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Interaction of Nonsteroidal Anti – Inflammatory Drug Naproxen Sodium with DNA by Electrochemical and Spectroscopic Methods

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Introduction

Naproxen sodium (NAPS), the sodium salt of (S)-6-methoxy-a-methyl-2-naphtalenacetic acid (Figure 1) is a non-steroidal anti inflammatory drug, which is used in the treatment of severe pain and inflammation. Naproxen blocks the enzyme that makes prostaglandins (cyclooxygenase), resulting in lower concentrations of prostaglandins [1,2].

Deoxyribonucleic acid (DNA) has a central role in life process since it contains all of the genetic information required for cellular function. However, DNA molecules are prone to be damaged under various conditions, especially by interaction with some molecules and this damage may lead to various pathological changes in living organisms. There is growing interesting exploring the binding of small molecules with DNA for the rational design and constructon of new and more efficient drugs targeted to DNA as well as in understanding how proteins recognize and bind to specific DNA sequences [3-5].

There are generally three interaction models about binding of small molecules to the DNA double helix: (1) electrostatic interaction, *i.e.* small

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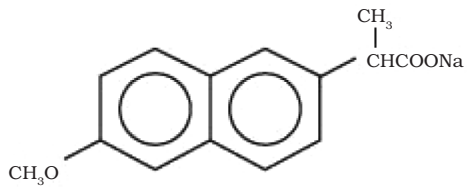

Figure 1
Chemical structure of naproxen sodium

molecules are electrostatically adsorbed on the phosphates of DNA chain, (2) intercalative binding, small molecules intercalate into the base pairs of the double stranded structure of DNA, (3) groove binding, *i.e.* small molecules act with the grooves of DNA chain. In the DNA double helix, there are two kinds of grooves, major groove and minor groove. The intercalative binding and the groove binding are related to these grooves, while the electrostatic binding can take place out of the groove [6]. The interaction of NAPS – DNA has been examined by various methods involving differential scanning calorimetry (DSC)[7], fluorescence[8]. In recent years, electrochemical methods have gained growing interests in the investigation of DNA – drug interaction [9-14].

There has not any report about the detection of NAPS – DNA interaction based on the electrochemical behaviours at gold electrode and especially on the change of various spectroscopic characteristics. Accordingly, in this work, detailed inverstigations of the electrochemical behaviour of NAPS upon addition of DNA were carried out. Morever, the changes in UV absorption spectra when NAPS binding to DNA were used to study the mode of such interaction. The agreement of the various methods is quite good. Thus it can be seen, there is a mutual complemet between electrochemical method and spectroscopy techniques, which can provide fruitful information about the mechanism of interaction.

Material and Methods

Calf thymus (pBR322 plasmid) DNA was obtained from Sigma (200 μg stock solution, 1 μg pBR322 plasmid: 4361 base pairs: 0.35 pmol: 2.1 x 10^{11} molecule) and used as received. Calf thymus (pBR322 plasmid) DNA solution (1 μM) was dissolved in water and stored at 4^0C. Reactions

were done in mixtures containing 50 mM NaCl + 5 mM Tris HCl buffer, pH 7.1 and solutions of DNA gave rations of UV absorbance at 260 and 280 nm (A_{260} / A_{280}) of approximately 1.9, indicating that the pBR322 plasmid DNA was sufficiently protein-free [15,16]. Solutions were incubated at 37 ^{0}C for 2 hours. NAPS stock solution (5.0 x 10^{-3} M) were prepared in MeOH and kept away from light to avoid photochemical decomposition. Working standard solutions were prepared daily by appropriate dilution of the stock standard solutions with 50 mM NaCl + 5 mM Tris HCl (1:1). If not specially stated, the supporting electrolyte was 50 mM NaCl + 5 mM Tris HCl (1:1) (pH 7.1). All reagents were analytical grade and aqueous solutions were prepared using doubly distilled deionized water.

Instrumentation

CV and DPV studies were carried out by CHI1230A (CH Instruments, USA). The three-electrode system consisted of a gold working electrode, an Ag/AgCl-saturated KCl reference electrode and a platinum wire counter electrode. All potentials were referred to the reference electrode. UV/Vis absorbance spectra were obtained by ND-1000 UV/Vis spectrophotometer equipped with a quartz micro-colorimetric vessel of 1 cm path length.

Results and Discussion

Interaction of NAPS with DNA

The electrochemical behaviour of NAPS at gold electrode was investigated employing CV and DPV. To prevent DNA from acidic or basic denaturing, pH 7.1 Tris HCl:NaCl (1:1) buffer chosen as supporting electrolyte. The CV behaviour of NAPS showed one oxidation peak at +1.125 V in Tris HCl:NaCl (1:1) of pH 7.1 with a scan rate of 100 mVs^{-1} (Figure 2).

The effect of scan rate (v) on the peak current (I_p) of NAPS have been studied. For this, we recorded CV of 5.0 x 10^{-5} M NAPS at gold electrode in the scan rate range of 50 – 500 mV s^{-1}. It is evident from Figure 2 that the peak current increased with increase in scan rate.

Both peak currents of drug and DNA complex were linerly dependent on the square root of the scan rate, suggesting that oxidation process

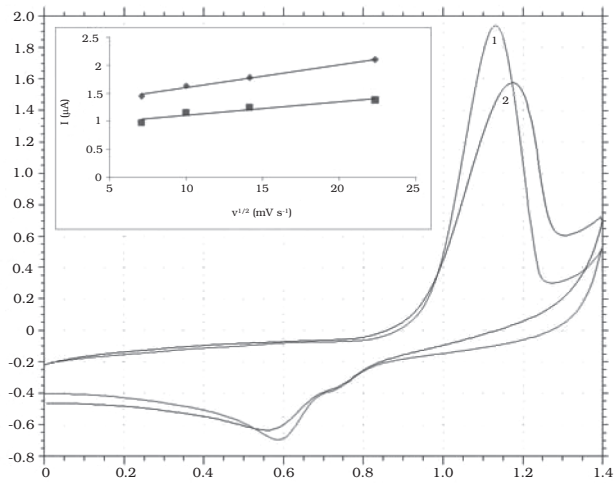

Figure 2

Cyclic voltammograms of 5.0 x 10-5 M NAPS in the (1) absence and (2) presence of 0.014 μM DNA. Supporting electrolyte: Tris HCl: NaCl buffer (1:1) pH 7.1, scan rate 100 mVs-1. Inset: relationship between the peak currents of the oxidation wave of NAPS in the absence (◇) and presence of (■) 0.014 μM DNA and the square root of scan rates

was controlled by diffusion of the electroactive species to the electrode surface [17]. In addition that, The plots of log I_p versus logv in the scan rate range of 50 – 500 mV s^{-1} yielded a straight line with slope of 0.532 for NAPS. These values are close to the therotical value of 0.500, which is expected for an ideal reaction condition for diffusion – controlled electrode process [17]. Furthermore, the smaller linear slopes of DNA complex demonstrated that NAPS could interact with DNA in solution, forming NAPS – DNA adducts with large molecular weight, resulting in a considerable decrease in the apparent diffusion coefficient [18]. Bard and co-workers [19] reported that positive shifts in the peak potential of intercalators were observed in the binding form via hydrophobic interactions (intercalation) while electrostatic interactions led to negative shifts. Based on this report, the positive shifts in the peak potential of NAPS upon binding to DNA should be as a result of intercalative interaction to DNA.

Current titrations were performed by keeping the constant concentration of the drug while varying the concentration of DNA using both DPV and CV at pH 7.1. The interaction of drug with DNA can be described using the following equation:

$$\text{drug} + \text{DNA} \leftrightarrow \text{drug} - \text{DNA}$$

An equation for amperometric titration can be deduced according to [9-11,19,20]

$$\log\left(\frac{1}{[DNA]}\right) = \log(K) + \log\left(\frac{I_{H\text{-}G}}{I_G - I_{H\text{-}G}}\right) \quad (1)$$

Where K is the apparent binding constant, I_G and $I_{H\text{-}G}$ the peak current of the free guest (G) and the complex (H-G), respectively. Under the assumption of diffusion – controlled electron transfer and the complex of drug with DNA (in nucleotide phosphate) is 1:1 association complex, then the plot of log (1 / [DNA]) versus log ($I_{H\text{-}G}$ / (I_G - $I_{H\text{-}G}$)) becomes linear with the intercept of log (K). The binding constant of this complex were evaluated according Eq. (1) and the results are listed in Table 1.

Linear range of DNA determination

The decrease in peak current of NAPS resulted from the addition of DNA into NAPS solution can be employed to determine the concentration of DNA. The peak current of DPV of NAPS at 1.072 V was used as the detection signal. Under the optimum experimental condition of Figure 3, the decreases in the DPV peak current were linearly related to DNA concentration in the range of 0 – 0.021 μM when NAPS concentration were fixed at 5.0 x 10^{-5} M, detection limit of DNA was found as 0.0025 μM. Typical calibration curves for DNA were in the inset of Fig. 3. The relative standard deviation (RSD) of six experiments performed at DNA concentration level of 0.015 μM was %2.4, indicating that the proposed method can provide a reproducible determination. The results suggested that the proposed method is simple and sensitive hence it can be applied to the determination of many kinds of DNA.

UV / Vis spectra

Figure 4 showed the UV/Vis absorption spectra of NAPS in the absence and presence of different concentrations of DNA. The maximum absorbance of NAPS was located around at 260 and 270 nm. It was observed that on the addition of DNA, NAPS showed an decrease in molar absorptivity with a red shift of 1 - 3 nm. This hypochromic effect is thought to be due to the interaction between the electronic states of the intercalating chromophore and those of the DNA bases [21]. So, the obviously large hypochromism observed in our experiments suggested the

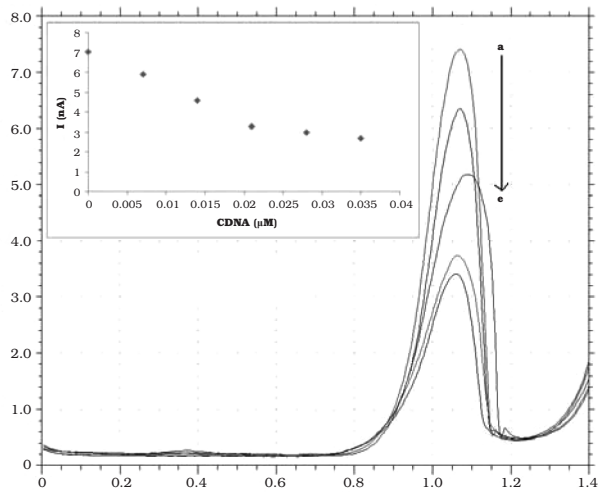

Figure 3

Differential pulse voltammograms of 5.0 x 10-5 M NAPS in the absence (a) and presence of (b) 0.007 (c) 0.014 (d) 0.021 μM DNA. Es = 0.004 V and pulse amplitude 0.05 V Inset: relationship between the DPV peak currents and the concentration of DNA at 5.0 x 10-5 M NAPS

close proximity of the NAPS chromophore to the DNA bases. The NAPS solution exhibited peculiar hypochromic effect and bathochromic shift in UV/Vis spectra upon binding to DNA, a typical characteristic of an intercalating mode [22].

Based on the variations in the absorbance spectra of NAPS upon binding to DNA, the binding constant (K) was calculated according to the equation (2) [23,24].

$$\frac{A_0}{A - A_0} = \frac{\varepsilon_G}{\varepsilon_{H\text{-}G} - \varepsilon_G} + \frac{\varepsilon_G}{\varepsilon_{H\text{-}G} - \varepsilon_G} \frac{1}{K\,[DNA]} \qquad (2)$$

where A_0 and A are the absorbances of drug in the absence and presence of DNA, ε_G and $\varepsilon_{H\text{-}G}$ are the absorption coefficients of drug and its complex with DNA, respectively. According to Eq. (2), the plot of A_0 / (A – A_0) versus 1 / [DNA] was constructed (figure not shown) using the data from the absorbance titrations and a linear fitting of the data yielded the binding constant (K) 1.02 x 10^4 M^{-1} for NAPS - DNA. These results are close to that from voltammetry (Table I).

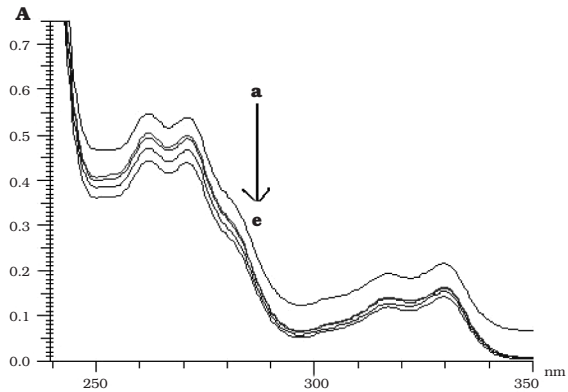

Figure 4

UV-VIS absorption spectra of 5.0 x 10-5 M NAPS in the absence (a) and presence of (b) 0.007 (c) 0.014 (d) 0.021 (e) 0.028 μM DNA

TABLE I

Binding constant of NAPS – DNA complex calculated from the results of voltammetry at pH 7.1

Complex	Cyclic voltammetry, K (M^{-1})	Differential pulse voltammetry, K (M^{-1})
NAPS-DNA	1.02×10^4	1.14×10^4

Conclusions

In this study, the interaction of NAPS with DNA was studied by cyclic voltammetry and differential pulse voltammetry especially by UV/Vis spectroscopy. The binding of NAPS to DNA resulted in a series of changes in the electrochemical behavior and spectra characteristics. Upon binding of NAPS to DNA, absorption spectra of NAPS showed hypochromic effect and bathochromic shift. From these experimental results, it could be affirmed that the interaction of NAPS with DNA through intercalative mode. These investigations showed that electrochemistry coupled with spectroscopy method could provide a convenient way to characterize both the binding mode and the interaction mechanism of NAPS to DNA. Differential pulse voltammetric results suggested that, it is feasible to apply the proposed method to quantitatively determine the concentration of DNA.

Summary

The interaction of NAPS, a nonsteroidal anti – inflammatory drug, with pBR322 plasmid DNA has been investigated by cyclic voltammetry (CV), differential pulse voltammetry (DPV) as well as UV/Vis spectroscopy. The interaction of NAPS with DNA could result a considerable decrease in the NAPS peak currents and a hypochromic effect and bathochromic shift in the maximum adsorption bands of NAPS. The variation in the electrochemical and spectral characteristics of NAPS indicated NAPS bind to DNA by intercalative mode. Binding constants were determined from voltammetric and spectroscopic data with addition of DNA. These studies are valuable for a better understanding the detailed mode of NAPS – DNA interaction, which should be important in deeper insight into the therapeutic efficacy of NAPS and design of new DNA targeted drugs.

Key Words: Naproxen sodium, Calf thymus DNA, Electrochemistry, UV/Vis spectroscopy, DNA Interaction

Özet

Non-steroidal Antienflamatuar İlaç Olan Naproksen Sodyumun pBR322 Plasmid DNA ile etkileşmesi

Dönüşümlü voltametri (CV), differansiyel puls voltametrisi (DPV) ve UV/Vis spektroskopisi yöntemleri kullanılarak incelendi. Naproksen sodyumun DNA ile etkileşmesi naproksen sodyumun pik akımında belirgin bir azalma ve maksimum absorpsiyon bandında hipokromik etki ve bathokromik kaymaya neden olmuştur. Naproksen sodyumun elektrokimyasal ve spektral özelliklerindeki değişimler; naproksen sodyumun DNA ile interkalasyon bir şekilde etkileştiğini belirtmektedir. Voltametrik ve spektroskopik veriler kullanılarak bağlanma sabitleri tayin edildi. Bu çalışmalar naproksen sodyum – DNA etkileşimini daha iyi anlamak, naproksen sodyumun tedavi edici etkisinin belirlenmesi ve ilerde yeni DNA hedefli ilaçların tasarlanması açısından değerlidir.

Anahtar Kelimeler: Naproksen sodyum, Kalf timüs DNA, Elektrokimya, UV/Vis spektroskopisi, DNA Etkileşmesi

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