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Intercalation of Epinephrine with Calf-thymus ds-DNA

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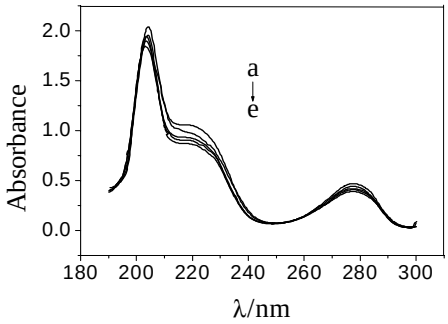
Abstract: A strong interaction between double stranded calf-thymus DNA (ds-DNA) and epinephrine but no interaction between single stranded calf-thymus DNA (ss-DNA) and epinephrine were observed by the use of UV-spectroscopy and cyclic voltammetry. It is suggested that the interaction leads to an intercalation of EP molecules into the groove of ds-DNA and the formation of ds-DNA$(EP)_n$ complex.

Keywords: DNA, epinephrine, UV-visible spectroscopy, cyclic voltammetry.

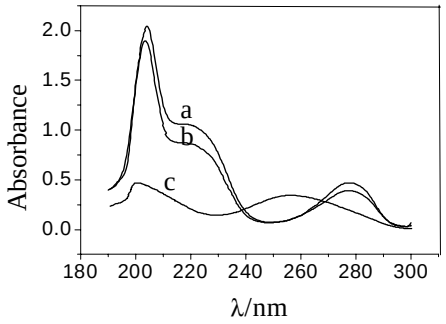
Interactions of DNA with various molecules are interesting because of its importance in gene expression process of living cells. Several models for the interaction between DNA and some small molecules have been proposed[1-4]. We are interested in the possibility of the interaction between DNA/RNA and neurotransmitters, such as dopamine and epinephrine, because these species are very important substances for keeping normal physiological functions of brain and neuro system. However, no report was found in the literature under these topics.

Figure 1 UV spectra of EP in the presence of ds-DNA



C_{EP}: 0.20 μmol/L. $C_{ds\text{-}DNA}$: a, 0; b, 0.074; c, 0.128,0.353; d,0.286; e, 0.207mg/mL

Figure 2 UV spectra of EP, ds-DNA and ds-DNA$(EP)_n$



a, 0.20 μmol/L EP; b, 0.20 μmol/L ds-DNA$(EP)_n$; c, 0.2mg/mL ds-DNA

Recently, the interaction system was studied in our group by using CT-DNA (Calf thymus DNA) and EP (epinephrine) as a model using cyclic voltammetry and UV-visible spectroscopic methods. Evidence showed that the double stranded CT-DNA (ds-DNA)

can strongly intercalate with EP molecules forming a sort of electrochemically inactive complex, CT-DNA(EP)$_n$, but not the corresponding single stranded CT-DNA (ss-DNA). This can be seen in **Figure 1** that in a 1 cm path-length cell and pH 7.0 phosphate buffer solution (PBS), 0.2 mmol/L EP showed a spectrum with free absorption peaks at 205, 220 and 277 nm, however the value of these absorption peaks was decreased with the addition of ds-DNA.

Actually, the ds-DNA showed two typical absorption bands at 201 and 258 nm in this solution, as shown in **Figure 2(c)**. The addition of the ds-DNA did not increase but reduce the absorption at these wavelengths, indicating obviously an occurrence of chemical reaction between ds-DNA and EP. Assuming the ds-DNA can complex strongly with n molecules of EP

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

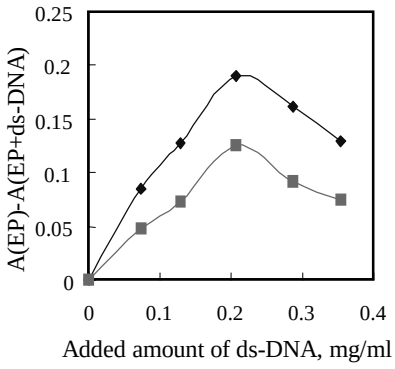
because $A^0_{EP}=\varepsilon_{EP}c^0_{EP}$ (2)

$$A_{EP+\ ds\text{-}DNA}=\varepsilon' c_{ds\text{-}DNA(EP)n}+\varepsilon_{EP}c_{EP}+\varepsilon_{ds\text{-}DNA}c_{ds\text{-}DNA} \quad (3)$$

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

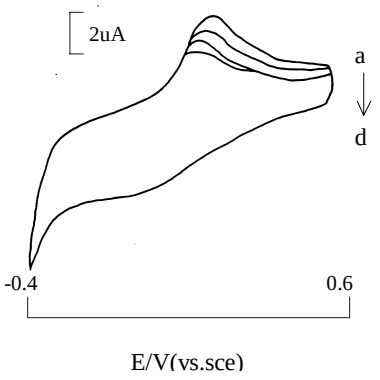
it leads to $f\!A=A^0_{EP}-A_{EP+ds\text{-}DNA}=(n\varepsilon_{EP}-\varepsilon')\,c_{ds\text{-}DNA(EP)n}-\varepsilon_{ds\text{-}DNA}c_{ds\text{-}DNA}$ (5)

Figure 3 Plot of $f\!A$ *vs.* the added amount of ds-DNA.



♦, at 277 nm; ■, at 204 nm. Experimental condition: the same as in **Figure 1**

Figure 4 Cyclic voltammograms of EP at GCE with addition of ds-DNA a, 0; b, 0.05; c, 0.10; d, 0.25 mg/mL.



Obtained at 100 mV/s in 0.02 mol/L pH 7.0 PBS

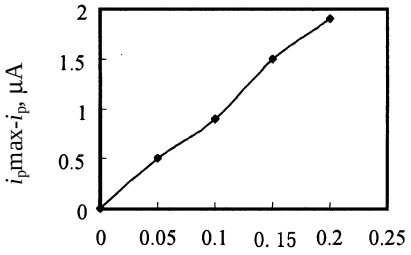
Certainly, the plot of experimental data, as seen in **Figure 3,** showed that the $f\!A$ value at both 204 nm and 277 nm increased proportionally with the increase of the amount of ds-DNA in the range of 0 to about 0.21 mg/mL. The decreasement of the $f\!A$ values for the further increase in the range of 0.21 to 0.35 mg/mL is also predictable. A sharp turning point appeared at about 0.21 mg/mL ds-DNA, indicating the formation constant of the complex should be quite large.

The same spectral experiments were carried out for ss-DNA, which was obtained from heat treatment of the ds-DNA. No interaction between EP and ss-DNA was found.

At a micro glassy carbon disc electrode (GCE) of 0.07 cm^2 formal surface area, Ep showed an irreversible voltammetric oxidation peak at E_{pa} of 0.23 V(*vs.* sce). Adding ds-DNA into the solution, the peak current decreased with increase of the amount of ds-DNA, and the peak potential shifted to the negative direction as shown in **Figure 4.** No new oxidation peak was observed within the potential window. It is in agreement with the fact that EP can strongly intercalate with the ds-DNA and led to a significant reduction of its diffusion current. The negative shifting of E_{pa} during ds-DNA addition is interesting. It may be attributed to the kinetic effect of the molecular interaction.

Assuming the oxidation peak current is generated by the free EP in the solution and the ds-DNA$(EP)_n$ complex is electrochemically inactive in the potential range or the diffusion coefficient is too small, the decrease of the peak current should be proportional to the amount of ds-DNA$(EP)_n$. The plot of these data approximately gives a straight line, as shown in **Figure 5**.

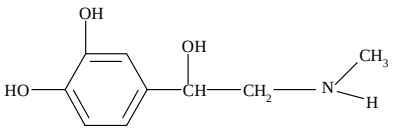
Figure 5 Plot of I^o_p-I_p for EP oxydation vs added amount of ds-DNA



Added amount of ds-DNA, mg/ml
Experimental condition: as in **Figure 4**

The same voltammetric experiments were carried out for ss-DNA addition. However, no change of the peak current and peak potential was found, suggesting no interaction between EP and ss-DNA.

This primary study of the DNA-EP system demonstrated that the EP molecule does interact with ds-DNA but not with ss-DNA. The mechanism of the interaction can be suggested as a complex formation of ds-DNA$(EP)_n$.Both electrostatic interaction and intercalation reaction between EP and ds-DNA may occur[5], because EP has NH and OH groups and a phenyl ring as shown below:



However, in the pH 7.0 PBS solution the neutral EP molecule is most likely to intercalate into the groove of ds-DNA with π~π interaction.

The number of EP molecules intercalated in a ds-DNA (n value) can not be estimated from our data because the molecular weight of CT-DNA was not well estimated. Synthesized oligo-DNA may be used for more accurate investigation.

Further study is being undertaken.

Acknowledgments

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References

1. L. H. Hurkey, D. Needham-Vam, *D. R. Acc. Chem.Res*., **1986**, *19*, 230.
2. K. Ikeda, Y. Shirota, T. Sakurai, *J. Electroanal.Chem.*, **1990**, *287*, 179.
3. D. Esppoddito, P. Del, *J. Am. Chem. Soc.*, **1997**, *119*, 2606.
4. C. L. Pinelopi, K. C. Theodore, *Anal. Chem.*, **1998**, *70*, 689.
5. J. P. Rehmann, J. K. Barton, *Biochemistry*, **1990**, *29*, 1709.

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