Electrochemical Comparison of the Interaction of 5-Nitrouracil with Single-or Double-Stranded DNA at mercury and glassy carbon electrodes

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Abstract

The interaction of the 5-Nitrouracil (5NU), with ss-, and ds-DNA was investigated electrochemically in absence and presence of copper ions by using cyclic voltammetry (CV) and differential pulse stripping voltammetry (DPSV) at hanging mercury drop electrode (HMDE) and glassy carbon electrode (GCE) surfaces. It was found that, in absence of copper ions, the addition of ss- or ds-DNA to a buffered solution of 5NU results in a decrease on the 5NU redox peak current with a remarkable change in the peak potential (ca. 150 mV vs. Ag/AgCl) at both electrodes. This means that, an interaction of 5NU molecules with both ss- and ds-DNA was observed. The results also demonstrate that a distinguish between ss-, and ds-DNA can be achieved in presence of copper ion through their interaction with 5NU. The binding constants of 5NU with ds-DNA at HMDE and GCE were determined through the changes on the 5NU redox peak currents (at HMDE, $1.45x10^5$ and at GCE, $2.65x10^5$). The calibration plot for the DNA determination was obtained through the corresponding decreases on the DPSV peak current of 5NU to different additions of DNA (ss- or ds-DNA) concentration levels at the optimum conditions.

Keywords: Voltammetry, 5-nitrouracil, interactions, DNA

1. Introduction

The bioactivity of 5-substituted uracils , a class of the substituted pyrimidines, induces exceptional interest in their biochemistry and pharmacology[1], and they are the most interesting and studied uracils. 5-substituted uracils are potential substitutes for thymine in the DNA, and are used to increase the sensitivity of the DNA to ionizing radiation[2]. The most prominent representatives are: 5-fluorouracil [3,4] and 5-bromouracil [5,6]. Apart from the halogen-modified nucleobases several aromatic nitro compounds also show promise as radiosensitizers for overcoming the radioprotection afforded some tumor cells by their lack of oxygen, 'hypoxia' [7]. Moreover, many derivatives of 5-nitrouracil have shown the following effects: antibacterial activity [8], antitumor activity on leukemia cells [9] inhibitory effect on macrophage [10], prime interest to the non-linear optical community [11] and relevance to the biological and pharmaceutical sciences [12]. In addition, the 5-nitrouracil has been known to inhibit thymidine phosphorylase [13]. The complexing ability of these ligands with some essential bioelements in serum (Fe(III), Cu(II) etc.), as well as with Al(III) and Mg(II) ions in antacids is of special interest.

DNA is quite often the major cellular target for studies with smaller molecules of biological importance such as carcinogens, steroids and several classes of drugs [14–16], particularly for the designing of new DNA-targeted drugs and the experience of these in vitro. Drug-DNA interactions have been studied by a variety of analytical techniques with their relative advantages and disadvantages such as luminescence [17], fluorescence [18], UV–vis

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spectroscopy [19], and voltammetric methods [20]. Among these electrochemical methods are widely used in drug–DNA binding studies due to their advantages like high sensitivity, efficient selectivity, cost affectivity, more reliability, extensive versatility and fast detection ability. So, in the recent years, growing interest has arisen in electrochemical investigations of interaction between nucleic-acid-binding molecules and DNA using especially mercury or carbon electrodes [21-24]. Furthermore, the voltammetric methods were used to probe the interaction (electrostatic or intercalative) of a number of metal chelates with calf thymus DNA [25]. Continuing our studies on the interaction of small molecules with DNA [26-29], the present paper is concerned with the voltammetric studies of the interaction of 5-nitrouracil with calf thymus ss- or ds-DNA in absence and presence of Cu(II).

2. Materials and Method

2.1 Chemicals and reagents

Single-stranded (ss) or double-stranded (ds) calf thymus deoxyribonucleic acid (DNA) and 5-nitrouracil (Fig. 1) were obtained from Sigma and were used without further purification. Stock solutions of DNA were prepared by dissolving an appropriate amount of the DNA in H_2O and stored at 4^oC. DNA concentration (per nucleotide phosphate, NP) was determined spectrophotometrically assuming ε_{260} = 6600 M^{-1} cm^{-1}. Stock solution of the 5NU was prepared by dissolving an appropriate amount of the 5NU in water. The supporting electrolyte was phosphate buffer prepared in the usual way by adding appropriate amounts of 1.0 M orthophosphoric acid to 0.25 M sodium phosphate. All chemicals were reagent grade (Merck, Darmstadt). Twice-distilled water was used to prepare the aqueous solutions. The pH was measured using pH-meter Model M64 (ORION) with accuracy to ± 0.05.

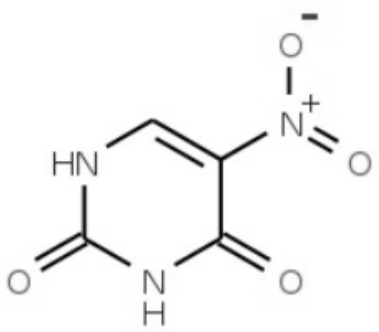

Figure 1.

2.2 Instrumentation

A PAR Model 264A polarographic analyzer/ stripping voltammetry coupled with a PAR Model 303A HMDE and X-Y recorder Model RE0151 were employed for differential pulse stripping voltammetric (DPSV) and cyclic voltammetric (CV) measurements. The area of the HMDE was 1.2x10^{-2} cm^2. A PAR glassy carbon electrode (GCE) for Model 303A was also used as the working electrode. An Ag/AgCl saturated KCl reference electrode and a Pt wire auxiliary electrode were used. All voltammetric measurements were carried out at the ambient temperature of the laboratory (23-25 ^oC), according to our previous work [27].

3. Results and Discussion

3.1 The interaction of 5NU with ds-DNA at HMDE

The interaction of 5NU with ds-DNA was investigated by CV and DPSV at the HMDE surface. By investigating the effect of pH value on the reaction of 5NU with ds-DNA, it was found that in the pH range from 3.0 to 12.0, the addition of ds-DNA to 5NU solution and subsequent scanning over the potential range produced no new peaks, but a decrease in reduction peak current with a big shift of the peak potential to a negative direction was observed (Fig.2). As reported previously, the shifted of the peak potential of CV waves to more positive or negative values, indicating that the action of 5NU with ds-DNA may be intercalation [30]. Strong interaction are observed at pH 3.0 and 10.0 phosphate buffer compared to media with other pH values. The latter behaviour reflects that the protonated and anionic species of 5NU are strongly interacted with ds-DNA.

According to these observations, it seems that the decreases of peak currents of 5NU after an addition of excess ds-DNA are caused by the intercalation of 5NU to the bulky, slowly diffusing ds-DNA, which results in a considerable decrease in the apparent diffusion coefficient. We suppose this intercalation masks the electroactive site of 5NU and in a way reduces the equilibrium concentration of 5NU. This behaviour confirmed by the dependence of the 5NU process at the HMDE on the scan rate (ν), in the absence and presence of an excess ds-DNA. The peak currents obtained for 5NU and 5NU-ds-DNA adduct show linear dependence on the square root of the scan rate up to 500 mV s^{-1}, indicating diffusional mass transport of the electroactive species to the HMDE surface at lower scan rate [31]. Furthermore, the slope of the linear of I_{pc} *versus* $\nu^{1/2}$ without ds-DNA was more than that with ds-DNA (Fig. 3), suggesting the diffusion coefficient of the free form of 5NU (D_f) was larger than that of the complexed form of 5NU with ds-DNA (D_b) as shown in Table 1.

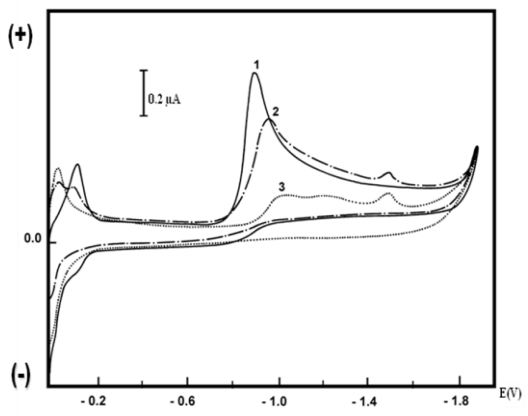

Figure 2.

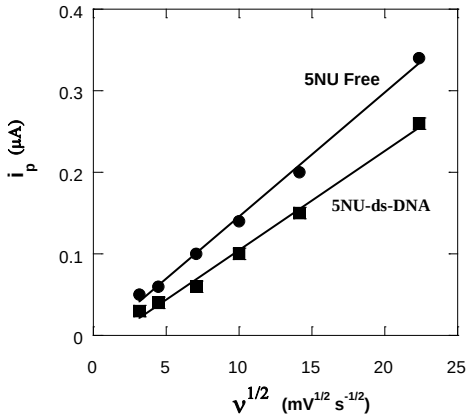

Figure 3.

3.2 The interaction of 5NU with ds-DNA at GCE

The interaction of 5NU with ds-DNA was also investigated by CV and DPSV at GCE. Typical differential pulse voltammetric behaviours of 5NU in the absence, and presence, of ds-DNA at the GCE are shown in Fig. 4. By investigating the effect of pH value on the reaction of 5NU with ds-DNA at GCE, it was found that in the pH range from 3.0 to 12.0, the addition of ds-DNA to 5NU solution and subsequent scanning produced no new peaks, but caused the peak current diminish considerably with a large shift of the peak potential to a negative direction. Strong interaction are observed in the acidic media of pH 3.0 phosphate buffer compared to media with other pH values. The latter behaviour reflects that the

protonated species of 5NU are strongly interacted with ds-DNA at GCE. Moreover, there is no significant obstruction of the glassy carbon surface via adsorption of DNA, as opposed to mercury electrodes, where adsorption of DNA at negative potentials is significant [32]. On one hand, the intercalation of 5NU into a large DNA double helix might increase the steric hindrance to the heterogeneous electron transfer. On the other hand, intercalation might induce some overlap between the large π-bond of the pyrimidine moiety of 5NU and the stacked base planes of ds-DNA [33]. The probable explanation of these experimental phenomena is that 5NU intercalated to ds-DNA forms an electroinactive complex with its diffusion coefficient decreasing responsible for the decrease of peak currents [34]. This behaviour confirmed by the dependence of the 5NU process at the GCE on the scan rate (ν), in the absence and presence of an excess ds-DNA. The slope of the linear of I_{pc} *versus* $\nu^{1/2}$ without ds-DNA was more than that with ds-DNA, suggesting the diffusion coefficient of the free form of 5NU (D_f) was larger than that of the complexed form of 5NU with ds-DNA (D_b) as shown in Table 1.

Table 1: Diffusion coefficients of 5NU and 5NU-ds-DNA complex calculated from the results of CV using the slope of the linear of i_p *versus* $\nu^{1/2}$ in absence and presence of ds-DNA at the HMDE and GCE at pH 3.0.

Electrode	Slope ($nA/mV^{1/2}\ s^{-1/2}$)		D_f ($cm^2\ s^{-1}$)	D_b ($cm^2\ s^{-1}$)
Species	5NU	5NU-ds-DNA	5NU	5NU-ds-DNA
HMDE	15.2	12.1	$1.87x10^{-7}$	$1.18x10^{-7}$
GCE	52.4	25.6	$2.22x10^{-6}$	$5.30x10^{-7}$

3.3 Calculation of the Binding constant (K)

Current titrations were performed by keeping the constant concentration of the 5NU while varying the concentrations of ds-DNA at HMDE or GCE using DPSV at pH 3 (Fig's. 4 and 5). The interaction of 5NU with ds-DNA can be described using the following equation:

$$5NU + \text{ds-DNA} \leftrightarrow 5NU\text{-ds-DNA} \qquad (1)$$

An equation for amperometric titration can be deduced according to references [9,35]. The current titration equation was described as follows

$$\log\frac{1}{[\text{ds - DNA}]} = \log K + \log\frac{I_{H\text{-}G}}{I_G - I_{H-G}} \qquad (2)$$

Where K is the apparent binding constant, I_G and I_{H-G} are the peak current of the free guest (G; here free 5NU) and the host-guest complex (H-G; 5NU intercalated into ds-DNA), respectively. Under the assumption of reversible, diffusion-controlled electron transfer and the complex of 5NU with ds-DNA (in nucleotide phosphate) is a 1:1 association complex, then the plot of log 1/[ds-DNA] vs. log I_{H-G}/I_G-I_{H-G} becomes linear with the intercept of log K. Based on the variations in the currents at the HMDE and GCE (Fig's. 4 and 5), the binding constants of these complexes were evaluated according to equation (2) and the results are listed in Table 2. The results indicate the large binding constant for 5NU-ds-DNA adduct at GCE than that obtained at HMDE.

The change in Gibbs free energy, ΔG°, is an important parameter which reflects the stability of the formed adduct. ΔG° can be derived from equation (3):

$$\Delta G° = -RT \ln K \qquad (3)$$

Where the *R* is the universal constant (8.31 $J\ K^{-1}\ mol^{-1}$), *T* the absolute temperature, and K the binding constant. The ΔG° values for the binding of 5NU with the ds-DNA were calculated at

both HMDE and GCE (Table 2). These values are negative indicating that the binding process is favourable and spontaneous.

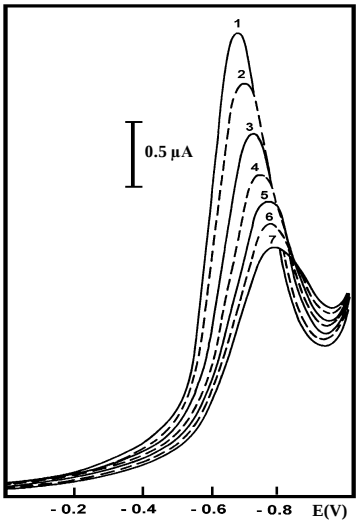

Figure 4.

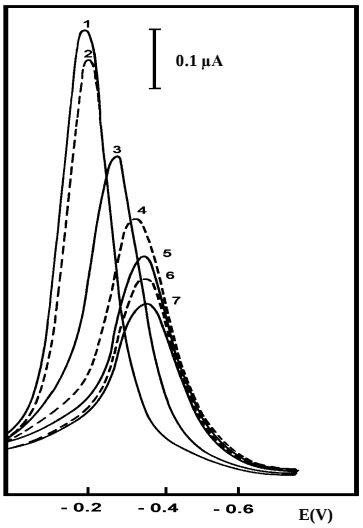

Figure 5.

Table 2: Binding constants of 5NU-ds-DNA complex calculated from the results of DPSV at the HMDE and GCE at pH 3.0.

Electrode	K (M^{-1})	$-\Delta G$ (kJ mol^{-1})
HMDE	$1.45x10^5$	29.46
GCE	$2.65x10^5$	30.95

3.4 Effect of Cu(II) on the interaction of 5NU with ss- or ds-DNA at HMDE

The differential pulse stripping voltammograms of 5NU, 5NU-DNA, and 5NU-DNA-Cu at pH 3.0 are shown in Fig's 6A and 6B. As increasing the ss- or ds-DNA concentration at constant 5NU concentration, a decrease in reduction peak current with a big shift of the peak potential to a negative direction was observed. This means that, an interaction of 5NU molecules with both ss- and ds-DNA was observed. On the other hand, the addition of Cu(II) ions decrease the interaction between 5NU and ss-DNA and a positive potential shift was observed (Fig. 6A), indicating that the action of 5NU with ss-DNA may be electrostatic interaction by which 5NU molecules bind electrostatically to the negatively charged deoxyribose-phosphate backbone of DNA. However, the interaction of 5NU with ds-DNA approximately unaffected by the addition of Cu(II) ions as show in Fig. 6B. This behaviour can confirm the suggested interaction mode of 5NU molecules with ds-DNA by intercalation (by which 5NU intercalated into the double helix of DNA). We suppose this intercalation masks 5NU molecules and in a way reduces the interaction between Cu(II) and 5NU. From these characteristics, it is apparent that intercalative and groove bindings are related with the grooves in the DNA double helix while the electrostatic binding can take place out of the groove or on the surface of the DNA molecule. Thus distinguish between the interaction mode of ss-, and ds-DNA can be achieved in presence of copper ions through their interaction with 5NU.

3.5 Linear Range of DNA Determination

The decrease in peak current of 5NU resulted from the addition of ss-DNA or ds-DNA into the 5NU solution can be employed to determine the concentration of ss-DNA or ds-DNA. The peak current of the DPSV peak of 5NU was used as the detection signal. Under the optimum experimental conditions of Fig's. 4 and 5, the decreases in the DPSV peak current were linearly related to ss-DNA or ds-DNA concentration in the range of 0 – 30 μM when the 5NU concentrations were fixed at $2.0x10^{-5}$ M, with detection limits of $1.23x10^{-6}$ M for ds-DNA and $2.66x10^{-7}$ M for ss-DNA. The procedure to handle a sample takes only 15-20 min. The proposed method is simple, sensitive and rapid and hence can be applied to the determination of many kinds of DNA.

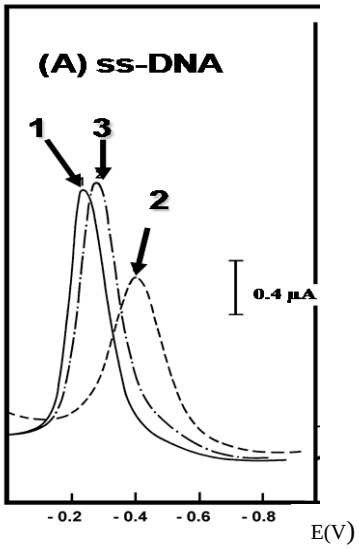

Figure 6A.

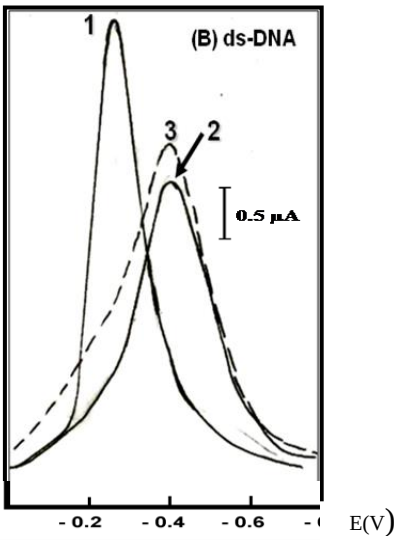

Figure 6B.

3.6 The nature of 5NU interacting with DNA

This work has shown experimental evidence of interaction of 5NU with DNA at HMDE and GCE, and may contribute to the understanding of the mechanism of action of this drug with ss- and ds-DNA in absence and presence of copper ions. In comparison to the HMDE, 5NU displayed high affinity for ds-DNA at the GCE. The large binding constant (K) observed for 5NU at GCE in compared to that at HMDE (Table 2) is attributed to a mode of binding that involves a strong stacking interaction between the 5NU chromophore and the base pairs of ds-DNA, i.e. the 5NU chromophore is in close proximity to the ds-DNA bases at the GCE surface. The results presented here show also that, the distinguish between the interaction mode of ss-, and ds-DNA can be achieved in presence of copper ions through their interaction with 5NU. The voltammetric changes unequivocally suggest that 5NU is intercalated into the base-stacking domain of the ds-DNA double helix, however, the interaction mode of 5NU molecules with ss-DNA is mainly by electrostatic (by which 5NU bind electrostatically to the negatively charged deoxyribose-phosphate backbone of DNA). It has been reported that the intercalation of DNA with small molecules commonly has stronger strength than electrostatic action [36], thus, the 5NU molecules should have stronger affinity with ds-DNA than ss-DNA. This results are consistent with that reported previously by Ortiz M. et al. [37] for the single-stranded DNA interaction with methylene blue using spectroscopic technique. In summary, the interaction is correlated to the planarity, hydrophobicity and electrostatic component of 5NU and the main effect on the interaction is the intercalation (by hydrophobic interaction) of 5NU to DNA. These investigation are of potential importance in understanding the mechanism of interaction and recognition of the drugs in the living body.

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The Figure Captions

Figure 1. Structure of the 5-nitrouracil**.**

Figure 2. Cyclic voltammograms of $3.85x10^{-5}$ M 5NU in the (1) absence and presence of (2) $2.20x10^{-6}$ and (3) $4.55x10^{-5}$ M ds-DNA. Supporting electrolyte: phosphate buffer pH 10, scan rate 200 mV s^{-1} at HMDE.

Figure 3. Relationship between the CV cathodic peak current (i_{pc}) at pH 3.0 of $3.85x10^{-5}$ M 5NU in the (•) absence and (■) presence of $2.26x10^{-5}$ M ds-DNA and the square root of scan rates ($\nu^{1/2}$).

Figure 4. Differential pulse voltammograms of $2.0x10^{-5}$ M 5NU in the absence (1) and presence of (2) 0.266; (3) 0.522; (4) 0.768; (5) 1.45; (6) 2.64 and (7) 32.7 μM ds-DNA. Scan rate 20 mV s^{-1}, Es= 0.0 V and pulse amplitude 100 mV. Supporting electrolyte: phosphate buffer pH 3.0, at GCE.

Figure 5. Differential pulse voltammograms of $2.0x10^{-5}$ M 5NU in the absence (1) and presence of (2) 1.23; (3) 2.26; (4) 3.36; (5) 10.40; (6) 19.30 and (7) 28.90 μM ds-DNA. Scan rate 20 mV s^{-1}, E_s= 0.0 V and pulse amplitude 100 mV. Supporting electrolyte: phosphate buffer pH 3.0, at HMDE.

Figure 6. (A): Differential pulse voltammograms of: (1) $1.0x10^{-5}$ M 5NU; (2) $1.0x10^{-5}$ M 5NU + 10 ppm ss-DNA (3) $1.0x10^{-5}$ M 5NU + 10 ppm ss-DNA + $1.0x10^{-5}$ M Cu(II); **(B):** Differential pulse voltammograms of: (1) $2.0x10^{-5}$ M 5NU; (2) $2.0x10^{-5}$ M 5NU+0.266 μM ds-DNA; (3) $2.0x10^{-5}$ M 5NU+ 0.266 μM ds-DNA +$1.0x10^{-5}$ M Cu(II). Scan rate 20 mV s^{-1}, Es= 0.0 V and pulse amplitude 100 mV. Supporting electrolyte: phosphate buffer pH 3.0, at HMDE.