Int. J. Electrochem. Sci., 10 **(2015)** 7320 - 7330

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Electrochemical Study on the Interaction Between Dopamine and Deoxyribonucleic Acid Using a Glassy Carbon Electrode Modified With Silver-Doped Poly Cysteine Membrane

Xueliang Wang[1,2], *Li Sun*[2], *Na Zou* [2], *Zhangyu Yu*[2,3,*]

[1]School of Chemistry and Chemical Engineering, Shandong University, Jinan 250010, Shandong, China
[2]Department of Chemistry and Chemical Engineering, Heze University, Heze 274015, China
[3]College of Chemistry and Chemical Engineering, Jining University, Qufu 273165, China
[*]E-mail: yuzywxl@163.com

Received: 31 May 2015 / *Accepted:* 7 July 2015 / *Published:* 28 July 2015

The interaction of dopamine (DA) with herring sperm deoxyribonucleic acid (ds-DNA) has been studied by cyclic voltammetry (CV) at a glassy carbon electrode (GCE) modified with silver-doped poly cysteine membrane (Ag NPs/Pcys/GCE). The modified electrode exhibited excellent catalytic properties for the redox reaction of DA in 0.1 mol/L phosphate buffered saline (PBS) of pH 7.0. As added DNA into the DA solution, the oxidation peak current of DA decreases as well as the peak potential shifts negatively and no new peaks appear. Based on the electrochemical results, some electrochemical parameters, such as the electron transfer coefficient (α), the standard rate constant of the electrode reaction (K_s) were calculated in the absence and presence of DNA. The binding ratio (m) and the apparent binding constant (β) were also calculated. The observed negative shift in peak potential of DA in the presence of DNA indicated that DA reacted with DNA via an electrostatic binding mode. Furthermore, the hyperchromic effect of DA in ultraviolet visible spectroscopy (UV-Vis) after it interacted with DNA confirmed above conclusions obtained from electrochemical method.

Keywords: dopamine; DNA; interaction; voltammetry

1. INTRODUCTION

Dopamine (3,4-dihydroxyphenyl ethylamine), as one of the important catecholamine neurotransmitters which is widely distributed in the mammalian central nervous system, plays a pivotal

role in the function of human metabolism, renal, cardiovascular, and hormonal systems, and is proposed to play a role in mood, motor function, and cognition[1]. A growing body of evidence indicates that extreme abnormalities of DA concentration is associated to several diseases and neurological disorders, e.g., Huntington's, Schizophrenia, Epilepsy, HIV infection and Parkinson's disease[2-6]. Therefore, the precise determination of the concentration or the simultaneous electrochemical detection of dopamine, ascorbic acid and uric acid in human brain, urine, tissue and blood has been one of the most important research focuses and many quantitative methods, including spectrophotometry, high performance liquid chromatography, capillary electrophores, chemiluminescence, fluorimetry and electrochemical techniques have been developed[7-10]. Among of these methods, electrochemical methods with chemically modified electrode have been certified to be efficient tools for studying the oxidative and reductive substances due to its high sensitivity and selectivity. Zhang and associates detected DA at an in-site functionalized self-assembled monolayer on a gold electrode[11]. Denis and co-workers developed an ionic liquid-based miniature electrochemical sensor for the voltammetric determination of catecholamines[12]. Carlos modified a glass carbon electrode with L-cysteine for the determination of DA[13].

In recent years, several researches have revealed that DA could promote the oxidative DNA damage in the presence of copper or ferric ions although catecholamines are potent radical scavengers[14-17]. For example, Miaomiao Chen studied the DNA damage induced by ferric ions catalyzed oxidation of DA[15]. Therefore, there exited many scientific questions on the interaction of catecholamines and DNA to be studied.

Deoxyribonucleic acid (DNA), an essential biological material, which is correlates with many kinds of biological phenomena, plays a vital role in the storage, replication, and transcription of the genetic information, and is always to be a major target for antibiotic, antiviral and anticancer drugs[18]. Recently, the interaction between small molecules and DNA has been aroused considerable interest because it is contacted with the mutation of genes, the replication and transcription of DNA in vivo. On the other hand, the interaction mechanisms are of great importance in drug designing and pharmaceutical development processes[19-23]. A variety of techniques, such as constant wavelength synchronous fluorescence spectroscopy (CW-SFS)[24], resonance Raman spectroscopy[25], circular dichroism[26], single-molecule force spectroscopy[27], UV-Visible spectrophotometry[28,29], fluorescence spectroscopy[29-31], and electrochemical methods[31-33], have been used to investigate the interactions. The electrochemical methods were widely used due to the electron transfer reactions that occur between solution and on electrode are similar to the redox reaction that take place in living cell in vivo. The changes in the electrochemical behaviors of the small molecules, either in an aqueous medium or on a DNA-modified electrode, can be monitored to elucidate the mechanism of the molecule-DNA interactions[34].

In the present work, the detailed electrochemical behavior of DA at a silver-poly (L-cysteine) modified electrode and the interaction with DNA were studied by voltammetric and spectroscopic techniques. This method utilized the electrooxidation of L-cysteine to its analogous radicals to form a

chemically stable covalent linkage between the nitrogen atom of the amine group and the edge plane sites at the carbon electrode surface[35], the poly L-cysteine modified GCE has been used to study the electrochemical behaviors of DA and its determination[13]. However, to the best of our knowledge, using silver doped poly (L-cysteine) modified electrode to study the interaction between DA and DNA has not been reported. It is found that the poly film showed good electrocatalytic activity for redox reactions of DA. The systems provided useful insight on the electrochemistry of DA oxidation as well as a novel method to introduce selectivity for sensitive sensing of DA. Further, binding modes of DA to DNA can be used as a model for DNA binding of anticancer drugs, and DA may be used as an indicator in the detection of DNA damage and hybridization.

2. EXPERIMENTAL

2.1. Reagents and apparatus

Herring sperm DNA (crude oligonucleotides < 50bp) was purchased from Beijing Biodee Biochnology, the purity can be described by the absorbance ratios (A_{260}/A_{280} and A_{260}/A_{230}). This ratio should be in the range of 1.8-1.9 to ensure that the DNA is sufficiently free of protein[36]. The molar concentration of the DNA was detected by UV absorption spectroscopy at 260 nm (ε = 6600 L $mol^{-1}cm^{-1}$)[37]. The DNA stock solution was prepared by dissolving DNA in triple-distilled water and was stored at 4ºC. The 10 mg/mL dopamine hydrochloride was available from Harvest Pharmaceutical Co., Ltd.(Shanghai, China). L-cysteine was provided by Sinopharm Chemical Reagent Co., Ltd.(Shanghai, China). A 0.1 mol/L PBS, prepared by mixing stock solution of 0.1 mol/L NaH_2PO_4 and 0.1 mol/L Na_2HPO_4 and the pH was adjusted with 0.1 mol/L H_3PO_4 or 0.1 mol/L NaOH, was used as supporting electrolyte for the determination of DA. Na_2HPO_4, NaH_2PO_4, H_3PO_4 and NaOH were all obtained from Tianjin Chemical Co., Ltd. (Tianjin, China). All other regents were of analytical grade and used without further purification. Triply distilled water was used throughout. All solutions under investigation were deaerated by bubbling highly pure nitrogen prior to measurements.

Electrochemical measurements were performed with CHI660C electrochemical workstation (Shanghai Chenhua Instruments Co., Ltd., China) with a conventional three-electrode configuration of a GCE (d = 3 mm) or modified GCE electrode as working electrode, a saturated calomel electrode (SCE) as the reference electrode and a platinum wire auxiliary electrode. UV spectroscopy was carried out on a TU-1810 spectrophotometer (Beijing, China). A model pHS-25 digital acidometer (Shanghai Leici Factory, China) was used for pH measurement.

2.2 Preparation of Ag-Pcys/GCE

Before modification, the bare GCE was pretreated according to the previous literature[38]. Then the GCE was treated with cyclic scanning in the potential range of -0.8~2.4V at 140 mV/s for 8 cycles in PBS (pH 7.0) containing 0.096 mmol/L of HNO_3, 1.0 mmol/L of $AgNO_3$, 0.05 mol/L of

KNO_3, and 4.0 mmol/L L-cysteine. The modified electrode could be used repeatedly after rinsed with triply distilled water to remove the physically adsorbed and unreacted species from the electrode surface and dried in the air at ambient temperature.

2.3 Experimental procedure

Cyclic voltammetry (CV) was performed with three electrodes in PBS. The cyclic voltammograms were recorded by cycling in the potential range of -0.3 ~ 0.6V at a scan rate of 160 mV/s. In addition, DNA and different concentrations of DA were mixed in 0.1 mol/L PBS of pH 7.0; then the CV was recorded after equilibration period of 13 minutes. Absorption spectra were recorded after each successive addition of DNA solution followed an equilibration period of 13 minutes.

3. RESULTS AND DISCUSSION

3.1 Voltammetric behavior of DA

The electrochemical behaviors of DA at a bare (curve a) and Ag NPs/GCE (curve b), Pcys/GCE (curve c) and Ag NPs/Pcys/GCE (curve d) were investigated employing cyclic voltammetry (CV). As shown in Fig.1, DA exhibited poor current response on the bare electrode (curve a), whereas on the modified electrode, especially the Ag NPs/Pcys/GCE (curve d), the current of DA was greatly enhanced, which may be ascribed to the good catalytic properties of L-cysteine for DA and the increase of specific surface area of Pcys/GCE due to the presence of Ag NPs. DA had an oxidation peak at 0.245V and a reductive peak at 0.11V with a scan rate of 160 mV/s. The separation between the cathodic and anodic peak potential ($\Delta E_p = E_{pa} - E_{pc} = 135$mV) and the current ratio (i_{pa}/i_{pc}) ≈ 1.4 indicated that the electrochemical process of DA at the Ag NPs/Pcys/GCE was quasi-reversible. Based on the formula $|E_p - E_{p/2}| = 56.5/n$ [39], the electron transfer number (n) of redox reaction can be calculated to be approximately 2.

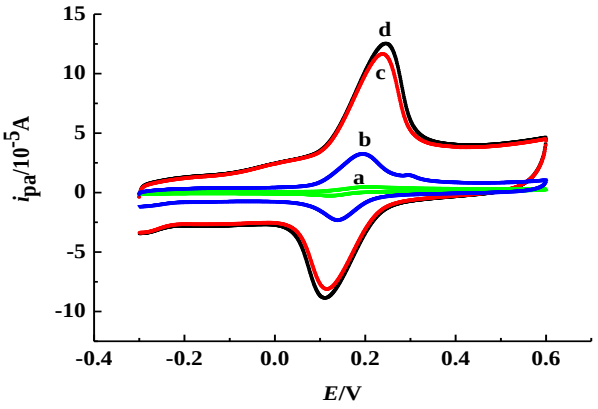

Figure 1. Cyclic voltammograms of 5.0×10^{-5} mol/L DA in PBS (pH = 7.0) at bare GCE(a), Ag NPs/GCE(b), Pcys/GCE(c), and Ag NPs/Pcys/GCE(d), Scan rate (v):160 mV/s

In order to obtain the best response of DA at Ag NPs/Pcys/GCE, the effect of the scan rate on the electrochemical behaviors of DA was discussed.

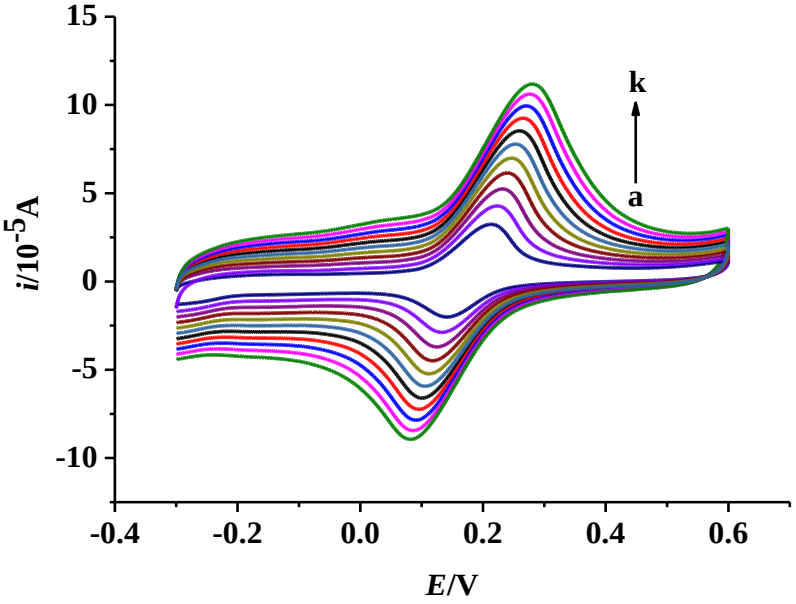

Figure 2. Cyclic voltammograms of 5.0×10^{-5} mol/L DA at the Ag NPs/Pcys/GCE with different *v*. Curves a to k are 40, 60, 80, 100, 120, 140, 160, 180, 200, 220 and 240 mV/s, respectively.

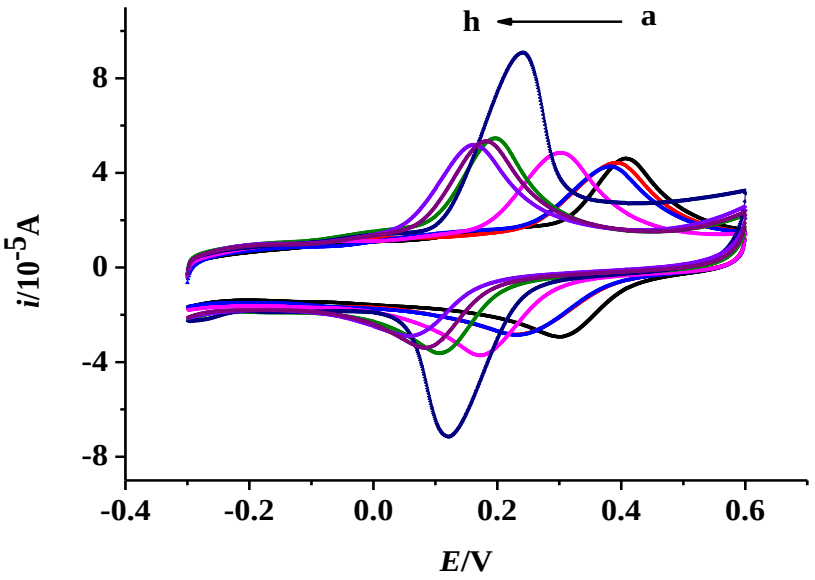

Figure 3. Cyclic voltammograms of 5.0×10^{-5} mol/L DA in PBS of different pH values (a to h) : 4.0, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0. *v*: 160 mV/s

It is evident from Fig. 2 that with the increasing of the scan rate, the peak current of DA increased, the plot of the oxidation peak current against the square root of the scan rate was obtained in the scan rate range of 40~240 mV/s with the linear regression equation of i_{pa} (10^{-5}A) = -0.9077 + 0.4992 $v^{1/2}$ (mV/s) (r = 0.9983), which suggested that the electron transfer reaction of DA on Ag NPs/Pcys/GCE was a diffusion-controlled process. In addition, the positive shifts in the anodic peak potential and the negative shifts in the cathodic peak potential confirmed the irreversibility of the reaction[32].The pH of supporting electrolyte was investigated within the range of 4.0~8.5. As shown in Fig. 3, both the oxidation and reduction peak currents increased with increase of the pH up to 7.0, and then decreased. Therefore, pH 7.0 was chosen for the subsequent experiments. The anodic peak potential of DA shifted negatively with increase of pH with a linear regression equation of E_{pa} (V) = 0.5001 - 0.0521 pH (r = 0.9980). The slope of 52.1 mV/pH showed that the uptake of electrons is accompanied by an equal number of protons in both electrode reactions[40, 41].

3.2 DA interacted with DNA in solution

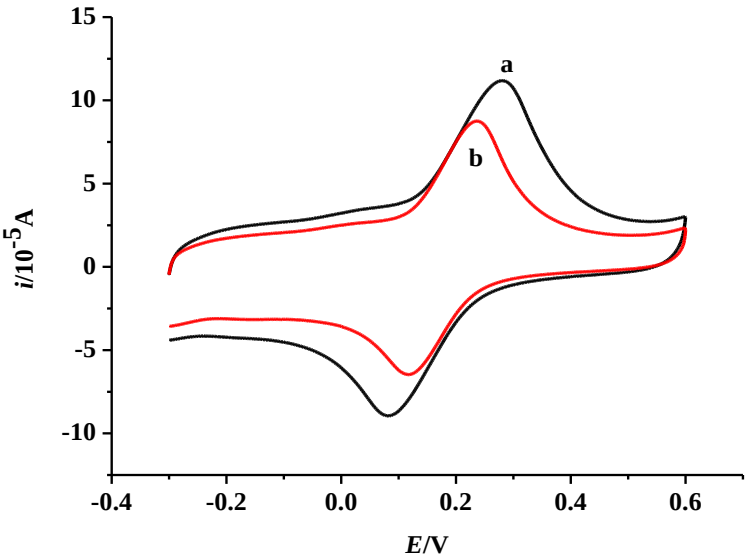

Figure 4. CV of 5×10^{-5}mol/L DA in absence (a) and presence of (b) 0.3 mg/mL DNA

Upon the addition of DNA to DA solution, appreciable decrease in anodic peak current of DA were observed and the formal potential shifted from 0.182 V to 0.176 V (see Fig. 4). This revealed that DA could interact with DNA. The decrease of peak current is explained with the formation of DA-DNA complex with large molecular weight, resulting in a considerable decrease of the diffusion rate. According to Bard and Xu[42], electrostatic interaction between small molecules and DNA led to negative shifts in formal potential after the addition of DNA. Therefore, DA binds with DNA via electrostatic mode. Further, the interaction of DA with DNA relates to time. In order to find out the

optimum interaction time, the CV of DA (10^{-5} mol/L) in presence of DNA (0.3 mg/mL) at different time intervals was investigated. The results showed that the peak current of DA leveled off and remained constant after 13 min. Therefore, in all the experiments, 13 min interaction time was used for interaction of DA with DNA.

The optimal pH value for the interactions was selected by detecting the difference of the oxidation peak current (Δi_{pa}) of DA ($5x10^{-5}$ mol/L) in different pH of PBS before and after it interacted with DNA (0.3mg/ml) for 13 min (see Fig. 5), indicating that DA could reach maximum binding in pH 7.0.

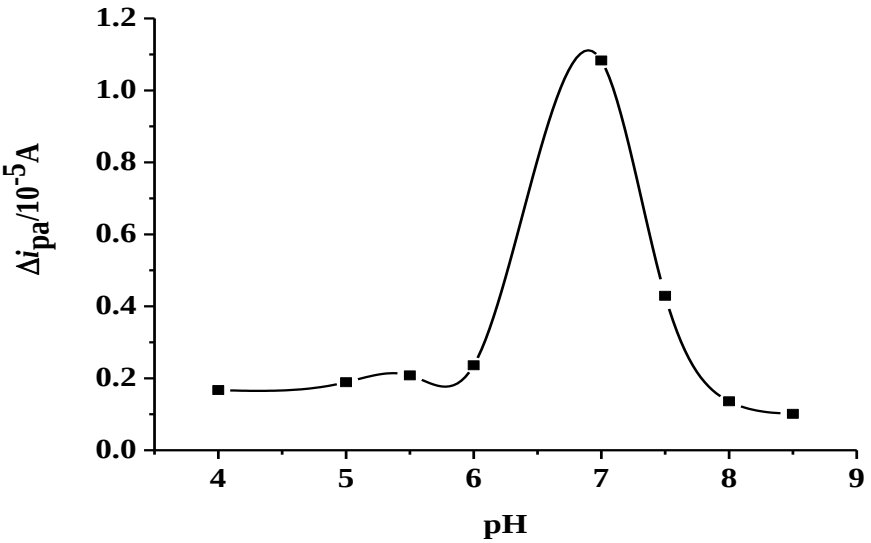

Figure 5. The influence of pH on Δi_{pa} (the peak current difference between the absence and presence of DNA). DA:5.0×10^{-5} mol/L; Reaction time: 13 min. Scan rate: 160mV/s.

The effect of scan rate on the oxidation peak current and peak potential of DA in the presence of DNA were also evaluated by CV. In a potential scanning rate range of 40~240 mV/s, i_{pa} was proportional to the square root of the scan rate, which suggesting that a diffusion-controlled process was involved in the redox reaction. The relationships between E_{pa} and ln$v$ were discussed as follows. Since the system studied belongs to that category where electrode reaction was a diffusion-controlled quasi-reversible reaction, we may use Laviron's equation [43]:

$$E = E_0 + \frac{RT}{\alpha nF}\ln\frac{RTK_s}{\alpha nF} + \frac{RT}{\alpha nF}\ln v \qquad (1)$$

where α is the electron transfer coefficient, F is Faraday constant, K_s is the apparent heterogeneous electron transfer rate constant, R is the universal gas constant (8.314 J K^{-1} mol^{-1}), T is the Kelvin temperature, n is the number of electron transfer and E_0 is the formal potential which can be obtained from the intercept of the E_{pa} vs. v plot by extrapolation to the vertical axis at v = 0. According

to Eq. (1), the plot of E_{pa} vs. ln$v$ should be linear. From its slope and intercept, the values of α and K_s can be determined.

Fig. 6 shows the plots of E_{pa} vs. ln$v$. In the absence of DNA, E_0 = 0.205V, the slop of E_{pa} *vs.* ln$v$ plot was 0.0376 and the intercept was 0.330V. Hence, α = 0.342 and K_s = 777.5s^{-1}. In the presence of DNA, E_0 = 0.193V, α and K_s were calculated to be 0.437 and 957.3s^{-1}, respectively. The changes of these electrochemical kinetics demonstrated that the interaction of DA with DNA took place.

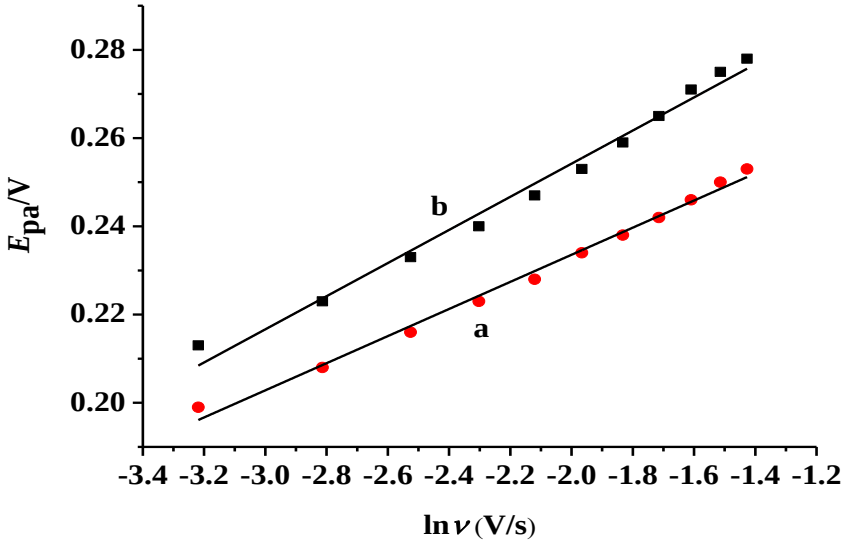

Figure 6. Semilogarithmic dependence of the oxidation peak potential (E_{pa}) on ln$v$ for: (a) 5.0×10^{-5} mol/L DA mixing with 0.3 mg/mL DNA, and (b) 5.0×10^{-5} mol/L DA.

The binding constant, β, and the binding ratio m, were evaluated, too. Assuming that the interaction of DA with DNA produces only a single complex, DNA-mDA, then the following equations can be used[44-46]:

DNA + mDA = DAN-mDA

where m is the binding ratio.

The equilibrium constant (β) can be deduced from the following equations:

$$\beta = [\mathrm{DNA-mDA}] / \{[\mathrm{DNA}][\mathrm{mDA}]^{m}\} \quad (2)$$

Because of

$$C_{\mathrm{DNA}} = [\mathrm{DNA-mDA}] + [\mathrm{DNA}] \quad (3)$$

$$\Delta i_{max} = K\, C_{\mathrm{DNA}} \quad (4)$$

$$\Delta i = K[\mathrm{DNA\text{-}mDA}] \quad (5)$$

Therefore:

$$\Delta i_{max} - \Delta i = K(C_{\mathrm{DNA}} - [\mathrm{DNA-mDA}]) \quad (6)$$

$$\Delta i_{max} - \Delta i = K[\mathrm{DNA}] \quad (7)$$

From Eqs. (2), (4), and (6) we get

$$1/\Delta i = (1/\Delta/_{max}) + \{[1/(\beta \Delta i_{max})] \times (1/[DA]^m)\} \quad (8)$$

or

$$\log[\Delta i/(\Delta i_{max} - \Delta i)] = \log\beta + m\log[DA] \quad (9)$$

Where Δi is the difference in peak current of DA in presence and absence of DNA, and Δi_{max} corresponds to the value when the concentration of DA is extremely higher than that of DNA. C_{DNA}, [DNA] and [DNA-mDA] are the total, free and bound concentrations of DNA in the solution, respectively.

If DNA and DA form a type of cooperative complex, the plot of log[Δi/ (Δi_{max} - Δi)] versus log[DA] for DA-DNA interaction shows linearity. The experimental data m and β can be deduced from the intercept and slope of the straight line, and these values are found to be 1.08×10^5 L/mol and 1, respectively. Thus, the formation of a stable 1:1 complex (DNA: DA) is proposed.

3.3 Study with ultraviolet visible spectroscopy (UV-vis)

For a detailed characterization of DA-DNA complex, the UV-Vis spectra were thoroughly analyzed. The maximum absorbance of DA was located around at 292 nm (Fig. 7, curve a). As evident from Fig. 7, the addition of DNA resulted in the tendency of hyperchromism and a very slight blue shift (curve b). The change of absorption spectra implied that there are intensive binding interactions between DA and DNA and a new DA-DNA complex is formed. In general, molecules which bind electrostatically with DNA may result in hyperchromism[47,48]. This conclusion is agreement with that obtained from electrochemical method.

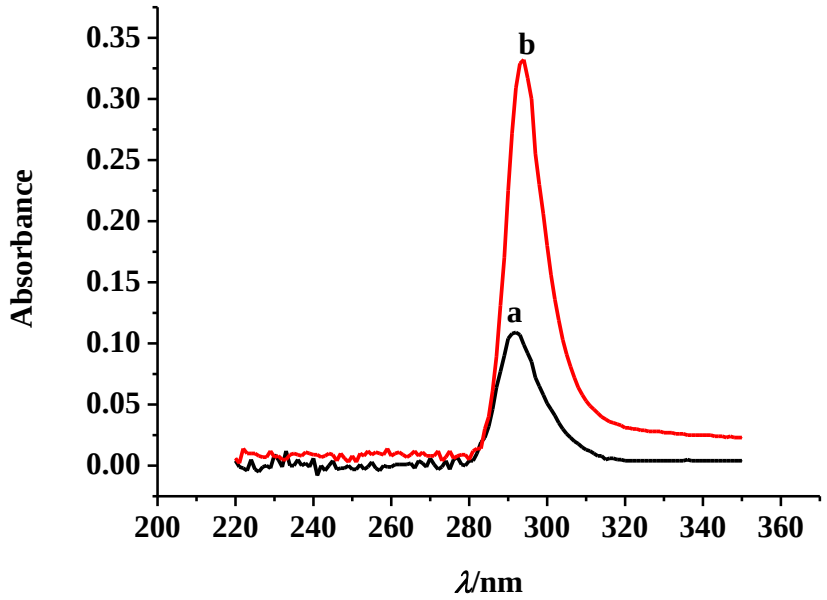

Figure 7. UV-Visible absorption spectra of 5.0×10^{-5} mol/L DA in the absence (a) and presence (b) of 0.3 mg/mL DNA in PBS at pH 7.

4. CONCLUSIONS

In this paper, the electrochemical behavior of DA at Ag NPs/Pcys/GCE and the interaction of DA and DNA are investigated and based on the experimental results, several electrochemical parameters are obtained. The peak current of DA remarkably increased under the optimum conditions at Ag NPs/Pcys/GCE compared with that on bare GCE or poly cysteine modified electrode. Utilizing the Ag NPs/Pcys/GCE, the interaction mechanism of DA with DNA is discussed in detailed by voltammetry. The binding of DA with DNA results in a series of changes in electrochemical behavior and spectral characteristics and the results showed that DA interacts with DNA by electrostatic force and formed a stable 1:1 complex. The spectroscopic methods confirmed the electrochemical conclusions. The fabricated electrode provided a new method for determination of DA and studying the interaction of DA and DNA. Most important of all, this study can be helpful for understanding the interaction mechanism of neurotransmitter amines agents with some macromolecules.

ACKNOWLEDGMENTS
The authors gratefully acknowledge the financial support of the National Natural Science Foundation of China (No. 21105023), China Postdoctoral Science Foundation Funded Project (2015M572039), Shandong Young Scientists Award Fund (No. BS2013HZ027).

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