Voltammetric study of interaction of $Co(phen)_3^{3+}$ with DNA at gold nanoparticle self-assembly electrode

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Abstract :

Modifying electrode surfaces on the molecule scale allow developing new electrochemical biosensors. A new strategy for the immobilization of calf thymus DNA on the surface of gold nanoparticles which are co-immobilized at a gold electrode through 4,4'-bis(methanethiol) biphenyl (MTP) molecule by assembly process is demonstrated. The DNA modified electrode was incubated in $Co(phen)_3^{3+}$ solution of an aqueous buffer or an acetonitrile (AN) solution, then it was rinsed and placed in a $Co(phen)_3^{3+}$ free buffer solution or AN solution, followed by cyclic voltammetric experiments. Clear redox peaks of $Co(phen)_3^{3+}$ were observed both in an aqueous and AN solutions. The concentration of supporting electrolyte on electrochemical behavior was discussed. It was found that the surface coverage value of DNA molecules on modified gold nanoparticle and the redox current of adsorbed $Co(phen)_3^{3+}$ were decrease with increasing the size of gold nanoparticles (6nm, 25nm, 42nm, 73nm, and 93nm). In aqueous solution, the electron transfer rate constant of $Co(phen)_3^{3+/2+}$ redox couple became slow with increasing the diameter of gold nanoparticle, and the speed almost had nothing to do with the diameter in nonaqueous solution. The surface concentration of $Co(phen)_3^{3+}$ adsorption on DNA modified electrode decreased and rate constant of adsorption kinetics increased with increasing the interactive temperature. In AN solution, the electrostatic interaction between DNA and $Co(phen)_3^{3+/2+}$ was greatly reduced, however, compare with in aqueous solution the interaction between DNA and reduced form of $Co(phen)_3^{2+}$ was more strongly than oxidized form $Co(phen)_3^{3+}$. The surface concentration of $Co(phen)_3^{3+}$ adsorption on DNA modified electrode reach maximum value when the interactive temperature about 20℃, and rate constant of adsorption kinetics nearly independent of the interactive temperature. The results show that the DNA can adsorb on the

modified electrode firmly and the $Co(phen)_3^{3+/2+}$ adsorbed on DNA give good electrochemical response both in aqueous and nonaqueous solutions. It was confirmed that the DNA modified electrode can be applied in a nonaqueous system and the modified electrode can be used to investigate the interaction between DNA and electroactive species both in aqueous and nonaqueous systems.

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Introduction

DNA-small molecule interactions play important roles in replication and transcription of DNA in vivo, DNA hybridization biosensors, mutation of genes and related variations of species in character, action mechanisms and determination of some DNA-targeted drugs, origins of some diseases, and action mechanisms of some synthetic chemical nucleases etc. [1-5]. As a result, the development of general and efficient assays of DNA-small molecule interactions is an important goal. There have been intensive studies to apply various analytical techniques in the interaction between DNA and some small molecules. Compared to spectroscopic methods, electrochemistry offers great advantages, such as rapid, simple, low-cost, and disposable.

The interaction between complex, antitumor drugs, and some small molecules with DNA have been investigated by electrochemical methods. Bard et al. [6, 7] have investigated the interaction (electrostatic or intercalative) of metal complexes, ML_3 (M=Co(III), Fe(III), Ru(III), and Os(II); L=1,10-phenanthroline, 2,2'-bipyridine), with DNA by voltammetric methods. The interaction between a cobalt(II) complex of five-coordinated chiral porphyrin and calf thymus DNA has been studied by U.V.–Vis. spectroscopy and cyclic voltammetry (CV) [8]. A DNA modified gold electrode has been used to study the interaction between DNA and metal complexes and antitumor drugs[1, 5, 9-13]. Ozsoz et al. used the CPE in combination with differential pulse voltammetry and CV to obtain information about the interaction of $Co(phen)_3^{3+}$, arsenic trioxide, and methylene blue with DNA[14-16]. J. J. Gooding et al. have studied an interaction of 2,

6-disulfonic acid anthraquinone with DNA as an intercalator [17]. This interaction can be used as a method of quantifying the amount of oligonucleotide that is immobilized onto an electrode surface.

Recent activity has focused on the development of nano-scaled particle applied in analytical chemistry for its special physico-chemical characteristics. Gold nanoparticle has been utilized in colorimetric detection of DNA by its surface plasma resonance absorption [18]. Nanoparticles combined with electrochemical methods have also been used to research in this field [19-21]. For example, Wang *et al*.[20] have developed a technique in which three kinds of nanoparticles, CdS, ZnS, and PbS, were used to probe hybrid events. These nanoparticles were attached to different DNA sequences probe. Probe-modified magnetic beads were hybridized with target DNA, and the nanoparticles were dissolved and analyzed by ASV. Three targets could be simultaneously probed by electrochemical method. On the other hand, colloid Au was also used to prepare modified electrode in order to enhance the DNA immobilization amount. The method has been applied in a detection of sequence-specific DNA [22, 23] and a determination of mifepristone [24].

The aim of this work is to investigate the effect of gold nanoparticle on the interaction between DNA and complex, $Co(phen)_3^{3+}$, and the characteristic of the interaction in a nonaqueous solution. In this paper, various size gold nanoparticles were assembled on gold disk electrode by rigid-rod dithiols molecule, 4,4'-bis (methanethiol)biphenyl (MTP). Calf-thymus DNA was adsorbed on the gold nanoparticle modified electrode. The interaction between DNA and $Co(phen)_3^{3+}$ both in aqueous and acetonitrile was studied by using cyclic voltammetry.

Experimental

Materials

Calf thymus DNA (D-1501, highly polymerized), was purchased from Sigma. Reagent grade 2-Amino-2-hydroxymethyl-1,3-propanediol (Tris), NaCl, H_2SO_4 H_2O_2, $HClO_4$, and hydrogen tetrachloroaurate(III) tetrahydrate were purchased from Wako Pure Chemical, Co. Ltd. and used

as received. Tetraethylammonium perchlorate (TEAP) was a polarographic grade product purchased from Nacalai Tesque. It was dry at 65 ^{o}C for 3 hours under a high vacuum with P_2O_5 before used. Acetonitrile was purified by the same method as that reported in the literature [25]. The tris(1,10-phenanthroline) Cobalt(Ⅲ) perchlorate, $Co(phen)_3(ClO_4)_3$, was synthesized as that reported in the literature [26]. The 4,4'-bis(methanethiol) biphenyl(MTP) was prepared in the same way as that reported elsewhere[27]. Colloidal gold sols with average particle diameters of 6, 25, 41, 72, and 97nm were prepared as previously reported [28-30].

The DNA solution was prepared with 50mM Tris-HCl (pH 7.00) and 20mM NaCl, stored at 4℃ and discarded after no more than 4 days. The concentration of DNA (NP) was determined by UV absorbance at 260nm. The extinction coefficient, ε_{260}, was taken as 6600$M^{-1}cm^{-1}$[31].

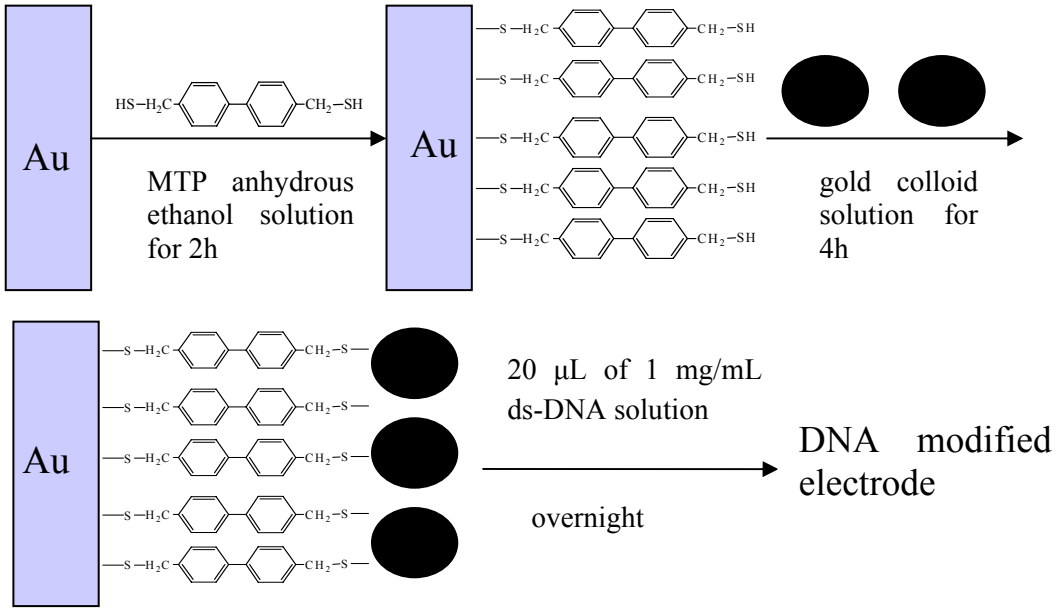
Electrochemical Measurement

Cyclic voltammetric measurements were performed using potentiostat/galvanostat combined with arbitrary function generator (HOKUTO DENKO) and an X-Y recorder at ambient temperature (23±2℃). A conventional three–electrode system was used. The working electrode was a DNA modified gold nanoparticle electrode and the reference electrodes were a Ag/AgCl/3M NaCl (from Bioanalytical System Inc.) for an aqueous system and a Ag^{+}(10mM)/Ag for AN system. The counter electrode was a platinum wire.

Preparation of DNA modified electrodes

The gold disk electrode was prepared by seal polycrystalline gold wire (2mm Φ) in a Teflon tube. The electrodes were carefully polished with emery paper (No.2500), followed by 0.05μm alumina slurry on microcloth pads. After removal of the trace alumina from the surface by rising with water, the electrodes were ultrasonicated for 10 min in fresh prinha solution (H_2SO_4: H_2O_2 (30 v/v%)=3:1). *Warning: Piranha solution reacts violently with organic solvents.* The electrode was then sonicated by water and anhydrous ethanol. The DNA gold nanoparticle modified gold disk electrode was then prepared as follows. Firstly the gold disk modified by incubation in 500μM MTP anhydrous

ethanol solution for 2 hrs. It was washed twice by ethanol and subsequently by distilled water prior to adsorption of gold nano-particle. The various size gold nanoparticles modified electrodes were obtained by immersing them into corresponding gold colloid solution for 4 hrs. Washing with water, the freshly electrode was modified by transfer a droplet of 20μL of 1 mg/mL ds-DNA solution onto the surface. It was dried by standing overnight at 4℃, followed by rinsing with water and Tris-HCl buffer to remove unadsorbed DNA molecules. The DNA modified electrode is illustrated in the scheme.



Scheme 1. Schematic diagram of the preparation of DNA modified electrode.

Results and discussion

The interaction between DNA and $Co(phen)_3^{3+}$ was performed at ambient temperature by immersing the DNA modified electrode in 0.5mM $Co(phen)_3(ClO_4)_3$ aqueous or AN solution for 300s. The electrode was rinsed with water or with AN for 20s and it was transferred to a $Co(phen)_3(ClO_4)_3$ free Tris-HCl buffer solution or AN solution for voltammetric experiments.

Cyclic voltammograms (CVs) exhibit a pair of cathodic peak and anodic counter peak as shown in Fig. 1.

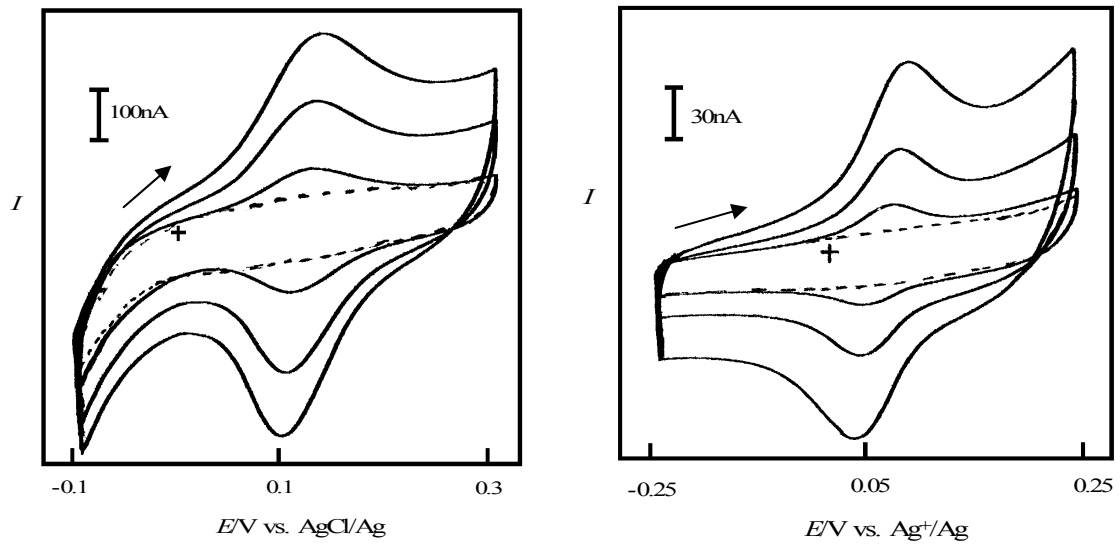


Fig.1 Cyclic voltammograms of $Co(phen)_3^{3+}$ adsorbed on DNA modified (6nm NG) electrode in aqueous and AN solutions. Left: aqueous system with 50mM NaCl + 20mM Tris-HCl (pH 7.0) as supporting electrolyte, potential scan rates v=10, 20, 40mv/s, reference electrode; Ag/AgCl . Right : AN system with 50mM TEAP as a supporting electrolyte, potential scan rates v=10, 20,40mv/s, reference electrode; 0.01M Ag^+/Ag. Dashed lines were obtained by using gold nanoparticle electrode (without ds-DNA) instead of DNA modified electrode under the same experimental conditions (v=10mV/s).

It can be seen that the redox peaks for $Co(phen)_3^{3+/2+}$ appear at an $E^{o'}$ (=(E_{pa}+E_{pc})/2, formal potential) value of 138mV(v.s. Ag/AgCl) with ΔE_p= 42mV(at scan rate of 20mV/s) in aqueous solution while the $E^{o'}$ value of 45mV(v.s. Ag^+/Ag) with ΔE_p= 48mV(at scan rate of 20mV/s) in AN solution. The peak current was directly proportional to the potential scan rate at low scan rate which was characteristic of a surface process. The full width half-maximum of 120mV, being great than the theoretical value for a single bound species, implies a distribution of $Co(phen)_3^{3+/2+}$ molecules in different environment.

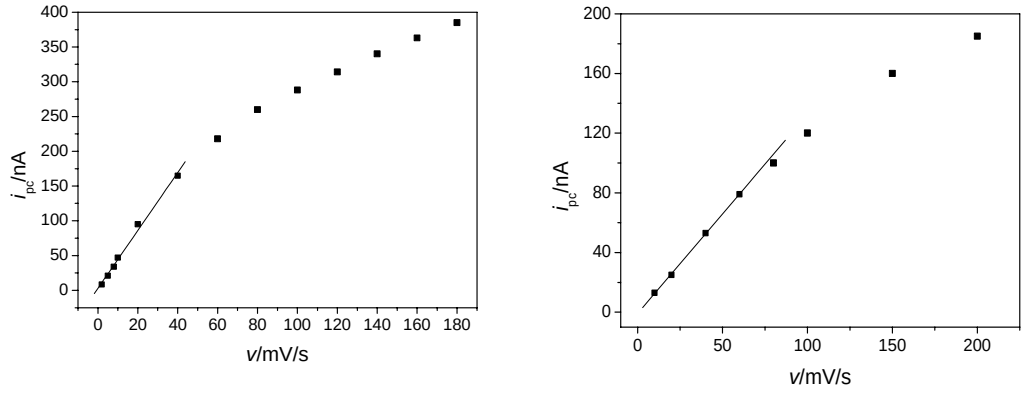


Fig. 2 Relationships between reduction peak currents of $Co(phen)_3^{3+/2+}$ and potential scan rate.

Left: in aqueous solution; Right: in AN solution. Other experiment conditions were the same as those in the caption for Fig. 1.

Using the slope of the line in Fig. 2, the concentration of $Co(phen)_3^{3+}$ on the modified gold electrode surface could be calculated according to the equation:

$$i_p=n^2F^2\Gamma vA/4RT$$

where n was the number of electrons transferred, F is the Faraday's constant, v was potential scan rate, A and Γ were the electrode area and the surface concentration of electroactive species, respectively. The geometric area of the bare gold electrode was applied to simulate calculation of Γ because it was difficult to measure accurately the total surface area of the modified electrode. The results were listed in Table 1. The deviation from linearity and the increase in ΔE_p with increasing scan rate were observed at high scan rate, which indicates that the electron transfer kinetics was slow. The electron transfer rate constants could be readily calculated using next equation of Laviron theory [32].

$$E_{pc}=E^{o'}-\frac{RT}{\alpha nF}\ln(\frac{\alpha nFv}{RTk_{et}})$$

$$E_{pa}=E^{o'}+\frac{RT}{(1-\alpha)nF}\ln(\frac{(1-\alpha)nFv}{RTk_{et}})$$

where E_{pc}, E_{pa}, and $E^{o'}$ are the cathodic, anodic peak potentials, and the formal potential,

respectively. The k_{et} is electron transfer rate constant and other symbols have their usual meanings. Figure 3 shows the relationship between the scan rate and relative peak potential, $\Delta E = E_p - E^{o'}$.

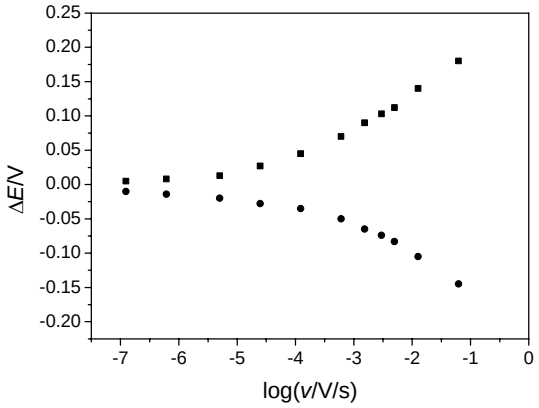


Fig. 3. The plot of ΔE *vs*. log(v) for cyclic voltammograms of $Co(phen)_3^{3+}$ intercalated into a DNA modified gold disk electrode in AN solution. The experimental conditions were the same as those in the caption of Fig. 1.

The electron transfer rate constants, k_{et}, could be derived from an analysis of the linear part of the plot at the high potential scan rates. Combining the values of the slope and the interception of the linear part, the k_{et} was found to be 0.53±0.06 s^{-1}.

The Effect of the Size of Gold Nanoparticle

In order to investigate the effect of the size of gold nanoparticle on CV character, several different size gold nanoparticles, 6nm, 25nm, 41nm, 72nm, and 97nm, were used to fabricate DNA modified electrode. Table 1 summarized the surface concentration of the adsorbed $Co(phen)_3^{3+}$ and electron transfer rate constants on the various sizes of gold nanoparticle modified electrodes. It can be found that the surface concentration $Co(phen)_3^{3+/2+}$, Γ, decreased with increasing the size of nano-particle diameter in both aqueous and AN systems.

Assuming the ratio of base pairs of the DNA to $Co(phen)_3^{3+/2+}$ on the gold nanoparticle modified electrode surface was 5 in aqueous[10], the DNA surface coverage value (in base pairs) could be determined. The results were also listed in Table 1. Pang and Abruna [5] investigated the

interactions between DNA adsorbed onto bare gold surface and redox-active molecules. The DNA surface concentration (in base pairs) on the bare gold electrode was about 1.1×10^{-10}mol/cm^2. On 6-nm gold modified electrode, 5.2×10^{-10}mol/cm^2 DNA molecules (in base pairs) were immobilized on the electrode surface. Comparing with bare gold electrode, about four times more DNA molecules could be adsorbed onto the surface of 6-nm gold nanoparticles modified electrode. However, when 97nm gold nanoparticles were used to fabricate modified electrode, the concentration of DNA adsorbed on electrode surface were almost same as bare gold electrode.

Table 1. Dependence of surface concentration of the adsorbed $Co(phen)_3^{3+}$ (c) and electron transfer rate constants (k_{et}) on the size of gold nano-particle

Size of nano-particle(/nm)		6	25	41	72	97
DNA concentration (in base pair)/ 10^{11}/mol/cm^2		52±4.0	30±3.0	18±1.0	13.5±1.8	10±1.0
In aqueous	$c/10^{11}\Gamma$/mol/cm^2	10.5±0.8	5.9±0.6	3.6±0.2	2.7±0.3	2.0±0.2
	k_{et}/s^{-1}	0.53±0.06	0.34±0.03	0.20±0.04	0.14±0.03	0.10±0.04
In acetonitrile	$c/10^{11}\Gamma$/mol/cm^2	3.4±0.3	1.8±0.3	1.1±0.1	0.95±0.07	0.76±0.06
	k_{et}/s^{-1}	0.51±0.05	0.43±0.06	0.45±0.04	0.41±0.06	0.48±0.08

Combining the values of DNA molecules and $Co(phen)_3^{3+/2+}$ adsorbed on the electrode surface, one can find that the ratio of base pairs of DNA to $Co(phen)_3^{3+/2+}$ was about 15 when the interaction between DNA adsorbed onto modified electrode and $Co(phen)_3^{3+/2+}$ was performed in AN solution. It means one complex molecule integrate with 2 times more base pairs in AN solution than in an aqueous system. This might be due to: 1. the conformation of DNA molecule in AN solution was different from it in aqueous; 2. the negative charge on DNA molecule greatly reduced in AN solution due to the suppression of proton dissociation compare with in aqueous. So electrostatic interaction between DNA and $Co(phen)_3^{3+/2+}$ became weak and even to the extent that the intercalative interaction between DNA and $Co(phen)_3^{3+/2+}$ was probably only existence.

The influence to the electron transfer speed of nano-particle diameter was different in these two kinds of solution systems. Increasing the diameter from 6nm to 97nm, the k_{et} became slow, and the speed almost had nothing to do with the diameter in non- aqueous solution.

The Effect of supporting electrolyte concentration

The effect of supporting electrolyte concentration on CVs was also investigated in both aqueous and AN systems. Cyclic voltammograms of $Co(phen)_3^{3+}$ adsorbed on the DNA-modified electrode in aqueous and AN solutions containing different concentrations of respective NaCl and TEAP are shown in Fig. 4. It can be found that the i_p and $E^{o'}$ varied with the concentration of supporting electrolyte in both solution systems. It is apparent that the ΔE_p (peak-peak potential separation) became decrease and i_p (peak current) enlarge with increasing the concentration. It was partly due to the effect of the concentration of supporting electrolyte on solution *iR* drops, however, the effect on $E^{o'}$ showed different character in aqueous and in AN solution. In aqueous, one can find that $E^{o'}$ moving to the positive direction with increasing supporting electrolyte concentration. It means that the reduced form of $Co(phen)_3^{2+}$ bond to DNA more strongly than oxidized form of $Co(phen)_3^{3+}$ in high ionic strength solution. That is consistent with the result that reported in literature [5]. The intercalative interaction is much more dominant than the electrostatic interaction in high supporting electrolyte concentration.

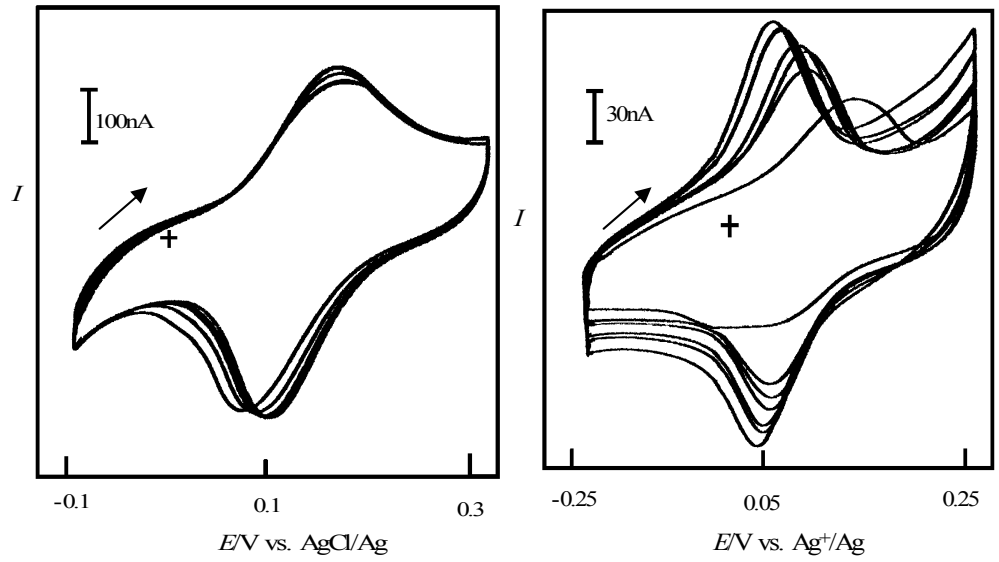


Fig. 4. Cyclic voltammograms of adsorbed $Co(phen)_3^{3+/2+}$ in different supporting electrolyte concentration. Left: in aqueous c_{NaCl}=0, 20,40,60, 80, 100mM (from left to right). Right: in AN solution c_{TEAP}= 0, 5, 10, 20, 40, 60, 80mM (from inner to outer). Scan rate 40mV/s.

The reverse trend, however, was observed in AN solution. Plots of $E^{o'}$ as a function of the supporting electrolyte concentration obtained from Fig. 4 and they are presented in Fig. 5.

At bare gold nanoparticles modified electrode (without DNA), a pair of redox peaks for $Co(phen)_3^{3+/2+}$ appeared at an $E^{o'}$ value of 31mV (*v.s.* 0.01mM Ag^+/Ag) in AN solution and the influence from the change in concentration of supporting electrolyte was neglected because of little difference in $E^{o'}$. From Fig.5 (right), it can be found that the $E^{o'}$ shift to the negative direction, from 68mV to 33mV, with increasing supporting electrolyte concentration from 0mM to 200mM (TEAP). This means that the reduced form of $Co(phen)_3^{2+}$ interacted with DNA more strongly than oxidized form of $Co(phen)_3^{3+}$ in AN solution in all over the range of supporting electrolyte concentration (0mM to 200mM). The result also suggests that oxidized form of $Co(phen)_3^{3+}$ bond to DNA was more strongly with increasing supporting electrolyte concentration in AN solution. This may be due to the weaker electrostatic interaction between $Co(phen)_3^{3+/2+}$ and DNA molecules in AN solution as discussed before. Moreover, the repulsion between

adsorbed $Co(phen)_3^{3+}$ and the $(C_2H_5)_4N^+$ ions around the electrode became strongly with increasing the concentration of TEAP in AN solution, as a result the interaction between $Co(phen)_3^{3+}$ and DNA might be more strongly in high TEAP concentration than that in low one.

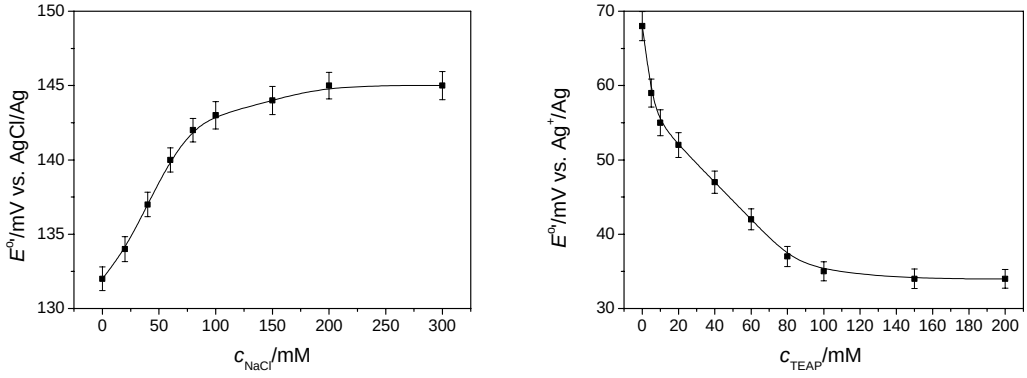


Fig. 5. The effect of supporting electrolyte concentration on E°'. Left: in aqueous; Right: in AN solution.

The Effect of interactive time, interactive temperature, and concentration of $Co(phen)_3^{3+}$

The additional information about the nature of the interaction between $Co(phen)_3^{3+/2+}$ and DNA can be obtained by investigating the effect of interactive time, temperature, and concentration of $Co(phen)_3^{3+}$ on CV behavior. In order to understand the effect of these factors, the CV experiments were executed in H_2O or AN blank solution at ambient temperature (23±2℃) while change the DNA modified electrode interactive time (in $Co(phen)_3^{3+}$ H_2O solution or AN solution), interactive temperature and the concentration of $Co(phen)_3^{3+}$ in H_2O solution or AN solution. Figure 6 shows the effect of concentration of $Co(phen)_3^{3+}$ on reduction peak current. It can be found that the peak current increase, either in H_2O or in AN solution, with increasing the concentration of $Co(phen)_3^{3+}$ in interactive solution. The $Co(phen)_3^{3+}$ saturated adsorption on DNA modified electrode were reached at the point of 0.1mM $Co(phen)_3^{3+}$ AN solution and 0.3 mM $Co(phen)_3^{3+}$ in H_2O system, respectively.

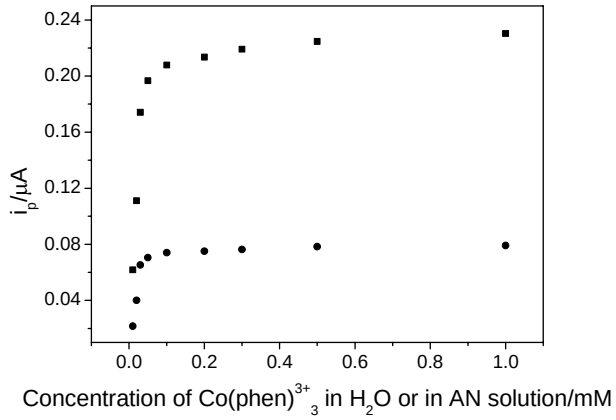


Fig. 6. The effect of $Co(phen)_3^{3+}$ on reduction peak current. DNA/MTP/Au electrode immersing in $Co(phen)_3^{3+}$ solution 300s at 25±0.5℃. Potential scan rate is 50mV/s and other experimental conditions were the same as those in the caption of Fig. 1. ■: in H_2O; ●: in AN solution.

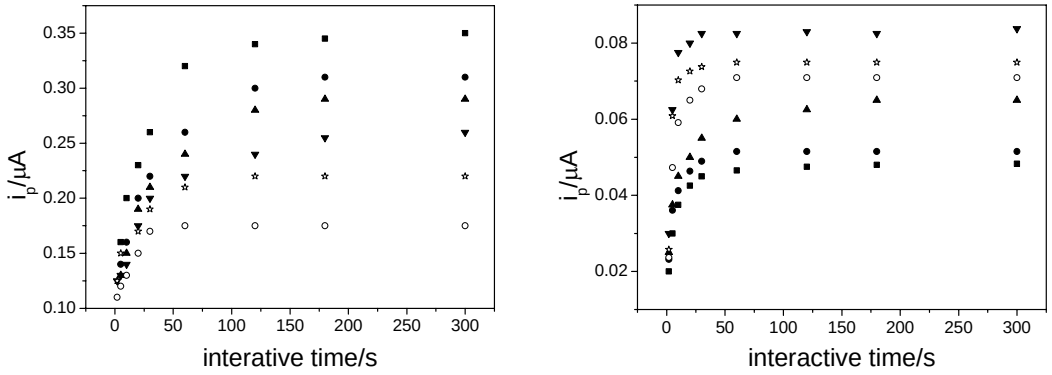


Fig. 7. The effect of interactive time and interactive temperature on reduction peak current. Left: in H_2O system; Right: in AN system. ■: 5℃; ●: 10℃; ▲: 15℃; ▼: 20℃; ☆: 25℃; ○:30℃; Potential scan rate: 50mV/s and other experimental conditions were the same as those in the caption of Fig. 1

Figure 7 shows the effect of interactive time and temperature on the interaction between $Co(phen)_3^{3+}$ and DNA . The concentration of $Co(phen)_3^{3+}$ was kept at 1mM. It is found, surface concentration of the adsorbed $Co(phen)_3^{3+}$ on DNA modified electrode decreased with increasing the interactive temperature in H_2O system. This may be due to the electrostatic interaction between $Co(phen)_3^{3+}$ and DNA weakening in high temperature. Using first-order Langmuir

isotherm model: $\theta(t) = 1 - \exp(-kt)$, where θ is the ratio of $Co(phen)_3^{3+}$ surface concentration to saturated concentration, *t* adsorption time (interactive time) , and *k* rate constant of adsorption kinetics. The values of *k* can be obtained based on figure 7, and the values were $0.022s^{-1}$, $0.026s^{-1}$, $0.031s^{-1}$, $0.037s^{-1}$, $0.042s^{-1}$, and $0.053s^{-1}$ for interactive temperature 5℃, 10℃, 15℃, 20℃, 25℃, and 30℃, respectively. In AN solution, surface concentration of the adsorbed $Co(phen)_3^{3+}$ on DNA modified electrode increased with increasing the interactive temperature from 5℃ to 20℃, and then decreased with further increasing the interactive temperature from 20℃ to 30℃. This may be due to: 1. In AN solution, only intercalative interaction between DNA and $Co(phen)_3^{3+/2+}$ exist, as discussed above; 2. The conformation of DNA helix in AN solution can adsorbed more $Co(phen)_3^{3+/2+}$ molecules through intercalative interaction around 20℃. The rate constant of the adsorption kinetics of *k* is about 0.3-0.5 s^{-1} in the range of 5℃-30℃. It is about ten times more than in H_2O system, and nearly independent of the interactive temperature.

Conclusion

We have demonstrated that a gold nanoparticle modified electrode is suitable for investigating the interaction between DNA and electroactive species not only in aqueous but also in nonaqueous (AN) solution. Surface concentration of DNA adsorbed on gold nanoparticle modified electrode varied with the size of nanoparticle. The difference of the ratio of base pairs of the DNA to $Co(phen)_3^{3+/2+}$ on the surface of modified electrode may be due to the existence of only intercalative interaction between DNA and $Co(phen)_3^{3+/2+}$ in AN system while both intercalative interaction and electrostatic interaction existence in aqueous solution.

In aqueous, the electron transfer rate of $Co(phen)_3^{3+/2+}$ redox couple adsorbed on DNA molecules became slow with increasing the diameter of gold nano-particle, the oxidized form of $Co(phen)_3^{3+}$ combining with DNA was more strongly than reduced form of $Co(phen)_3^{2+}$ in low supporting electrolyte concentration, while the contrary result was observed in high supporting electrolyte

concentration solution. The surface concentration of $Co(phen)_3^{3+}$ adsorbed on DNA modified electrode decreased and rate constant of adsorption kinetics increased with increasing the interactive temperature. In AN solution, however, the electron transfer rate almost had nothing to do with the diameter of gold nanoparticle, the interaction between reduced form $Co(phen)_3^{2+}$ and DNA was more strongly than that's of oxidized form $Co(phen)_3^{3+}$ in all over the range of supporting electrolyte concentration (0mM to 200mM). The surface concentration of $Co(phen)_3^{3+}$ adsorbed on DNA modified electrode reached maximum value when the interactive temperature was about 20℃, and rate constant of adsorption kinetics nearly was independent of the interactive temperature. It also suggested that the electrostatic interaction between DNA and redox species was greatly weaken and the intercalative interaction became dominator in AN solution.

Acknowledgement

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