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Voltammetric, UV Absorption and Viscometric Studies of the Interaction of Norepinephrine with DNA

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Voltammetric, UV-vis spectroscopic and viscometric methods were used to investigate the interaction between norepinephrine and DNA. The results of square wave voltammetric and UV-absorption measurements have shown that norepinephrine was bound to DNA. Besides the electrostatic interaction between DNA and norepinephrine, hydrogen bonds and intercalation may also exist. Viscosity measurements also suggest that NE is bound to DNA via an electrostatic mode of interaction at lower norepinephrine concentrations. However, at higher concentrations of norpinephrine, the increase in the relative viscosity of DNA indicated that the intercalation is also involved in the binding. The binding constant and the binding site size for the interaction of norepinephrine with DNA were $K = 3.3\ (\pm\ 0.18) \times 10^3 \mathrm{M}^{-1}$, $s = 2.89\ (\pm\ 0.05)$ base pairs and $K = 5.15\ (\pm\ 0.38) \times 10^3 \mathrm{M}^{-1}$, $s = 2.41\ (\pm\ 0.04)$ base pairs for the limiting conditions of static and mobile binding equilibrium, respectively. The binding site size determined shows that norepinephrine covers more than 2 base pairs upon binding to DNA. The standard Gibbs free energy change ($\Delta G^0 = -RT \ln K$) comes out to be approximately -21.17 kJ/mol at 25 °C, which indicates the spontaneity of the binding of NE with DNA.

Key Words: DNA, norepinephrine, voltammetry, UV absorption, binding constant, binding site size.

Introduction

There is growing interest in the binding of small molecules to DNA. The interaction of small molecules with DNA may be summarised as an electrostatic interaction that extends the negatively charged phosphates outside the DNA double helix, an interaction with grooves of DNA, and an intercalation model in which the base pairs of DNA unwind to accommodate the intercalating agent[1,2]. DNA plays an important role in the life process since it contains all the genetic information for cellular function. However, DNA molecules are prone to be damaged under various conditions including, e.g., interactions with some molecules. This damage may lead to various pathological changes in living organisms[3]. The binding interaction of small molecules with DNA is of interest for both therapeutic and scientific reasons[4,5]. These interactions may also be used for conformational recognition to find new structures of DNA and sequence-specific differences along

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the helix of a DNA molecule[6−8]. In addition to the potential therapeutic applications of many drugs, due to their electrochemical behaviour their interactions with DNA are anticipated to be useful in electroanalytical applications[9−12].

DNA binding interactions have been studied by several analytical methods including electrophoresis[13], calorimetric titration[14], luminescence[15], UV absorption[16], fluorescence[17] and voltammetric methods[18−22]. It has been shown that the binding constants for redox active species can be obtained from voltammetric experiments in which the DNA is titrated against the redox active molecule. In voltammetric experiments, the measurement of peak currents in the presence of excess nucleic acid has shown that the diffusion coefficient of DNA-bound species was more than one order of magnitude lower than that of the free species[10,23].

Norepinephrine (NE) (Figure 1) is one of the most important biochemical messengers in mammalian central nervous systems, existing in the nervous tissue and biological body fluid[24]. Many diseases and the caducity process are related to changes in its concentration. Thus, its interaction with DNA is of importance scientifically to determine the chemical factors controlling the binding of NE to DNA. In this paper, voltammetric, spectroscopic and viscometric methods were applied to investigate the interaction between DNA and NE.

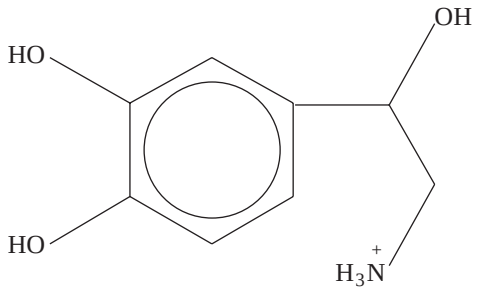

Figure 1. Chemical structure of norepinephrine (NE).

Experimental

Materials and instrumentation

Type XIV Herring Testes ds-DNA sodium salt was purchased from Sigma. Solutions of DNA were prepared fresh before each experiment using doubly distilled water containing 0.1 M Tris-HCl buffer, pH 7. The concentration of DNA solutions expressed in moles of nucleotide phosphate [NP] was determined by UV-vis spectrophotometry at 260 nm using a value of 6600 M^{-1} cm^{-1} for the absorption coefficient[25]. The purity (freedom from bound protein) was assessed from the ratio of the absorbances at 260 nm and 280 nm[26]. In general, the commercial DNA preparation was found to be free of protein ($A_{260nm}/A_{280nm} = 1.9$) according to this criterion and no further purification was attempted. Norepinephrine (NE) was purchased from Fluka**.** All other reagents were of analytical grade or equivalent and obtained from Merck or Sigma.

Voltammetric experiments were performed in a single compartment cell with a 3- electrode configuration on an EcoChemie Autolab PGSTAT-12 potentiostat/galvanostat with the electrochemical software package GPES 4.9. A 3-electrode system was used: a 2 mm sized Pt disc working electrode, an Ag/AgCl reference electrode and a Pt wire counter electrode. The electrodes were purchased from Metrohm. A known volume of buffered DNA solution was added to the cell and voltammograms were recorded as aliquots of NE solution were added. The procedure was then repeated using a buffer solution containing no DNA to

generate a current-concentration calibration plot. The working electrode was polished with 0.05 μm alumina on a polishing pad and washed with doubly distilled water prior to each experiment. Oxygen-free nitrogen was bubbled through the solution for 5 min before each experiment.

Absorption spectra were measured on a Perkin-Elmer Lambda 45 spectrophotometer. A quartz cuvette of 1 cm was used. Titration of the drug with herring sperm DNA solution was performed by adding a small aliquot of a concentrated DNA solution to a drug solution at constant concentration. Solutions were allowed to equilibrate for 5 min before measurements were obtained. All experiments were carried out at 25 °C.

Viscosity measurements were conducted on an Ubbelodhe viscometer, immersed in a thermostated water bath maintained at 25 °C. Titrations were performed by the addition of aliquots of the NE solution into a constant concentration of DNA solution in the viscometer. Data were presented as (η/η_o) versus the concentration of NE, where η is the viscosity of DNA in the presence of NE and η_o is the viscosity of DNA alone.

Data Analysis

A simple site binding model was used to fit the experimental data acquired from the interaction between NE and DNA[18].

$$C_b/C_f = K\{[\text{free base pairs}]/s\} \tag{1}$$

where s is the binding site size in terms of base pairs. The concentration of DNA was measured in terms of [NP], and therefore the concentration of base pairs is [NP]/2. C_f and C_b are the concentrations of free and DNA-bound species respectively. Using the mass balance equations for the concentration of nucleotide phosphate and NE, C_f can be expressed in terms of total concentration of NE, C_T, total concentration of nucleotide phosphate, $[\text{NP}]_0$, K and s [18]:

$$KC_f^2 + (1 - KC_T + \frac{K[NP]_0}{2s})C_f - C_T = 0 \tag{2}$$

The ratio of bound to free NE is obtained from the peak currents in the absence of DNA, i, and in the presence of DNA, i_{DNA}[27]:

$$\frac{C_b}{C_f} = \frac{(i - i_{DNA})}{i_{DNA}} \tag{3}$$

Voltammetric experiments were analysed using both static equilibrium, in which the binding kinetics of NE/DNA system are slow, and mobile equilibrium, in which the kinetics of the NE/DNA binding equilibrium are fast, compared to the experimental time scale[18]:

$$i_{DNA} = BD_b^x C_b + BD_f^x C_f (\text{static equilibria}) \tag{4}$$

$$i_{DNA} = BC_T(D_b\frac{C_b}{C_T} + D_f\frac{C_f}{C_T})^x (\text{mobile equilibria}) \tag{5}$$

where B is the appropriate collection of constant and $x = 0.5$. D_b and D_f are the diffusion coefficients of bound and free NE.

In this work, we computed $C_b/C_f$and obtained K and s by the least squares regression of C_b/C_fon C_T. The correlation between K and s was reduced by the application of a non-linear fitting model based upon the minimisation of the sum of squares with K and s as the only adjustable parameters.

Results and Discussion

Square wave voltammetric studies of the interaction of NE with DNA

Figure 2 shows the voltammograms of 0.1 mM NE in 0.1 M Tris-HCl buffer, pH 7.0, in the absence and presence of DNA. The peak current of NE decreased with the addition of DNA, indicating that NE was bound to DNA. The current decreases further with increasing amount of DNA. In the presence of nucleic acids, the current is mainly due to free species, as the diffusion rate of bound species is small[23]. The peak current decreased because the apparent diffusion coefficient and concentration of electroactive species decreased[10,18].

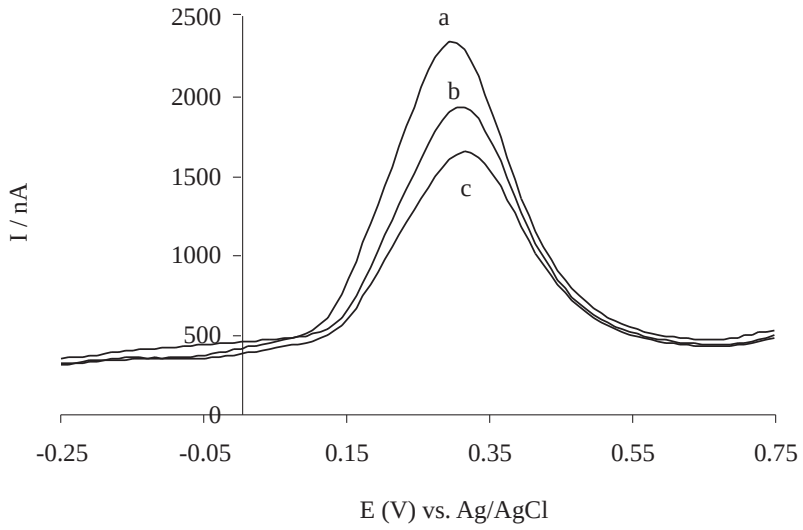

Figure 2. Square wave voltammograms of 0.1 mM NE in 0.1 M Tris-HCl buffer at pH 7.0 (a) without DNA (b) in the presence of 0.25 mM DNA and c) 2 mM DNA. Equilibrium time: 10 s, Frequency: 10 Hz, Step Potential: 5 mV, Amplitude: 25 mV.

UV absorption studies

UV absorption titration was also used to investigate the binding of NE to DNA. Figure 3 shows the UV spectra of the NE/DNA system. The absorbance of NE at 278 nm increased with the addition of DNA. A slight blue shift was observed with increasing concentrations of DNA. The absorbance of the mixture was different from the sum values of each component. The calculation of the free NE and added DNA demonstrated that NE was bound to DNA since $A_{NE} + A_{DNA}$ is larger than A_{NE-DNA}. The data were indicative of a hypochrome effect between NE and DNA[3].

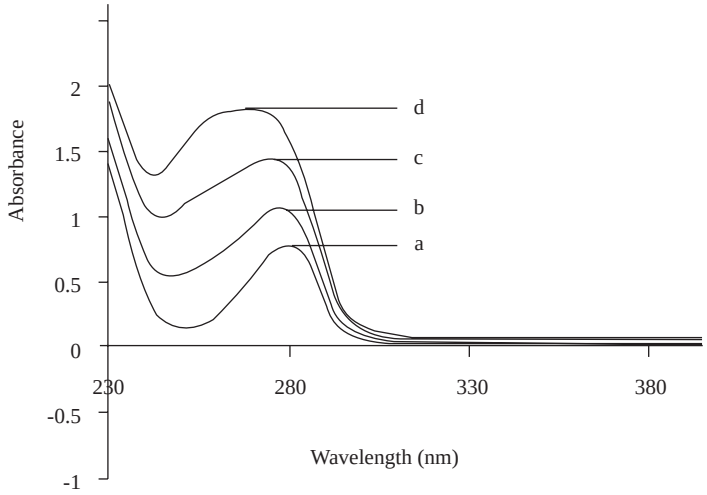

Figure 3. UV absorption spectra of 0.3 mM NE in 0.1 M Tris-HCl buffer, pH 7.0 (a) without DNA, (b) in the presence of 0.25 mM DNA (c) 0.5 mM DNA and (d) 1.0 mM DNA.

Determination of the binding constant of NE to DNA

The voltammetric titration of DNA with NE was used to determine the binding constant and the binding site size. The titration data were analysed using both static and mobile binding equilibria. A plot of C_b/C_fvs. C_T for NE in the presence of 2.0 mM DNA is given in Figure 4. The binding constant and binding site size obtained for a mobile binding equilibrium were $K = 5.15\ (\pm\ 0.38) \times 10^3\mathrm{M}^{-1}$, $s = 2.41\ (\pm\ 0.04)$ base pairs. A least-squares fit of C_b/C_fvs. C_T for NE in the presence of 2.0 mM DNA computed from the experimental data to equation 2 gave values of $3.3\ (\pm\ 0.18) \times 10^3\mathrm{M}^{-1}$ for K and $s = 2.89\ (\pm\ 0.05)$ base pairs for a static binding equilibrium. Two approaches have been used to determine the binding constant of the NE/DNA system. The experimental conditions under which each limiting case is justified were given by Evans[28]. The assumption of mobile inter-conversion yields values of K larger than those predicted by the static limit[18]. In the NE/DNA system, the 2 limiting cases give experimental K values which differ by a factor of about 2. However, it is difficult to determine exactly the more appropriate model for the measured current, since the electrochemical processes of the free and bound materials are not well resolved[18]. Thus, we presented results for calculations of both limiting cases. However, another binding model was utilised to determine the binding constant for a comparison. In this case the binding constant of the NE-DNA interaction was obtained using the following equation[29]:

$$Ip^2 = \frac{1}{K[DNA]}(Ip_0^2 - Ip^2) + Ip_0^2 - [DNA] \tag{6}$$

where Ip_0 and Ip are the peak currents of NE in the absence and presence of DNA respectively. The plot of Ip^2vs. $(Ip_0^2\text{-}Ip^2)/[DNA]$ gave a straight line with a binding constant of $K = 5.29\ (\pm\ 0.7) \times 10^3\mathrm{M}^{-1}$ (Figure 5). This value is very close to the K obtained by the mobile approach. Thus, the mobile approach may be more reasonable and appropriate for our system of the determination of the binding constant of the NE-DNA interaction. The binding site size determined shows that NE covers more than 2 base pairs upon binding to DNA. The standard Gibbs free energy change ($\Delta G^0 = -RT \ln K$) comes out to be approximately -21.17 kJ/mol at 25 °C, which indicates the spontaneity of the binding of NE with DNA.

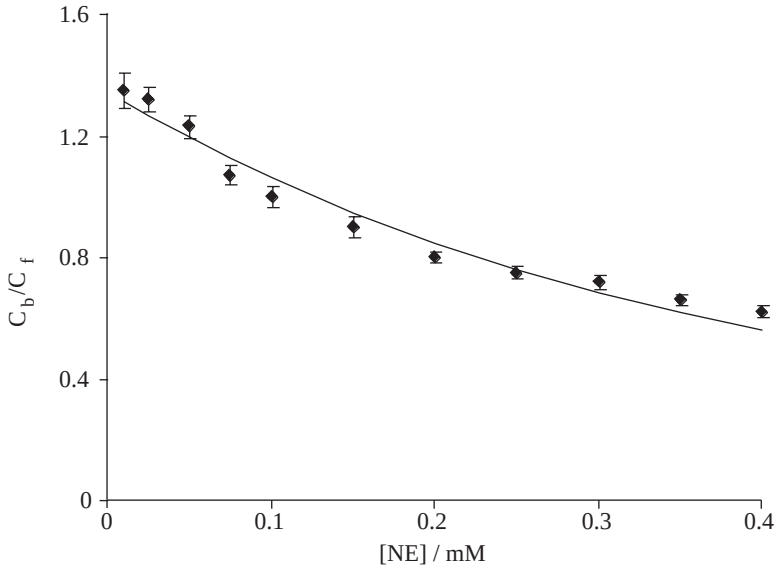

Figure 4. A plot of the ratio of C_b/C_f vs. C_T from the square wave voltammetric titration of NE against 2.0 mM DNA. The solid line shows a least squares fit to the data. Error bars were calculated at 95% confidence intervals from 5 different runs.

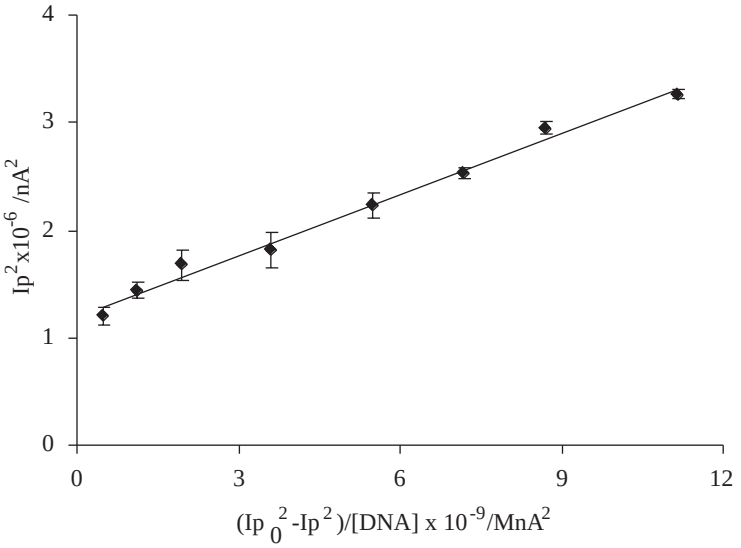

Figure 5. The plot of Ip^2 vs. $(Ip_0^2\text{-}Ip^2)/[DNA]$ used to calculate the binding constant. Error bars were calculated at 95% confidence intervals from 5 different runs.

An investigation of the ionic strength dependence on the binding constant

The effect of ionic strength in solution on the affinity of NE for DNA was tested by the addition of NaCl to determine the possible binding mode. It has been shown that when a charged ligand is added to a polyelectrolyte solution its binding constant, K, depends on the total counterion concentration, $[Na^+]$, as[30,31]:

$$\frac{d\log K}{d\log[Na^+]} = \Delta r = -z\Psi \tag{7}$$

where z is the charge on the ligand molecule, Ψ is the fraction of counterions bound to DNA and Δr is the number of counterions released upon the binding of the ligand with charge z. The theoretical slope of the log$K$ vs. log[Na^+] relation is -0.88 for a singly charged ligand bound to the B form of DNA in aqueous solutions at 25 °C[31]. The binding constant drops with the addition of NaCl to the solution containing NE and DNA. The plot of log$K$ vs. log[Na^+] was linear with a slope of -0.80. A comparison of the experimentally determined value of the slope with that predicted by the theory indicates that the predominant mode of the binding of NE to DNA is electrostatic, with a minor component of non-electrostatic attractions such as hydrogen bonds and intercalation since NE is a planar molecule.

Viscosity Measurements

Viscosity measurements were also carried out to provide clues for a binding model between NE and DNA. The values of relative specific viscosity (η/η_o) of DNA in the presence of NE are plotted against the concentration of NE (Figure 6). In the presence of lower concentrations of NE, no significant changes were observed in the relative viscosity of DNA. Ligands that do not intercalate or bind to DNA electrostatically typically cause less pronounced changes (positive or negative) or no changes in the DNA solution viscosity.[32] However, at higher concentrations of NE, the relative specific viscosity of DNA is increased. In general, a classical intercalation mode causes an increase in the viscosity of DNA solution due to the increased separation of base pairs at intercalation sites and, hence, an increase in overall DNA length[33]. This behaviour suggests that intercalation may also exist in the binding interaction between DNA and NE.

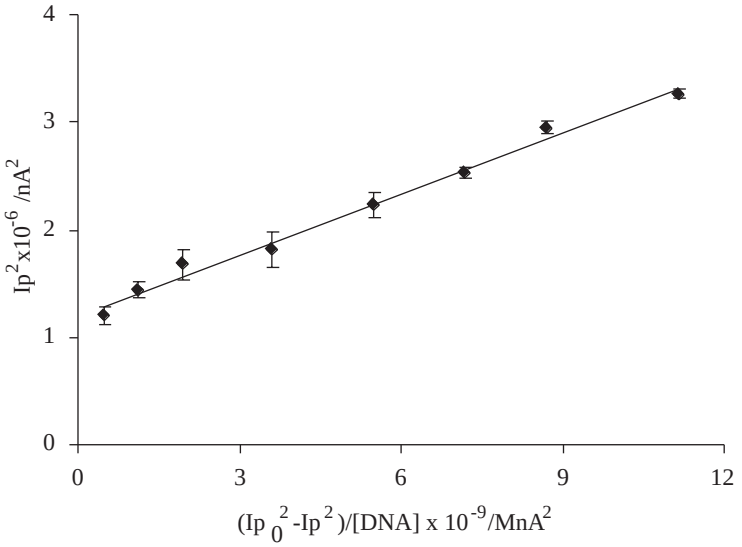

Figure 6. A plot of the ratio of the specific viscosity of DNA to that of free DNA in the presence of increasing concentration of NE at 25 °C.

Conclusion

A general conclusion from this study is that the neurotransmitter NE binds to herring sperm DNA with a binding constant of $K = 3.3\ (\pm\ 0.18) \times 10^3 \mathrm{M}^{-1}$, $s = 2.89\ (\pm\ 0.05)$ base pairs and $K = 5.15\ (\pm\ 0.38) \times$

$10^3 M^{-1}$, $s = 2.41$ ($\pm$ 0.04) base pairs for the limiting conditions of static and mobile binding equilibrium, respectively. However, the results showed that the binding constant obtained by the mobile approach is more reasonable. The binding site size determined shows that NE covers more than 2 base pairs upon binding to DNA. The standard Gibbs free energy change comes out to be approximately -21.17 kJ/mol at 25 °C, which indicates the spontaneity of the binding of NE with DNA. Besides the electrostatic interaction between DNA and NE, hydrogen bonds and intercalation may also exist since NE is a planar molecule.

Acknowledgements

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