Spectral and Electrochemical Investigation of Intercalations of Adrenaline and CT-DNA†

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A strong interaction between double stranded calf-thymus DNA (ds-DNA) and adrenaline in solution, but no interaction between single stranded calf-thymus DNA (ss-DNA) and adrenaline was observed by the use of UV-visible spectroscopy and voltammetric techniques. It is suggested that the interaction leads to an intercalation of adrenaline molecules into the groove of ds-DNA and the formation of ds-DNA(adrenaline)$_n$ complex. The binding site size of the interaction of adrenaline with CT-DNA in nucleotide phosphate [NP] has been determined as 25. The interaction of different concentration adrenaline with DNA modified GCE shows that the DNA modified GCE can be a good tool to detect lower concentration adrenaline.

Keywords DNA, adrenaline, UV-visible spectroscopy, cyclic voltammetry, differential pulse voltammetry

Introduction

Interactions of DNA with various molecules are interesting because of its importance in gene expression process of living cells. Several models for the interaction between DNA and some small molecules have been proposed.[1-4]

Adrenaline belongs to a group of compounds known as catecholamines which play a particularly important role in the regulation of physiological processes in living systems. Catecholamines serve as carriers for the nervous system, influencing the constriction of blood vessels and controlling tissue metabolism by increasing the levels of glucose and lactic acid. They have been used as medicines for the treatment of some diseases. Electrochemical studies of adrenaline and analogues have been reported for their chemical reactions *in vivo* processes and their determination in biological samples.[5-7] Several studies of electrochemical redox processes of adrenaline have been carried out.[8,9]

We are interested in the possibility of the interaction between DNA/RNA and neurotransmitters, such as dopamine and adrenaline, because these species are very important substances for keeping normal physiological functions of brain and neuro system, and these species can also help to develop the new DNA probe. Although the interaction of catecholamines with 2′-deoxyguanosine (dG) is a known phenomenon in biomedical community,[10] none has reported about the interaction of adrenaline with long double-stranded DNA.

Experimental

Chemical and solutions

Calf thymus DNA (CT-DNA, ds-DNA) (sino-American Biotechnology) and Adrenaline (Shanghai Chemical Reagent factory, China) were used without purification. Other Chemicals were of analytical reagent grade. Double distilled water and high purity N_2 gas were used.

Instrumentation

A CHI 832 electrochemical analyzer (Electrochemical Instruments Inc. Shanghai, China) was used for electrochemical acquisition with a three-electrode system, which consisted of an SCE reference electrode, Pt counter electrode and a testing electrode. A glassy carbon disk electrode (GCE) with a geometric surface area of 0.07 cm^2 was used as the basic testing electrode. This electrode was polished with fine sandpapers to a mirror surface and then cleaned ultrasonically in 95% ethanol and water for 5 min, successively, before use.

A UV265 UV-Vis-NIR spectrophotometer (Shimadzu Inc. Japan) was used for spectral measurement.

Procedure

Preparation of stock solutions

Concentrated stock DNA solutions of 1 mg/mL were prepared in 0.02 mol/L pH = 6.8 phosphate buffer solutions (PBS). These solutions were deoxygenated by N_2

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Received November 12, 2002; revised March 19, 2003; accepted May 8, 2003.
Project supported by the Live Science Research Base of University of Science and Technology of China (Grant No. KY1212) and the Key project of University of Science and Technology of China (Grant No. KY2216)
†Dedicated to Professor ZHOU Wei-Shan on the occasion of his 80th birthday.

bubbling for 10 min and then stored at 4 ℃. The CT-DNA concentration in nucleotide phosphate [NP] was determined spectrophotometrically, according to the known absorption coefficient $\varepsilon = 6600\ mol^{-1} \cdot cm^{-1}$ at 258 nm.[11]

The single-stranded DNA (ss-DNA) was produced by heating the native ds-DNA in boiling water bath for about 5 min, followed by rapid cooling in an ice bath. An adrenaline stock solution of 10 μmol/L was prepared in 0.02 mol/L pH = 6.8 PBS too, deoxygenated by N_2 for 10 min just before use.

Solution-phase interaction

Certain amount of adrenaline stock solution with different amount of DNA stock solution were mixed in a flask, then diluted to 10.0 mL by adding 0.02 mol/L pH = 6.8 PBS, mixing for 10 min before measurement. Electrochemical detections with cyclic voltammetry (CV) or differential pulse voltammetry (DPV) methods can be carried out directly in the mixed solution at freshly prepared GCE.

Interaction at DNA *modified electrode*

A ds-DNA-modified GCE (ds-DNA/GCE) was prepared by dropping a portion of 20 μL 0.2 mg/mL ds-DNA on a freshly prepared GCE and to allow evaporating at room temperature to dryness, which is similar to the method used by Pang *et al*.[12] After cleaned by PBS for 10 s, the ds-DNA/GCE was transferred to 0.2 μmol/L adrenaline solution for a desired time to allow reaction proceeding. Then, the electrode was rinsed carefully with PBS for a short time, CV and DPV measurements were carried out in a blank pH = 6.8 PBS.

Results and discussion

Absorption spectra of ds-DNA *and adrenaline*

Spectral evidence for a strong interaction between the ds-DNA (CT-DNA) and adrenaline is shown in Fig. 1.

As seen in this figure, 0.2 μmol/L adrenaline has a spectrum with three absorption peaks at 204, 220 and 277 nm (spectrum a), and the ds-DNA has two typical absorption bands at 201 and 258 nm (spectrum b). Mixing these two solutions while keeping the formal concentration of adrenaline unchanged should result in the summation of these two spectra, if no chemical reaction occurs. However, the experimental result shows that the absorption peaks at 204 nm in the mixture decreased and shifted red a little with increase of the ds-DNA concentration (spectra c, d, f). It indicates obviously formation of new species.

Assuming the ds-DNA can complex strongly with n molecules of adrenaline and only one product was formed, as shown in reaction (1).

$$\text{ds-DNA} + n(\text{adrenaline}) = \text{ds-DNA(adrenaline)}_n \quad (1)$$

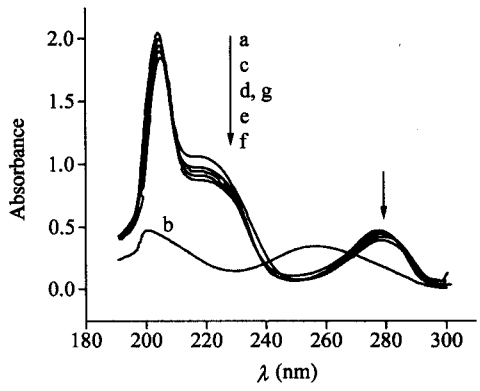

Fig. 1 UV spectra of ds-DNA and of adrenaline in the presence of ds-DNA. a: 0.20 μmol/L adrenaline; b: 0.207 mg/mL CT-DNA; c: 0.20 μmol/L adrenaline and 0.074 mg/mL CT-DNA; d: 0.20 μmol/L adrenaline and 0.128 mg/mL CT-DNA; e: 0.20 μmol/L adrenaline and 0.285 mg/mL CT-DNA; f: 0.20 μmol/L adrenaline and 0.208 mg/mL CT-DNA; g: 0.20 μmol/L adrenaline and 0.353 mg/mL CT-DNA.

Because

$$A^0_{\text{adrenaline}} = \varepsilon_{\text{adrenaline}}\, c^0_{\text{adrenaline}}, \quad (2)$$

where the superscript of 0 indicates the original values before the mixing,

$$A_{\text{adrenaline}} + \text{ds-DNA} = \varepsilon' c_{\text{ds-DNA(adrenaline)}_n} + \varepsilon_{\text{adrenaline}}\, c_{\text{adrenaline}} + \varepsilon_{\text{ds-DNA}}\, c_{\text{ds-DNA}} \quad (3)$$

$$c_{\text{adrenaline}} + n c_{\text{ds-DNA(adrenaline)}_n} = c^0_{\text{adrenaline}} \quad (4)$$

Definning $$\Delta A = A^0_{\text{adrenaline}} - A_{\text{adrenaline + ds-DNA}} \quad (5)$$

leads to

$$\Delta A = (n\varepsilon_{\text{adrenaline}} - \varepsilon')\, c_{\text{ds-DNA(adrenaline)}_n} - \varepsilon_{\text{ds-DNA}}\, c_{\text{ds-DNA}}. \quad (6)$$

The plot of experimental data is shown in Fig. 2. As

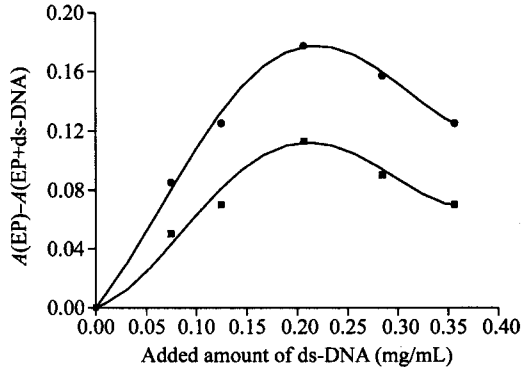

Fig. 2 Plot of ΔA vs. the added amount of ds-DNA. ■, at 277 nm; ●, at 204 nm. Experimental conditions are the same as those in Fig. 1.

seen in this plot, the ΔA values at both 204 nm and 277 nm increase proportionally with increase of the amount of ds-DNA in the range of 0 to about 0.21 mg/mL, however, a decrease of ΔA appeared for the further increase of ds-DNA in the range of 0.21 to 0.35 mg/mL. This is in a good agreement with the prediction of Eq. (6).

The sharp turning point appeared at about 0.21 mg/mL ds-DNA, indicating the formation constant of the complex should be quite large and the formation of ds-DNA(adrenaline)$_n$ is complete at this point. Under this assumption, the binding site size (s) of adrenaline $(NP)_s$ is calculated as about 25 at 0.21 mg/mL CT-DNA, which was determined as 5 μmol/L in nucleotide phosphate [NP] ($s = [NP]/c_{adrenaline} = 5\ \mu mol/L/0.2\ \mu mol/L$).

A similar spectral experiment was carried out for ss-DNA, which was obtained from the heat treatment of the ds-DNA. However, no interaction between adrenaline and ss-DNA phenomenon was observed.

Cyclic voltammetry of adrenaline and ds-DNA

Cyclic voltammograms (CVs) of 0.2 μmol/L adrenaline at bare GCE is as shown in Fig. 3. An irreversible oxidation step is appeared at E_m(I) of 0.08—0.18 V (vs. SCE). A reversible redox step can be seen at E_m(II) of −0.28—−0.20 V, which is assigned to reactions of electrogenerated species at oxidation (I). On the positive scan, adrenaline (A) was oxidized to an open-chain quinone (B) at about 0.18 V (peak 1) and most of the open-chain quinone (B) was cyclized quickly to adrenochrome (C). On the successive negative scan, the open-chain quinone (B) and adrenochrome(C) were reduced to adrenaline (A) and leucoadrenochrome (D) respectively at about 0.08 V (peaks) and −0.28 V.

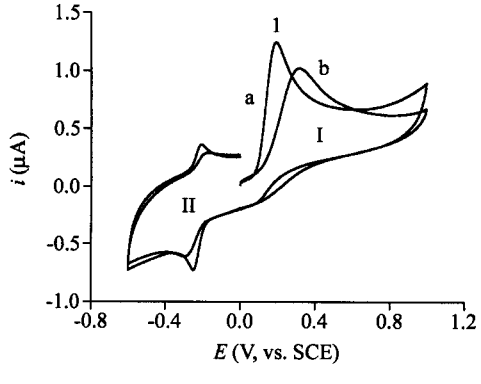

Fig. 3 Cyclic voltammograms (CVs) of the 0.2 μmol/L adreraline (a) and 0.2 μmol/L adreraline with 5 μmol/L CT-DNA in [NP] (b).

In the presence of 5 μmol/L [NP] calf thymus ds-DNA, the peak current of peak 1 decreased and the peak potential shifted to 0.32 V, as shown in Fig. 3b. As indicated above, the adrenaline should be completely coordinated to the ds-DNA, the CV peak at 0.32 V corresponds to the oxidation of adrenaline in the complex ds-DNA(adrenaline)$_n$.

Fig. 4 shows that the CV peak current of adrenaline at GCE decreases rapidly upon the addition of calf thymus ds-DNA to the solution (0 < concentration of nucleotide phosphate, [NP] < 5 μmol/L). It is reasonable because the diffusion coefficient of adrenaline in the combination state should be smaller than that in the free solution state. It is in agreement with the fact that the adrenaline in the combination state is less reversible than that in the free solution state.

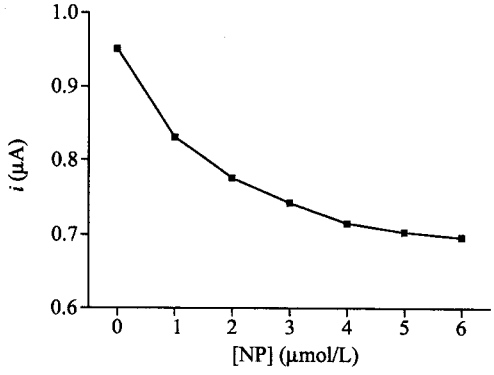

Fig. 4 Titration curves of peak 1 current (as shown in Fig. 3) of adrenaline with different concentration CT-dsDNA (0 < concentration of nucleotide phosphate [NP] < 5 μmol/L).

Regularly there are two modes when DNA interacts with other moleculars, one is the electrostatic mode, and another is intercalation mode.[13-15] A net negative E_m shift, is usually considered as evidence of electrostatic interaction of a complex with DNA via the anionic phosphate residues.[16,17] But in our case, the adrenaline exhibited a gradual positive E_m shift, *e.g.*, by 86 mV for 0.2 μmol/L adrenaline and 5 μmol/L NP in pH = 6.8 PBS. So we thought that the mode of DNA interacting with adrenaline is intercalation.

$E^{0'}$ of the adrenaline (A)/open-chain quinone (B) reaction can be estimated approximately by use of the E_m values measured on the CV curves in the presence of different amount of ds-DNA, which is listed in Table 1. The shift of $E^{0'}$ can be suggested due to the difference in binding strength for adrenaline and open-chain quinone with ds-DNA:

$$E^{0'}_{bound} - E^{0'}_{free} = 0.059 \lg (K_{open\text{-}chain\ quinone\ (B)}/K_{adrenaline\ (A)}) \quad (7)$$

where $E^{0'}_{bound}$ and $E^{0'}_{free}$ are the formal potential of the bound

Table 1 E_m and ΔE_m of peak 1 of adrenaline with different concentration of ds-DNA

	[NP] (μmol·L^{-1})					
	0	1.0	2.0	3.0	4.0	5.0
E_m(V)	0.145	0.162	0.179	0.196	0.215	0.231
ΔE_m(V)	0	0.017	0.034	0.051	0.070	0.086

and free adrenaline forms respectively. From the experimental data, the ds-DNA$(A)_n$ complex is about 10 times more stable than ds-DNA$(B)_n$.

Similar voltammetric experiments were carried out for ss-DNA addition. However, no change in the current and potential of peak 1 was found, suggesting that there is no interaction between adrenaline and ss-DNA.

Interactions at DNA *modified electrodes*

It is interesting if adrenaline also interacts with immobilized DNA at electrode surfaces. A stable ds-DNA/GCE was prepared. The ds-DNA/GCE exhibited much higher background charging currents than the original GCE, depending on the thickness of the modified DNA layer. But no other difference was found.

For better resolution and sensitivity DPV was used for the analysis of time effect and concentration effect of adrenaline interaction at ds-DNA/GCE. Fig. 5 shows the time effect. It can be seen from this figure that the i_{pl} of adrenaline increases with time in the range of 0 to 10 min. After 10 min, the i_{pl} keeps constant. It is interesting to note that 10 min is needed for the surface accumulation of adrenaline on ds-DNA/GCE to proceed completely under the experimental conditions.

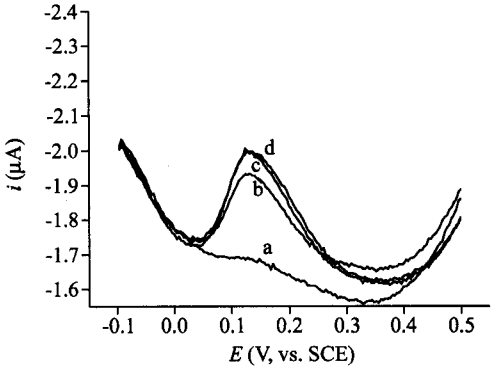

Fig. 5 Time effect of interaction of DNA modified GCE with 0.2 μmol/L adrenaline. a: 1 min; b: 5 min; c: 10 min; d: 15 min.

Recent efforts to develop an electrochemical DNA hybridization sensor[18] are among the most important trends in the electrochemistry of DNA. Quite recently the selectivity of the hybridization has been greatly improved by replacing the DNA probe with peptide nucleic acid one.[19] On the other hand, improvements of hybridization indicators, which may help to increase the sensitivity of electrochemical methods are needed. Our results show significant changes in E_{pl} when adrenaline binding to DNA (Fig. 3). So adrenaline can be thought as a new DNA hybridization indicator.

On the other hand, we also found that the ds-DNA/GCE can be a sensitive probe for detection of adrenaline due to the surface accumulation. A preliminary study gave a DPV curve with different concentration of adrenaline at ds-DNA/GCE as shown in Fig. 6A. The DPV peak current was plotted against the concentration as shown in Fig. 6B. It can be seen that the peak current of adrenaline is a linear function of adrenaline concentration in the range of 0.05 μmol/L to 0.3 μmol/L with a slope of about 5.5 μA per μmol/L. However, another linear response might be found in the concentration range of 0 to 0.03 μmol/L with a much larger response slope of about 25 μA per μmol/L. The relative error of current value in every experiment is smaller than 5%. What's more, the ds-DNA/GCE is very stable. The detection current of this electrode decreases only 10% when this electrode was put in the PBS solution (pH = 6.8) for 5 d. So the ds-DNA/GCE seems to be suitable for the detection of the concentration range of adrenaline based on the interaction with ds-DNA.

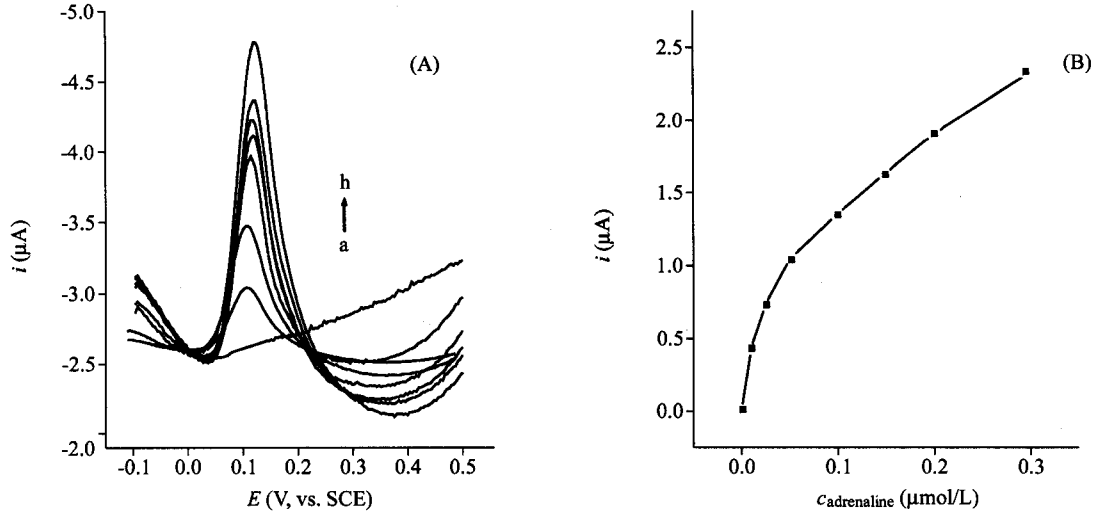

Fig. 6 Concentration effect of adrenaline with DNA modified GCE. A: Differential pulse voltammetrys (DPVs) (adrenaline concentration: a, 0; b, 0.01; c, 0.025; d, 0.05; e, 0.1; f, 0.15; g, 0.2; h, 0.3 μmol/L); B: Plot of concentration effect of adrenaline.

Conclusion

A strong interaction between double stranded calf-thymus DNA (ds-DNA) and adrenaline in solution and at the ds-DNA modified GCE but no interaction between single stranded calf-thymus DNA (ss-DNA) and adrenaline were observed. The mode of this interaction has also been proposed by the cyclic voltammogram and the binding site size also has been calculated.

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(E0211122 ZHAO, X. J.; LU, Z. S.)