 to 1.0000 with the *S* from 0.85 to 1.04, indicating the adsorption-controlled reaction. Based on the ΔE_p values and Equation 3, the calculated k_s values of MB ranged from 2.26 to 7.79 s^{-1}. The $\Gamma_{m(CC)}$ values of MB labeled ds-DNA SAMs on gold with different R_f ranged from 5.54 × 10^{-12} to 10.94 × 10^{-12} mol cm^{-2} (see Figure 3D) and $\Gamma_{m(CV)}$ was 17% ~ 63% of $\Gamma_{m(CC)}$. All the ΔE_p, k_s, $\Gamma_{m(CC)}$ and $\Gamma_{m(CV)}/\Gamma_{m(CC)}$ values were not changing monotonically with increasing R_f values (see Table 1). Furthermore, the $\Gamma_{m(CV)}/\Gamma_{m(CC)}$ values were also not changing monotonically with increasing $\Gamma_{m(CC)}$ (see Table 1). As shown in Table 1, some $\Gamma_{m(CV)}/\Gamma_{m(CC)}$ values differed much although the $\Gamma_{m(CC)}$ values were close. These indicated that the reproducibility of $\Gamma_{m(CC)}$ and $\Gamma_{m(CV)}/\Gamma_{m(CC)}$ values for MB labeled ds-DNA SAMs on gold electrodes pretreated by *RM+C+E* was worse than by *M+E*, *M+C(piranha)*, *M+C(dilute aqua regia)*, *M+C+E(piranha)* and *M+C+E(dilute aqua regia)*.

It was noteworthy that the k_s values (2.26 ~ 7.79 s^{-1}) of MB labeled ds-DNA/MCH mixed SAMs on gold pretreated by *RM+C+E* differed not so much.



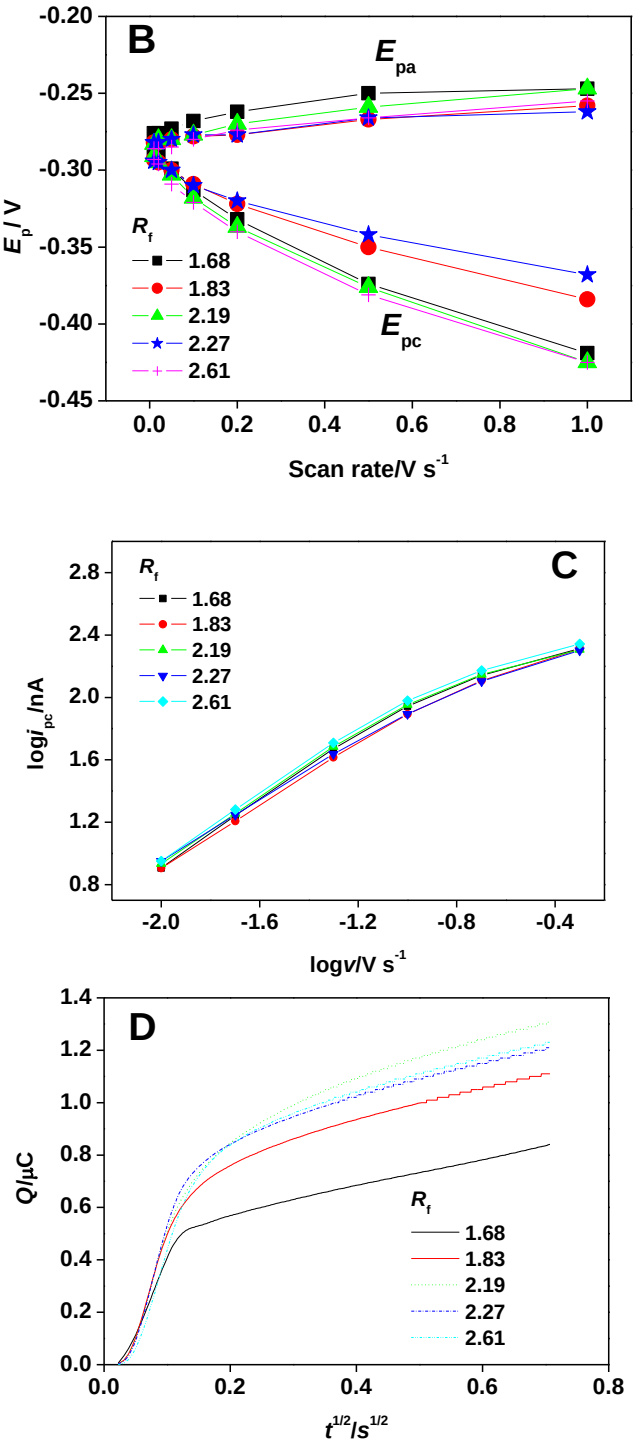


Figure 3. (A) Cyclic voltammetry (*CV*) plots of methylene blue (MB) labeled double stranded DNA (ds-DNA)/mercaptohexanol (MCH) mixed SAMs on gold pretreated by *RM+C+E(piranha)*. The scan rate v was 0.1 V s^{-1} and the electrolytical solution was 50 mM NaCl and 5 mM sodium phosphate buffer solution (pH 7.0). (B) Plots of redox peak potential E_p (E_{pa}, oxidation peak potential; E_{pc}, reduction peak potential) for MB with scan rate v. (C) Plots of logi_{pc} (reduction peak current) with log v. (D) Chronocoulometry (*CC*) plots (background subtraction) in 50 μM $Ru(NH_3)_6^{3+}$ and 10 mM tris-HCl (pH 7.4) solution. In order to show the plots clearly, the data of six electrodes (R_f = 1.68, 1.83, 2.19, 2.27 and 2.61) were used to draw the plots.

Table 1 showed that the k_s of MB labeled ds-DNA/MCH mixed SAMs on gold pretreated by *M+C+E(piranha)* were 4.76 ± 2.68 s^{-1} although R_f of the gold electrodes were almost the same for 1.16 ± 0.12. Thus, gold surface roughness was not the key factor to make the difference of MB electron transfer rate.

3.3 Effect of pretreatment procedures (M+E, M+C(piranha), M+C(dilute aqua regia), M+C+E(piranha), M+C+E(dilute aqua regia)) on electron transfer rate (k_s) of MB through MCH SAMs on gold

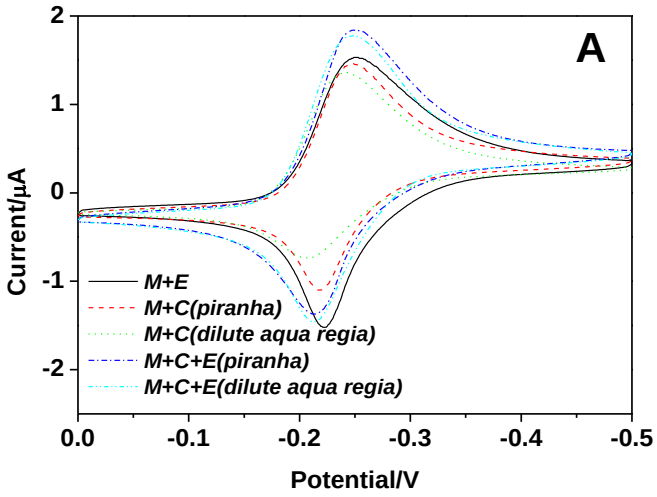
In order to explore the reason arousing the difference of k_s for MB labeled ds-DNA/MCH mixed SAMs on pretreated gold, we investigated MB electron transfer rate through the only MCH SAMs.

The *CV* plots of MCH SAMs on pretreated gold at 0.1 V s^{-1} in 0.1 mM MB, 50 mM NaCl and 5 mM sodium phosphate buffer solution (pH 7.0) were showed in Figure 4A. A couple of MB redox peaks appeared and the $E^{o'}$ values were about −0.230 V for all the plots. All the ΔE_p of MB at v from 0.01 to 0.2 V s^{-1} were about 30 mV, indicating $2e^-$ Nernst response and a diffusion controlled reaction. These plots of E_{pa} and E_{pc} with v (see Figure 4B) were almost superposed, indicating that k_s of MB through MCH SAMs on gold were almost the same and independent of gold pretreatment procedures. Furthermore, the linear relationships of logi_{pc}~log v plots were good with S values for about 0.50 (see Figure 4C), indicating the diffusion-controlled reaction. The diffusion coefficient D of MB (cm^2 s^{-1}) was calculated to be about 4.7×10^{-6} cm^2 s^{-1} based on Equation 6:

$$i_p = 2.69\times10^5 n^{3/2} D^{1/2} v^{1/2} AC \quad (6)$$

Where i_p was the peak current (A), n was electron transfer amount, v was scan rate (V s^{-1}), A was the real area of gold electrode (cm^2) and C was MB concentration (mol mL^{-1}).

According to the linear slopes of E_p ~ ln$v$ plots at higher v, Equation 4 and Equation 5, the k_s of MB through the only MCH SAMs on gold was 2.46×10^{-2} cm s^{-1}.



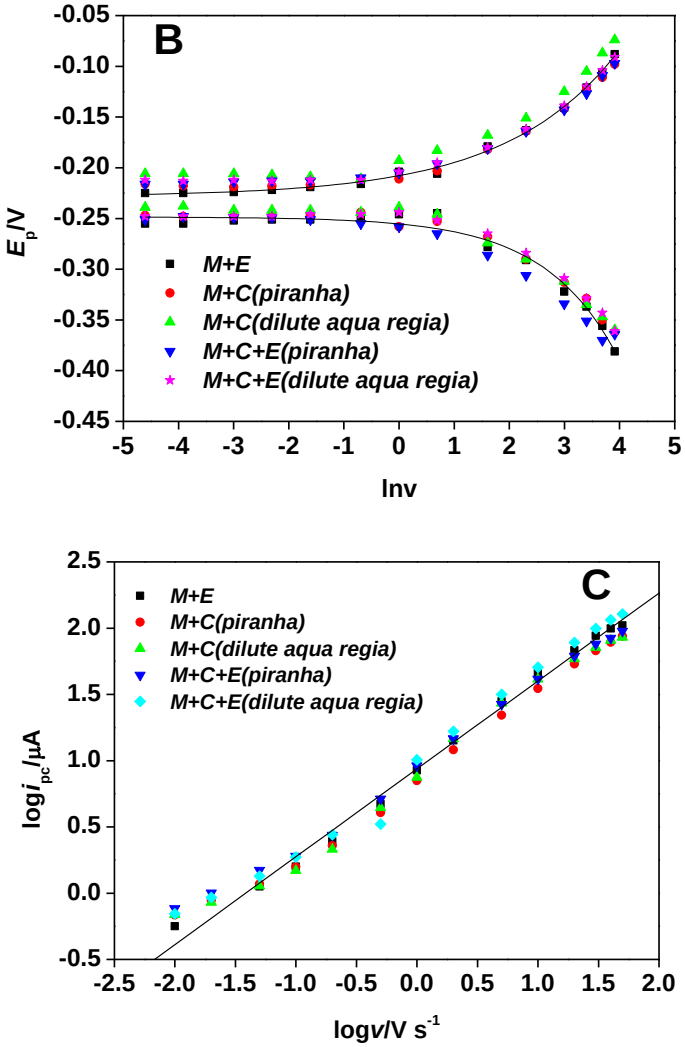


Figure 4. (A) Cyclic voltammetry (*CV*) plots of MCH SAMs on gold pretreated by different procedures including *M+E*, *M+C(piranha)*, *M+C(dilute aqua regia)*, *M+C+E(piranha)* and *M+C+E(dilute aqua regia)*. The scan rate *v* was 0.1 V s^{-1} and the solution was 0.1 mM MB, 50 mM NaCl and 5 mM sodium phosphate buffer solution (pH 7.0). (B) Plots of redox peak potential E_p (E_{pa}, oxidation peak potential; E_{pc}, reduction peak potential) for MB with ln*v*. (C) Plots of logi_{pc} (reduction peak current) with log *v*.

3.4 Exploration for the reason arousing the difference of electron transfer rate (k_s) for MB labeled ds-DNA/MCH mixed SAMs on gold

According to the hexanol packing model for thiol labeled ds-DNA SAMs on Au(111)[19], the ds-DNA molecules could lie flat on gold with tilted angle φ for 90° when Γ_m of ds-DNA was smaller than 1.2×10^{-11} mol cm^{-2}. Because the applied substrate potential was negatively charged (the $E^{o'}$ of labeled MB, −0.295 V), ds-DNA SAMs ($\Gamma_m \approx 8.0 \times 10^{-12}$ mol cm^{-2}) on gold in our work might not lie flat on gold due to the strong electrostatic repulsion between the negatively charged gold substrate and DNA phosphate backbone[20,21]. Electron transfer of MB labeled ds-DNA/MCH mixed SAMs mainly included two paths: 1) MB was intercalated into ds-DNA duplex and electron transferred through the interior of ds-DNA duplex. The electron transfer reaction was adsorption-controlled [7,22]; 2) MB was free from ds-DNA duplex and electron transferred by the bending of ds-DNA

duplex towards the mixed MCH SAMs [4,5]. The electron transfer reaction was diffusion-controlled. In our work, the slopes S of $\log i_{pc}$~$\log v$ plots for MB labeled ds-DNA/MCH mixed SAMs on pretreated gold ranged from 0.70 to 1.04 (S values for v = 0.01~0.2 V s^{-1}), indicating that MB electron transfer reaction was mainly adsorption-controlled and dominated by the path 1. Furthermore, the ΔE_p values of MB at smaller v (0.01 or 0.02 V s^{-1}) ranged from 10 to 15 mV (see Figure 2B and Figure 3B), also indicating the adsorption-controlled redox reaction. Moreover, the reduction peak current of MB decreased greatly when we changed MB labeled ds-DNA SAMs to be MB labeled single stranded DNA (ss-DNA) SAMs with almost the same Γ_m, further proving that the path 1 dominated MB electron transfer.

Barton et al [22] obtained that k_s of daunomycin (DM) labeled ds-DNA SAMs on gold was limited by electron transfer through the tether length, which was a semiclassical superexchange process. The k_s of DM through the interior of ds-DNA duplex was about 10^8~10^9 s^{-1}, much bigger than the k_s (4.4 ~ 733 s^{-1}) through the total ds-DNA SAMs on gold with different tether lengths. If this was the case, in our work the k_s of MB labeled ds-DNA SAMs on gold should be independent of the pretreatment procedures because the tether lengths were the same. However, in our work it was certain that the k_s values for MB labeled ds-DNA SAMs on gold pretreated by *M+C(piranha)* and *M+C(dilute aqua regia)* were smaller than those by *M+E*, *M+C+E(piranha)*, *M+C+E(dilute aqua regia)* and *RM+C+E(piranha)* although the k_s values differed not so much (see Table 1). Thus, electrochemical polishing was an especially important step in gold pretreatment procedures, which might influence the k_s of MB. Furthermore, the difference of k_s values for MB labeled ds-DNA SAMs on gold pretreated by *RM+C+E(piranha)* (see Table 1) was possibly due to the difference of gold surface conditions and R_f was not the key factor to make the difference of k_s, as discussed in Section 3.2.

The reason that electrochemical polishing influenced the k_s of MB labeled ds-DNA/MCH mixed SAMs on gold was confusing. Scanning electron microscopy (*SEM*) images [11] showed that some pits and continuous ridgelike structures appeared for gold pretreated by *M+C(dilute aqua regia)* and *M+C+E(dilute aqua regia)*, which were obviously different from the surface structures (some scratches and pits) of gold pretreated by *M+E*, *M+C+E(piranha)*, *M+C+E(dilute aqua regia)* and *RM+C+E(piranha)*. We considered that the difference of gold surface structures might not the main reason arousing different k_s because k_s of MB labeled ds-DNA/MCH mixed SAMs on gold pretreated by *M+C(piranha)* and *M+C(dilute aqua regia)* or *M+C+E(piranha)* and *M+C+E(dilute aqua regia)* were almost the same although their surface structures differed much. The k_s values of MB through the only MCH SAMs were almost the same and independent of pretreatment procedures (see Figure 4B), also indicating that the difference of gold surface structures was not the key factor influencing MB electron transfer rate. However, MB redox reaction on MCH SAMs was diffusion controlled and the diffusion of free MB molecules from bulk solution to the surface of MCH SAMs on gold was the rate-limiting step, which masked the effect of gold pretreatment procedures on the k_s of MB. X-ray photoelectron spectroscopy (Xps) measurement [23] indicated that the elemental composition of gold surfaces pretreated by *M+C(piranha)* and *M+C(aqua regia)* was different from that by *M+E* and the elemental contents (O, C, N and Cl) on gold surface were reduced when electrochemical polishing was applied to pretreat the gold surfaces. The difference of elemental contents on pretreated gold surfaces

possibly led to different k_s of MB. As reported in literatures [24,25], gold oxide might be encapsulated by alkanethiol SAMs.

It was noteworthy that the linear slopes *S* of logi_{pc}~log$v$ plots for MB labeled ds-DNA/MCH mixed SAMs on gold pretreated by *M+C(piranha)* and *M+C(dilute aqua regia)* were close to 0.70 (v = 0.01~0.2 Vs^{-1}), which indicated a mixed controlled reaction (diffusion and adsorption of MB existed simultaneously). On the contrary, the *S* values of MB on gold pretreated by *M+E*, *M+C+E(piranha)*, *M+C+E(dilute aqua regia)* and *RM+C+E(piranha)* were close to 1.0, indicating an ideal adsorption reaction. Thus, the existing diffusion controlled step of MB might slow down the k_s. Essentially, the difference of gold surface conditions possibly led to the difference of MB redox reaction mechanism (mixed controlled reaction or only adsorption controlled reaction). We summarized the reports from literatures about studying electron transfer of MB labeled ds-DNA SAMs on gold (see Table 2). The k_s values of MB reported by Barton et al [6] were close to those obtained by us. Furthermore, Barton et al [6,22] obtained that the electron transfer of MB was an adsorption controlled reaction, consistent with our experimental report. Different from our report, Ferapontova et al [4,5] obtained that electron transfer of MB was a diffusion controlled reaction and MB labeled ds-DNA SAMs on gold could bend itself to the surface of MCH SAMs for reaction.

Table 2. Electrochemical parameters of methylene blue **(**MB) labeled double stranded DNA (ds-DNA)/mercaptohexanol (MCH) mixed SAMs on gold from literatures and our experimental reports

Molecular structures of MB labeled ds-DNA/MCH mixed SAMs[a] (MB-C_n-ds-DNA_m)	*The formal potential of MB* $E^{o'}$/V vs.SCE[b]	*Redox peak potential difference of MB* ΔE_p/mV[c]	*Surface coverage of ds-DNA* Γ_m/ 10^{-12} mol cm^{-2}		*Electron transfer rate of MB* k_s[c]
			CV[c]	*CC*	
MB-5'-C_{14}-ds-DNA_{20}/MCH[4]	−0.332	~ 92 (0.05 V s^{-1})		4.1	Diffusion controlled
MB-5'-C_{10}-ds-$DNA_{16,20,22}$/MCH[5]	~ −0.282	~24(DNA_{16}) ~34(DNA_{20}) ~27(DNA_{22})	2.3(DNA_{16}) 1.8(DNA_{20}) 1.7(DNA_{22})		4.7×10^{-6}~10.3×10^{-6} cm s^{-1}
MB-3'-C_{10}-ds-$DNA_{16,20,22}$/MCH[5]	~ −0.282	~27(DNA_{16}) ~27(DNA_{20}) ~29(DNA_{22})	1.8 (DNA_{16}) 2.2(DNA_{20}) 2.5(DNA_{22})		5.4×10^{-6}~8.9×10^{-6} cm s^{-1}
MB-5'-C_{14}-ds-DNA_{17}/MCH[6]		140 (LS) 30 (SB)	3.24(LS), 0.1 M $MgCl_2$		3.2 s^{-1} (SB) 0.63 s^{-1} (LS) 3.2 s^{-1} (SB), 0.1 M $MgCl_2$ 2.51 s^{-1} (LS),0.1 M $MgCl_2$
MB-5'-C_n-ds-DNA_{20}/MCH[17]	−0.250	~55(0.05 V s^{-1})	30	50	
MB-3'-C_9-ds-DNA_{25}/MCH (This work)	−0.295 ± 0.002	18 ± 6 (0.05 V s^{-1}) 30 ± 8	3.59 ± 0.24	7.86 ± 2.32	4.76 ± 2.68 s^{-1}

(a) The subscript n of C_n (n = 9, 10, 14) represented the amount of bonded atoms (including C and N) by framework model (For the convenience of the study, we took the N atom as C atom to count the n values).The bigger the n value was, the farther the distance linking MB and DNA was. The n value was not pointed out in Ref [17]. The subscript m of ds-DNA_m represented the amount of base pairs.

(b) These formal potential $E^{o'}$ of MB reported in literatures were all converted to the potential versus SCE based on the following rule: Ag/AgCl (3 M KCl)[4,5], −0.032 V vs. SCE; Ag/AgCl (saturated KCl) [6], −0.045 V vs. SCE.

(c) The ΔE_p was obtained at 0.1 V s^{-1} by *CV* scans unless specifically specified. The "LS" and "SB" in Ref. [6] indicated the low salt buffer (5.0 mM phosphate, 50 mM NaCl, pH 7) and spermidine buffer (5.0 mM phosphate, 50 mM NaCl, 4 mM $MgCl_2$, 4 mM spermidine, 50 μM EDTA, 10% glycerol, pH 7) respectively used for electrochemical measurement. The DNA SAMs were assembled on gold with the assembly electrolytes (LS) with or without 0.1 M $MgCl_2$ [6]. Some ΔE_p values have not been provided directly by literatures, which were obtained by manual measurement from the figures of the literatures.

4. CONCLUSIONS

Effect of different pretreatment procedures on the electron transfer rate (k_s) of methylene blue (MB) labeled double stranded DNA (ds-DNA)/mercaptohexanol (MCH) mixed SAMs on gold was investigated. The k_s values of MB on gold surfaces pretreated by *M+C(piranha)* and *M+C(dilute aqua regia)* were smaller than those by *M+E*, *M+C+E(piranha)*, *M+C+E(dilute aqua regia)* and *RM+C+E(piranha)*, indicating that electrochemical polishing was an important pretreatment step, which might influence the k_s of MB. Furthermore, gold surface roughness was not the key factor to make the difference of MB electron transfer rate. We considered that the difference of elemental composition on gold surface possibly led to different k_s of MB.

Although DNA electron transfer based on redox probes labeled ds-DNA SAMs on gold was studied by some research groups, electron transfer mechanism through ds-DNA SAMs has not been explored clearly. For example, diffusion or adsorption controlled reaction of MB was proposed for electron transfer through ds-DNA SAMs on gold (see Table 2). Further work would be done in our lab for exploring DNA electron transfer mechanism: 1) Change the chain length, the types (5' or 3' phosphate group or DNA bases) or the position covalently linking ds-DNA and redox probes; 2) Change the types of redox probes (intercalation or non-intercalation) and mixed thiols; 3) Besides electrochemical techniques, spectroscopic and microscopic techniques should be applied to characterize surface structures of ds-DNA SAMs on gold in order to explore DNA electron transfer paths in depth.

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