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ORIGINAL ARTICLE

Voltammetric studies of the interaction of 6-mercaptopurine with cucurbit[7]uril and DNA

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Abstract The interaction of 6-mercaptopurine (6-MP), an antitumor drug, with cucurbit[7]uril (Q[7]) and DNA in an acetate buffer solution was studied by differential pulse voltammetry (DPV) and cyclic voltammetry(CV). The electrochemical data indicated a 1:1 complex formation of 6-MP with Q[7] and DNA. The formation constants of these complexes were determined based on the variations in the current. Moreover, the interactions of the 6-MP-Q[7] inclusion complex with DNA have been investigated by means of voltammetry. The results suggested that 6-MP displayed a high affinity for Q[7] and that the inclusion complex did not decompose when it bound to DNA. It can be inferred from the experimental data that the binding model of 6-MP to DNA may be 'electrostatic binding'. In addition, the formation of inclusion complexes between Q[7] and 6-MP was confirmed by UV-Vis spectroscopy and the ^{1}H NMR technique.

Keywords Voltammetry · 6-mercaptopurine · Cucurbit[7]uril · DNA

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Introduction

Deoxyribonucleic acid (DNA) binding studies are important for the rational design and construction of new and more efficient drugs targeted to DNA [1]. A variety of small molecules interact reversibly with double stranded DNA, primarily through three modes: (i) electrostatic interactions with the negatively charged nucleic sugar-phosphate structure, which are along the external DNA double helix and do not possess selectivity; (ii) binding interactions with the two grooves of the DNA double helix; and (iii) intercalation between the stacked base pairs of native DNA.

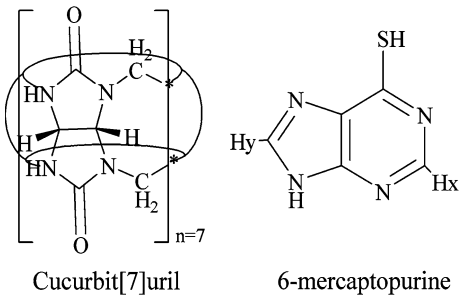
The DNA binding compound 6-mercaptopurine (6-MP) is an antiviral agent of the purine series, which has been used extensively since the 1950s in remission maintenance therapy for patients suffering from acute lymphoblastic leukemia [2, 3]. It is also used as an immunosuppressive drug in the treatment of acute lymphoblastic leukemia, acute myoblastic leukemia, chronic myelocystic leukemia and Crohn's disease [4–6]. The drug is known to inhibit the synthesis of both DNA and RNA by disturbing purine metabolization in the body. Biochemical studies demonstrated that this drug competes with the DNA template primer in the polymerase system [7]. The negative aspects of 6-MP are its side effects, which include marrow restraint and jaundice, nausea and vomiting. These side-effects limit the clinical use of the drug. Thus, contributions to a deeper knowledge of the mechanism of interaction of this drug with DNA are important for a better understanding of the therapeutic efficacy of the drug. Lately, the interactions of 6-MP or its metal derivatives with DNA have been studied using electrochemical methods and electronic absorption spectroscopy [8–10]. The results are valuable in understanding the mode of the complex binding to DNA,

examining the sustained release of 6-MP and laying the foundation for the rational design of DNA structure probes and antitumor drugs. Moreover, much attention has been paid to the binding of small molecules, for example planar dye molecules and small drug molecules, with cyclodextrin (CD) and DNA by various physico-chemical methods [11–16]. These studies contribute to understanding the modes of the binding of small molecules to DNA and the factors that can affect the binding in general.

Cucurbituril [17] and its homologues (Q[n]s) [18, 19] are a new family of synthetic receptors that have been widely studied in recent years. The Q[n]s have characteristic features in common, such as a hydrophobic cavity and two opening hydrophilic portals. In addition, the varying cavity and portal sizes lead to the formation of inclusion or exclusion complexes with different organic or inorganic species through a combination of dipole-ion, hydrogen bonding and hydrophobic interactions [20]. Q[n]s have found widespread applications in the pharmaceutical industry [21–23]. In our previous study, several Q[n]-purine derivative inclusion systems were investigated using different methods [24, 25].

Both DNA and cucurbiturils have a hydrophilic coat and a hydrophobic core structure. Thus, an aromatic ring stacking between nucleobase pairs and it incorporated into the Q[n] cavity are the main driving forces for the binding of an intercalator into double-stranded DNA or a guest molecule into Q[n], respectively. Moreover, a study of the binding model of a nucleotide drug with DNA by the Q[n] system was not found in the literature. These reasons motivated us to investigate the complexing properties of Q[n] and DNA as hosts for an antitumor drug substance (6-MP was used as a guest model), which is useful in understanding the modes of the binding of small molecules to DNA.

In this paper, the interaction of 6-MP with Q[7] (Fig. 1) in an acetate buffer solution was studied by differential pulse voltammetry (DPV) and cyclic voltammetry (CV). The experimental results were further confirmed by UV absorption spectroscopy and ^{1}H NMR analysis. Moreover,



Cucurbit[7]uril 6-mercaptopurine

Fig. 1 Structures of cucurbit[7]uril and 6-mercaptopurine

the interactions of the 6-MP and 6-MP–Q[7] inclusion complexes with DNA were investigated by means of voltammetry. From the decrease in the peak current or from the variation in the absorption spectra, the formation constants (K_f) of the 6-MP–Q[7] and 6-MP–DNA interactions were obtained.

Experimental

Materials

Cucurbit[7]uril was prepared and purified according to the method developed in our laboratories. Calf thymus deoxyribonucleic acid (DNA) and 6-mercaptopurine were obtained from Sigma and were used without further purification.

Voltammetric measurements

Differential pulse voltammetry (DPV) and cyclic voltammetry (CV) were carried out using a BASi-Epsilon E2-440 electrochemistry analyzer in conjunction with a hanging mercury drop electrode(HMDE), the 1100(HMDE-1110). An Ag/AgCl saturated KCl reference electrode and a Pt wire auxiliary electrode were used. All measurements were carried out at 25.0 ± 0.5 °C.

Stock solutions of 6-MP were prepared by dissolving an appropriate amount of the drug in 0.2 M NaOH, Calf thymus DNA (DNA) were prepared by directly dissolving them in twice-distilled water and storing at 4 °C. The concentration of the stock solution of DNA (5.43×10^{-5} M) in nucleotide phosphate, NP) was determined by UV absorbance at 260 nm using the molar extinction coefficient of 6600 M^{-1} cm^{-1}. The concentration of the analyte was determined by voltammetry or spectroscopy in the buffer solution and remained constant for at least 12 h. Stock solutions of Q[7] were prepared by dissolving the desired weights in distilled water. The supporting electrolyte was a 0.20 M acetate buffer. All chemicals were reagent grade and double distilled water was used to prepare the solutions. The pH was measured using a S-3C pH meter, with an accuracy of ±0.05.

The test solution was reacted in a water bath pot for 90 min at 37 °C, then it was placed in an electrochemistry cell and de-oxygenated by passing nitrogen for 15 min. The voltammetric response was obtained using a accumulation time of 70 s, pulse repetition time of 0.2 s with an amplitude of 50 mV and a scan rate of 20 mV s^{-1} (or 100 mV s^{-1} for CV).

Current or absorption titrations were performed by keeping the concentration of 6-MP constant and varying

the concentrations of Q[7] or DNA. The current titration equation was described as follows [11]:

$$\frac{1}{C_H} = K_f \frac{1-A}{1-i/i_0} - K_f \quad (1)$$

where, C_H is the concentration of Q[7] or DNA, K_f is the apparent formation constant, i_0 and i are the peak currents without and with Q[7] or DNA, and A is the proportionality constant. The conditions of using Eq. 1 are that a 1:1 association complex is formed and C_H is much larger than the total concentration of 6-MP in solution. In addition, the formation constant can be evaluated using spectrophotometry according to the following equation [11]:

$$\frac{A_0}{A-A_0} = \frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G} + \frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G}\frac{1}{K_f[Q[7]]} \quad (2)$$

where A_0 and A are the absorbances of the free guest and the apparent guest, respectively, and ε_G and $\varepsilon_{H\text{-}G}$ are the absorption coefficients of the guest and complex, respectively. Thus, if Eqs. 1 and 2 fit with the experimental data, the complexes of 6-MP with Q[7] or DNA may be 1:1 association complexes.

UV measurements

The ultraviolet and visible absorption spectra were obtained using an Agilent-8453 spectrophotometer. All measurements were carried out at 25.0 ± 0.5 °C.

^{1}H NMR measurements

To study the host–guest complexation of Q[7] and 6-MP, 2.0–2.5 × 10^{-3} mmol samples of Q[7] in 0.5–0.7 mL D_2O with increasing concentrations of 6-MP were prepared. The corresponding ^{1}H NMR spectra were recorded at 25 ± 0.5 °C on a VARIAN INOVA-400 spectrometer.

Results and discussion

Effect of accumulation time

The adsorption behavior of 6-MP at hanging mercury drop electrode was tested (Fig. 2). The current peaks exhibit an initial increase and a later decrease, and then slightly change after 70 s, illustrating that adsorptive equilibrium of 6-MP on the hanging mercury drop electrode surface was achieved. The peak current of 6-MP–Q[7] and 6-MP–Q[7]–DNA exhibited slightly change in peak current upon addition of amounts of Q[7] or DNA. Thus the accumulation time of 70 s was used for further studies.

DPV measurements

The inclusion phenomena were investigated using cyclic voltammetry and differential pulse voltammetry, techniques that permit the collection of excellent data at low concentrations of the electroactive guest [26]. Due to the involvement of catalytic hydrogen current at low pH values and the closeness of waves I and II, the most useful pH range for the determination of 6-MP with an acetate buffer is pH 3.5–5.7 [27].

The differential pulse voltammogram of free 6-MP gave a cathodic peak at −0.06 V at pH 3.6. Addition of cucurbit[7]uril to aqueous solutions of 6-MP causes changes in the voltammogram of 6-MP (Fig. 3). With the increase in the amounts of cucurbit[7]uril, the cathodic peak potentials shifted in a positive direction, and the cathodic peak current decreased. These results suggest that the inclusion complexes were formed between 6-MP and the given Q[7]s. The decrease of the peak current can be explained by the smaller diffusion coefficients of the inclusion complexes.

The formation of the inclusion complexes was markedly affected by the pH value of the solution. Strong interactions were observed in a pH 3.6 acetate buffer compared with other pH values. The cathodic peak current decreased to about 40, 12 and 4% of the peak current in the absence of Q[7] for pH values of 3.6, 4.4 and 5.5, respectively. This behaviour reflects the strong interaction of the cationic species of 6-MP with Q[7].

The following equation was obtained for the decrease of the peak currents with the concentrations of Q[7]: $1/C_{Q[7]} = 2.44 \times 10^4/(1 - i/i_0) - 3.49 \times 10^4$ with a linear correlation coefficient (r) of 0.9952. This reveals that the inclusion

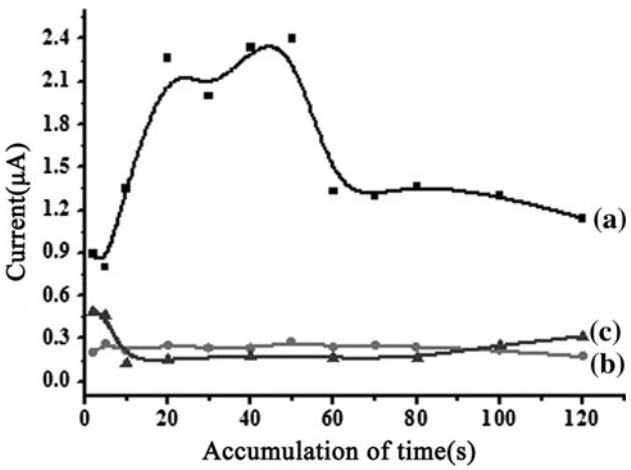


Fig. 2 Effect of accumulation time using cyclic voltammetric measurements in HMDE for 6.4 × 10^{-5} M 6-MP solution (*a*) 6-MP only (*b*) *a* + 6.4 × 10^{-5} M Q[7] and (*c*) *b* + 6.5 × 10^{-5} M DNA

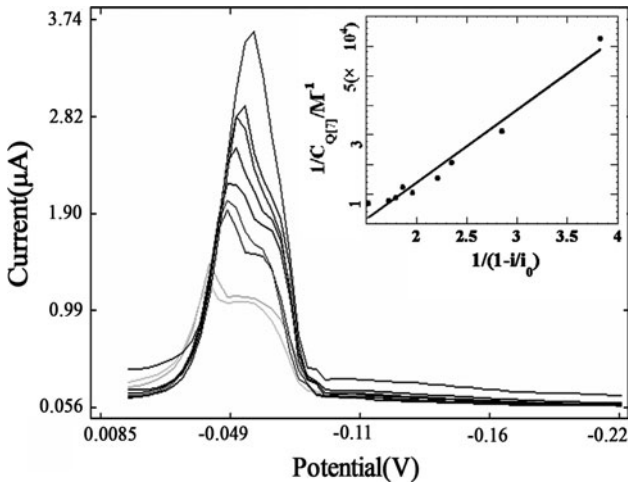


Fig. 3 Differential pulse voltammograms of 6-MP in the presence of increasing concentrations of Q[7] at HMDE in acetate buffer (pH 3.6). *Inset*: the plot of $1/C_{Q[7]}$ to $1/(1 - i/i_0)$

complex of 6-MP with Q[7] is a 1:1 association complex and that the formation constant (K_f) is $(3.49 \pm 0.01) \times 10^4$ M^{-1} for 6-MP-Q[7] in pH 3.6, as calculated from the *y*-intercept.

CV measurements

The inclusion phenomena of the electroactive 6-MP were also studied using cyclic voltammetry. Typical cyclic voltammetric behaviours of 6-MP in the absence and presence of Q[7] at pH 3.6 are shown in Fig. 4. Summaries of the cyclic voltammetric results are given in Table 1. The cyclic voltammetric behaviour of pure 6-MP includes one cathodic peak and a corresponding oxidation peak in the potential range of −0.0 to −0.6 V at the HMDE (curve 1 in Fig. 4). The separation of the anodic and the cathodic peak

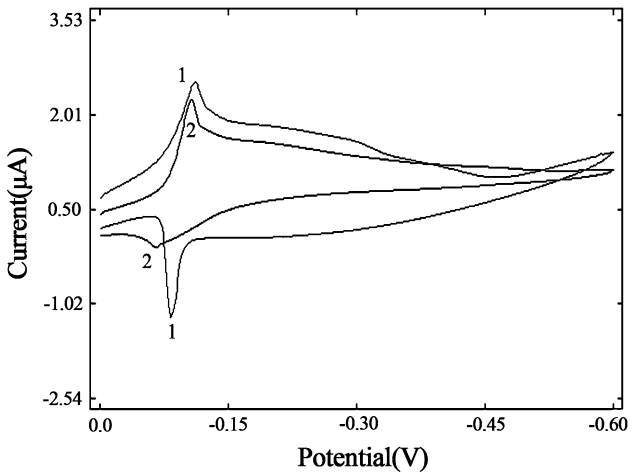


Fig. 4 Cyclic voltammograms for 6.4×10^{-5} M 6-MP solution obtained in acetate buffer (pH3.6) using a scan rate of 100 mV s^{-1} and accumulation potential of −0.2 V. (*1*) 6-MP only, (*2*) 1 + 6.4×10^{-5}M Q[7]

Table 1 Cyclic voltammetric parameters of 6-MP in absence and presence of Q[7] and DNA

Sample	E^a_{pc}/mV	E^a_{pa}/mV	$E^{0\prime}$/mV	ΔE_p/mV
6-MP	−111	−83	−97	28
6-MP–Q[7]	−107	−66	−86	41
6-MP–DNA	−113	−86	−99	27
6-MP–Q[7]–DNA	−108	−50	−79	58

[a] All values are average of three measurements ($E \pm 3$) mV

potentials, ΔE_p, was 28 mV at 100 mV s^{-1}, indicating a quasireversible redox process. The formal potential, $E^{\circ\prime}$, taken as the average of E_{pc} and E_{pa}, was −97 mV in the absence of Q[7] [11].

The addition of Q[7] to the 6-MP solution caused the cathodic peak currents to diminish (curve 2 in Fig. 4). The solutions of the complexed guests in the presence of Q[7] yielded cathodic peak potentials that were shifted in a positive direction. $E^{\circ\prime}$ shifted to a more positive potential by 10.5 mV in the presence of Q[7], which revealed that the reduction of 6-MP molecules was easy when they were included in the cavity of Q[7] as part of an inclusion complex[11]. In the presence of Q[7], the difference between the anodic and cathodic peak potentials, ΔE_p, was 41 mV,indicating that the irreversibility of the electron-transfer process was maintained under this condition. The decrease of the peak current observed upon addition of Q[7] is due to the lower diffusion coefficient of cucurbituril complexes compared to that of the free guest.

CV peak potentials were independent of scan rates (v) in the range of 50–200 mV s^{-1}, and the peak currents were proportional to the square root of scan rates both without and with Q[7] ($r = 0.9926$ and $r = 0.9935$, respectively). The slope of the linear i_p versus $v^{1/2}$ relationship for the tests without Q[7] (14.2 nA $mV^{-1/2}$ $s^{1/2}$) was larger than that for the tests with Q[7] (12.3 nA $mV^{-1/2}$ $s^{1/2}$), suggesting that the diffusion coefficient of the free form of 6-MP was larger than that of the form of 6-MP complexed with Q[7].

UV spectroscopy measurements

The formation of an inclusion complex between 6-MP and Q[7] can be further confirmed by a spectroscopic experiment. The UV spectra obtained with aqueous solutions containing a fixed concentration of the guest (40 μM) and variable concentrations of Q[7] are shown in Fig. 5. The absorption spectra of the 6-MP exhibited a progressively lower absorbance and a slight red shift with increasing concentrations of Q[7] and also contained an isosbestic point at 337 nm. According to an $A_0/(A - A_0)$ versus 1/Q[7] plot (Fig. 5 insert), a fit to Eq. 2, a 1:1 complex of

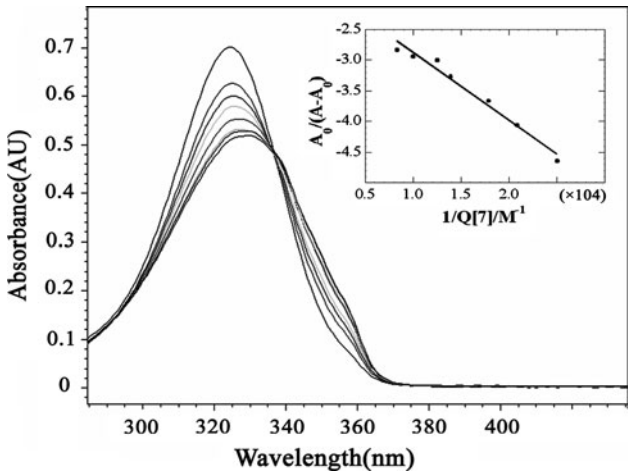


Fig. 5 UV absorption spectrum of 6-MP in the presence of increasing concentrations of Q[7] in acetate buffer (pH 3.6). Insert : the plot of $1/C_{Q[7]}$ to $A_0/(A - A_0)$

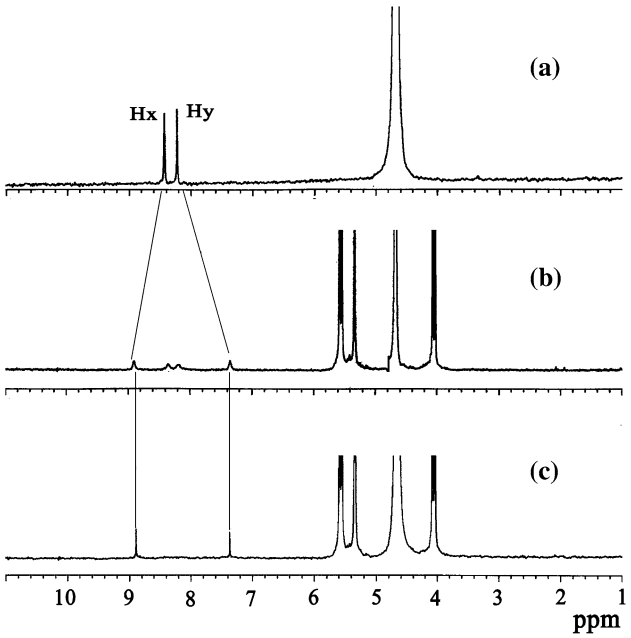


Fig. 6 ^{1}H NMR spectra (400 MHz, D_2O) of 6-MP in the absence (**a**) and in the presence of 0.4 equiv (**b**) and 1.0 equiv (**c**) of Q[7]

6-MP and Q[7] was formed. The ratio of the intercept to the slope gives the value of the formation constant (K) to be $(2.58 \pm 0.02) \times 10^4$ M^{-1} for 6-MP at Q[7] ($r = 0.9971$).

^{1}H NMR measurements

Evidence for the formation of the 6-MP–Q[7] complex can be also obtained from the ^{1}H NMR spectra. A spectrum for a complex would show changes in the chemical shifts of the guest. Figure 6 shows the ^{1}H NMR spectra of 6-MP in the absence (a), in the presence of 0.4 equiv (b) and 1.0 equiv of Q[7] (c). As can be seen in the spectra, the chemical shift of proton Hx on the pyrimidine of the guest 6-MP moves downfield by δ0.55, while the proton Hy on the imidazole ring showed an upfield shift of δ0.85, suggesting that Q[7] can partially include 6-MP. The imidazole moiety of the 6-MP is inserted into the cavity of Q[7] and the pyrimidine moiety of the 6-MP is located at the deshielding portal of the host.

Interaction of 6-MP with DNA

The typical cyclic voltammograms of 6-MP without and with DNA are shown in Fig. 7a. In the presence of DNA, the peak currents decreased and the peak potentials showed almost no change, indicating that DNA does not affect the electrode surface behaviour. The reason for the decrease of the peak current could be the lower apparent diffusion coefficient of the inclusion complex of 6-MP-DNA [28] and the lower apparent concentrations of the electroactive species. The lack of change of the peak potentials reveals that the interaction of the reduced form of the drug molecule with DNA was the same as that of its oxidized form [28]. The peak potentials (E_{pc}, E_{pa}) were independent of the scan rates (v) in the range of 50–200 mV s^{-1} and the peak currents (i_{pc}, i_{pa}) were linear with the square root of scan rates ($v^{1/2}$) in the presence of DNA. ΔE_p was 27 mV in the presence of DNA, indicating that the DNA did not affect the electron transfer process of 6-MP molecules. Moreover, the experimental data showing a decrease in the DPV peak current with the addition of DNA corresponded well to Eq. 1. According to the experimental data (at pH 3.6), the following equation was obtained with $r = 0.9988$: $1/C_{DNA} = 2.36 \times 10^3/(1 - i/i_0) - 1.69 \times 10^4$, revealing that the binding complex of a 6-MP molecule with DNA was a 1:1 association complex. The binding constant of 6-MP to DNA was calculated from the y-intercept and found to be $(1.69 \pm 0.014) \times 10^4$ M^{-1}, as shown in Fig. 7b.

Interaction of the 6-MP–Q[7] complex with DNA

The effect of the addition of DNA to the complexed 6-MP with Q[7] was investigated using cyclic voltammetry (Fig. 8). It was observed that, in presence of DNA, the cathodic peak potential of the 6-MP–Q[7] complex shifted to a more negative value, but the anodic peak potential shifted to a more positive value. In addition, the peak currents decreased. If the inclusion complex had decomposed, the addition of DNA would have changed the redox potential. In the experiments, the redox potential showed a small change when DNA was added. This suggests that the decomposition of the inclusion complex of 6-MP–Q[7] was not obvious when it interacted with DNA. The results also

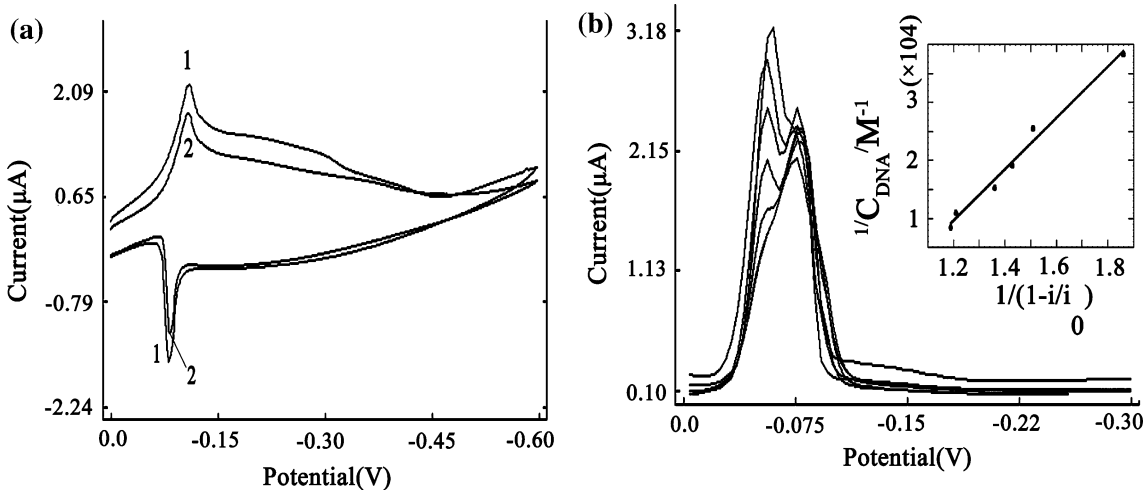


Fig. 7 **a** Cyclic voltammograms for 6.4×10^{-5} M 6-MP solution in the absence (*1*) and presence of (*2*) 6.5×10^{-5} M DNA; **b** Differential pulse voltammograms of 6-MP in the presence of increasing concentrations of DNA at HMDE in acetate buffer (pH 3.6). *Inset*: the plot of $1/C_{DNA}$ to $1/(1 - i/i_0)$

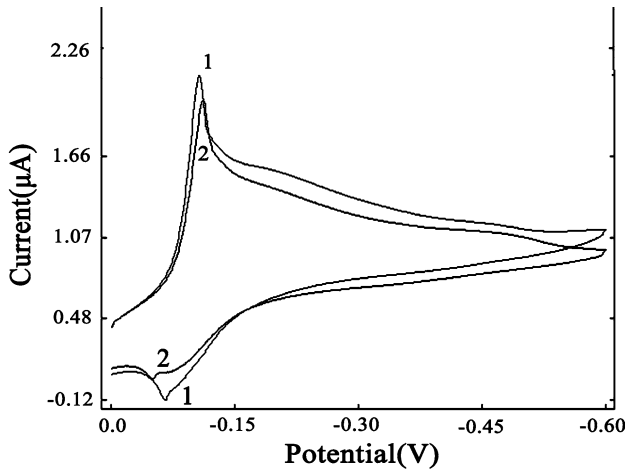


Fig. 8 Cyclic Voltammograms for (*1*) 6.4×10^{-5} M 6-MP + 6.4×10^{-5} M Q[7] and (*2*) 1 + 6.5×10^{-5} M DNA

indicate that the ΔE_p values for 6-MP–DNA and 6-MP-Q[7]–DNA are 27 and 58 mV, respectively, which suggests that the existence of Q[7] affects the interaction of 6-MP with DNA and that the interaction between 6-MP and Q[7] or DNA is a competitive process. This is reasonable due to the slightly higher formation constants of the 6-MP–Q[7] complexes than the 6-MP-DNA complexes.

In addition, the electrochemical method can be used to judge whether Q[7] interacted with DNA in the solution. The experimental results indicate that Q[7] did not interact with DNA in the acetate buffer.

Conclusions

In this paper, the interaction of 6-MP with Q[7] in an acetate buffer solution was studied by differential pulse voltammetry (DPV) and cyclic voltammetry (CV). The experimental results were further confirmed by UV absorption spectroscopy and ^{1}H NMR analysis. Moreover, the interactions of the 6-MP and the 6-MP–Q[7] inclusion complex with DNA have been investigated by means of voltammetry. The electrochemical experiments demonstrated that the 6-MP molecules formed simple complexes with Q[7] and DNA and the formation constant was calculated using the DPV method. The results also showed that 6-MP displays a high affinity for Q[7], the existence of Q[7] affects the interaction of 6-MP with DNA, and the inclusion complex does not decompose when it binds to DNA. That is to say, imidazole moiety of the 6-MP that was included in the cavity of Q[7] did not interact with DNA. It can be estimated that the most likely binding model of 6-MP–DNA under our experimental conditions is electrostatic binding. The binding is formed through the cationic group -SH binding with the PO_4^{3-} of the sugar-phosphate backbone in DNA. These investigations are of importance in understanding the mechanism of interaction of and recognition of drugs in the living body.

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