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Electrochemical studies of danthron and the DNA–danthron interaction

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Abstract

Danthron is an important natural occurring component in laxative drugs. In this paper, electrochemical investigation of danthron and its interaction with DNA is reported. Via the electrochemical approach assisted by ultraviolet–visible (UV–Vis) spectroscopy, we have proved that danthron intercalates into DNA strands forming some nonelectroactive complexes, which results in the decrease of redox peak currents of danthron. In addition, the decrease of the peak currents is proportional to the concentration of DNA. The difference between the interaction of danthron with double-stranded DNA (dsDNA) and with single-stranded DNA (ssDNA) has also been studied. This character implies the potential of danthron to discriminate dsDNA and ssDNA.
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Keywords: Danthron; Electrochemistry; DNA; UV–Vis spectroscopy; Intercalation

1. Introduction

Hydroxyanthraquinones, present in laxatives, fungi imperfecti, Chinese herbs and possible vegetables, are of great importance in phytochemistry and pharmacy. Most of them exist as pharmacologically inactive glycosides in plant extract which can be activated [1]. Danthron (1,8-dihydroxy-anthraquinone), one kind of naturally occurring anthraquinones, is an active principle in many plant-derived drugs, such as laxatives form senna (*Cassia senna*), aloe and frangula bark (*Rhamnus frangula*) [2]. Many analytical methods have been established for detecting varieties of anthraquinone derivatives, such as HPLC, reversed-phase HPLC (RP-HPLC), capillary chromatography, liquid chromatography-mass spectrometry (LC-MS), etc. [3–5]. Because of the remarkable oxidation–reduction properties of anthraquinones, electrochemical methods may be employed in the analysis for convenience. In this paper, the detailed electrochemical property of danthron has been well studied and the analytical protocol is simple and sensitive.

Meanwhile, the interaction of small molecules with nucleic acids is an actively investigated aspect. Some work has been contributed to illustrating the interaction of some organic chemicals with DNA [6–8]. Lately, the investigation of the interaction between a component of traditional Chinese herbal medicine and DNA has also been well studied [9]. Recently, much attention has been drawn to the carcinogenic potent of anthraquinones according to their supposed interaction with nucleic acids. It has been known that danthron can cause an increased rate of intestinal tumors in rats and adenomatous hyperplasias with cystic glands of the caecum and liver tumors in mice under certain conditions [10,11]. An increased relative risk for colorectal cancer in humans has also been reported among users of 1,8-dihydroxy-anthraquinone containing laxatives [12,13]. Furthermore, some researchers have proved that danthron will induce chromosomal damage in human lymphocytes in vitro or in V79 cells [14,15]. In this work, the mechanism of the interaction between danthron and DNA is elucidated through electro-

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chemical technique supported by UV–Vis method. The results have demonstrated the carcinogenic potential of danthron, and the danthron–DNA binding parameters may provide some helpful information about the appropriate dose of danthron in laxatives.

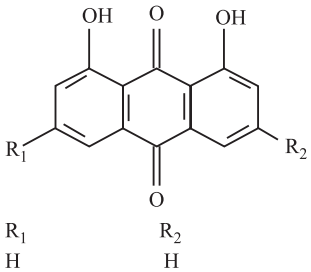
2. Experimental

2.1. Reagents

Danthron (1,8-dihydroxy-anthraquinone, Scheme 1) was synthesized by Shanghai NO. 1 pharmaceutical plant. Its stock solution was prepared by dissolving known amounts of danthron in 10 ml ethanol, with the final molecular concentration of 1.0×10^{-3} M. Fish testes DNA (sodium salt) was purchased from Amresco. The purity of the DNA solution (10 mM Tris–HCl/1 mM EDTA, pH 8.0) was measured by UV–Vis absorption, which produced A_{260}/A_{280} of 1.8–1.9, suggesting that the DNA sample was free of proteins. The stock solution of DNA (ca. 1.0 mg/ml) was stored at 4 °C. Denatured ssDNA was produced by heating a dsDNA solution in a water bath at 100 °C for 5 min, immediately followed by rapid cooling in an ice bath. Other reagents used were of analytical grade. Triply distilled water was used in all experiments.

2.2. Apparatus

Cyclic voltammetric experiments (CV) were performed on a PAR 263 Potentiostat/Galvanostat (EG&G, USA). A three-electrode configuration was employed. The working electrode was a pyrolytic graphite (PG) disk electrode (A=5.35 mm^2). The substrate of PG electrode was first polished using rough and fine sand papers, and then polished to mirror smoothness with alumina (particle size of about 50 nm)/water slurry on silk. After that, it was ultrasonicated in water and ethanol for about 2 min, respectively. A saturated calomel electrode (SCE) was used as the reference electrode and all potentials reported here were referred to this electrode. A platinum wire electrode served as the counter electrode. UV–Vis absorption spectroscopy was performed at a UV-1601 spectrophotometer (Shimadzu, Japan).



Scheme 1. The molecule structure of danthron (1,8-dihydroxy-anthraquinone).

3. Results and discussion

3.1. Electrochemical behavior of danthron

As shown in Fig. 1, with the addition of danthron to the buffer solution, danthron gives rise to a pair of well-defined reversible peaks, with the anodic and cathodic peak potentials (E_{pa}, E_{pc}) at about −317.0 and −337.2 mV, respectively. The apparent standard potential ($E^{0'}$) of danthron in pH 2.2, 0.05 M glycine–HCl buffer, at the scan rate of 100 mV/s, has been calculated to be −327.1 mV, estimated from its midpoint potential ($E_{1/2}$=(E_{pc}+E_{pa})/2). The peak currents, both anodic and cathodic, have been proven to be linearly proportional to the scan rates (Fig. 2), which indicates that the electrode reaction of danthon is controlled by the adsorption process [16]. The linear equations for the anodic and cathodic peak currents are y=1.9625−0.031x, R=0.9969 and y=−1.7715+0.0289x, R=0.9962, respectively.

The solution pH significantly alters the apparent standard potential of danthron. In alkaline condition, the peak currents of danthron decrease with a larger peak separation than that in acidic media. The electron transfer process of danthron has proven that the uptake of electron is accompanied by an equal number of protons, according to the slope ($\Delta E/\Delta$pH) ca. 49.18 mV/pH ($E^{0'}$=−49.18 pH−252.54), approximately to the Nernstian value of −59 mV.

3.2. DNA–danthron interaction

Above studies reveal that danthron can exhibit fine electrochemical response. On the other hand, it has a supposed planar structure. Therefore, danthron may interact with DNA and can be well studied through electrochemical technique. As shown in Fig. 3, a pair of stable redox peaks of danthron can be obtained upon repetitive potential scans; however, significant decrease of its peak currents is observed after the addition of dsDNA. With the presence of dsDNA, there is no appearance of new redox peaks and no shift of the peak potentials. Note that the peak currents decrease along with the increase of the dsDNA concentrations.

There are several probable mechanisms responsible for the peak attenuation while almost no change of peak potential. One possible explanation is based on the competitive adsorption between danthron and DNA, since the competitive adsorption between DNA and danthron on the electrode surface can induce the currents decrease of danthron [17]. The surface coverage (θ) is a useful criterion for measuring the molecular ratio adsorbed on the electrode. In addition, the value of θ can be gained

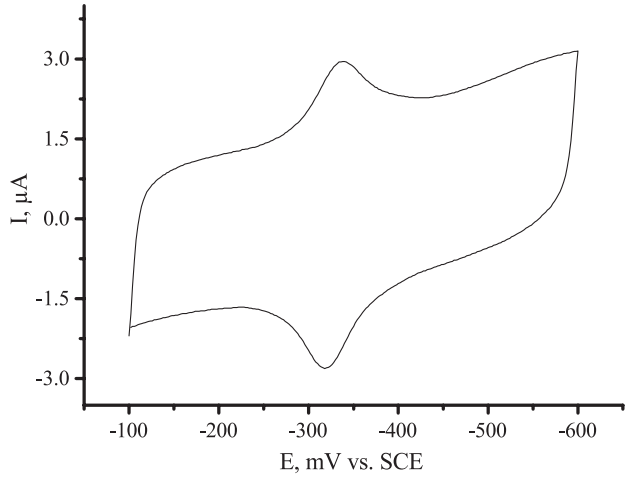


Fig. 1. Cyclic voltammogram of 4.0 μM danthron in 0.05 M glycine–HCl buffer with pH 2.2. Accumulation time: 2 min; scan rate: 100 mV/s.

from the ratio of the molecules adsorbed on electrode at different accumulation times (Γ, Γs). According to the following equation (Eq. (1)), we can obtain the value of Γ [6].

$$\Gamma = nFAQ \tag{1}$$

$$\theta = \Gamma/\Gamma s \tag{2}$$

In this system, danthron needs 5 min (t_s) to reach its saturation adsorption. The corresponding reduction charge (Qs), obtained by calculating the reduction peak area of voltammogram, is 32.65 μC. At accumulation time (t_a) of 2 min before DNA is added into the danthron solution, the reduction charge (Q) is 11.24 μC. Based on Eq. (2), θ=Q/Qs can be deduced, where A stands for the electrode area; Γ and Γs are the number of molecules adsorbed on electrode within t_a and t_s, respectively. The surface coverage of danthron θ, at 2 min, is

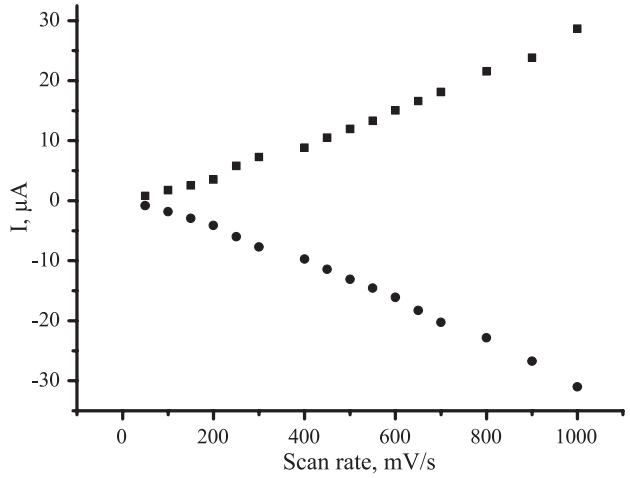


Fig. 2. Plot of the anodic (dots) and cathodic (squares) peaks currents versus scan rates. Other conditions are the same as in Fig. 1.

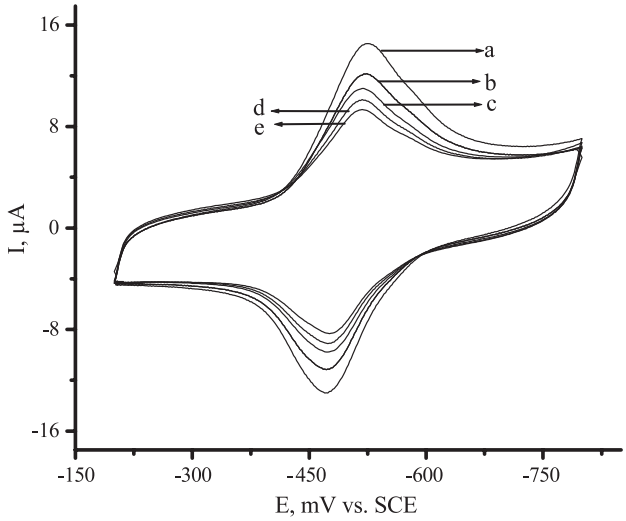


Fig. 3. Cyclic voltammograms of 4.0 μM danthron in the absence (a) and presence of (b) 50.0 μg/ml; (c) 100.0 μg/ml; (d) 150.0 μg/ml; (e) 200.0 μg/ml dsDNA in 0.2 M NaAc–HAc buffers with pH 5.0. Other conditions are the same as in Fig. 1.

calculated to be 0.344, which is far from forming a monolayer of danthron at PG surface. Therefore, at the time of 2 min to add DNA into the danthron solution, there are sufficient adsorption sites on the electrode surface for the adsorption of DNA. Thus, the phenomenon of competitive adsorption between DNA and danthron is not remarkable. It can be concluded that competitive adsorption between DNA and danthron cannot fully account for the significant drop of the peak currents at such a low surface coverage.

Another possibility is that the presence of DNA may change the electrochemical kinetics of the electroactive molecules. The major electrochemical kinetic parameters of danthron, either in the absence or in the presence of dsDNA, can demonstrate whether DNA influences the electrochemical kinetics of danthron or not. The electron transfer coefficient α and the standard rate constant k_s can be determined via the theory of Laviron and his co-workers [16,18,19], from Eq. (3) as follows,

$$E_{Pa} = E^{0\prime} - \frac{RT}{(1-\alpha)nF}\ln\frac{RTk_s}{(1-\alpha)nF} + \frac{RT}{(1-\alpha)nF}\ln v \tag{3}$$

where v stands for the scan rate; R, T, F have normal meanings. Plot of E_{pa} vs. ln$v$ produces a linear range at high scan rates; α has been estimated to be 0.38 for the danthron redox reaction in the absence of dsDNA. k_s can be calculated according to Eq. (4) (valid when $n\Delta$Ep <200 mV),

$$k_s = \alpha nFv/RT \tag{4}$$

from which k_s has been determined to be 3.13 s^{-1} (at a scan rate of 100 mV/s). Similarly, α and k_s for the

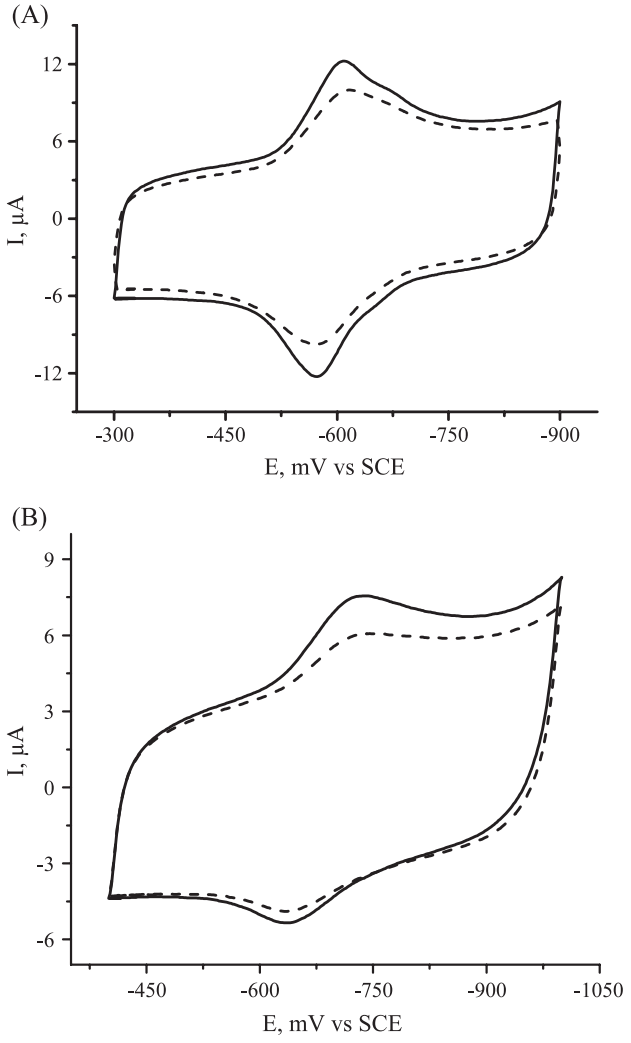


Fig. 4. Cyclic voltammograms of 4.0 μM danthron in the absence (solid line) and presence of dsDNA (dash line) at two different pH buffers: (A) pH 7.0, 0.2 M Na_2HPO_4–NaH_2PO_4 buffer; (B) pH 9.0, 0.05 M glycine–NaOH buffer.

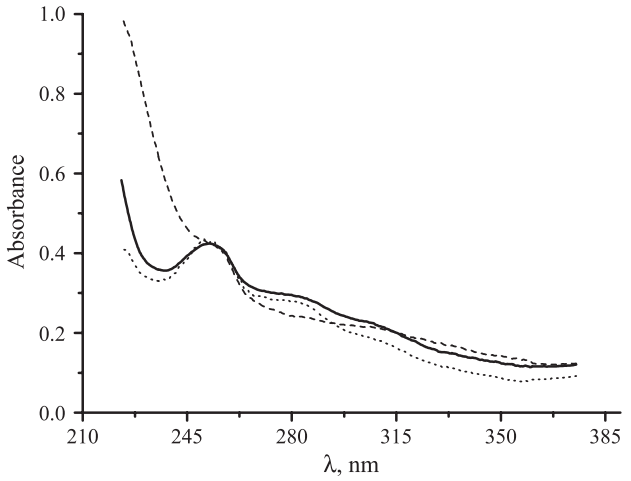


Fig. 5. UV absorbance spectra of 0.8 μM danthron in the absence (solid line) and presence of 40.0 μg/ml dsDNA/ssDNA (dash line/dot line) in 0.2 M NaAc–HAc buffers with pH 5.0. In order to compare directly, the absorbance of DNA has been subtracted in the spectra for danthron of dsDNA/ssDNA.

danthron redox reaction in the presence of dsDNA have been calculated to be 0.34 and 2.80 s^{-1}, respectively. Obviously, the presence of dsDNA does not significantly alter the kinetics of the danthron redox reactions at PG electrode surfaces.

The third possible explanation is electrostatic attraction between danthron and DNA. We have observed that the peak currents decrease with the addition of DNA under acidic, neutral and alkaline media (Figs. 3 and 4). So, electrostatic charge of danthron does not affect its interaction with DNA. Thus, the possibility of electrostatic attraction can be excluded.

Finally, we propose the most plausible mechanism for the binding and complexation between danthron and DNA. UV–Vis absorption spectrum provides potent evidence for

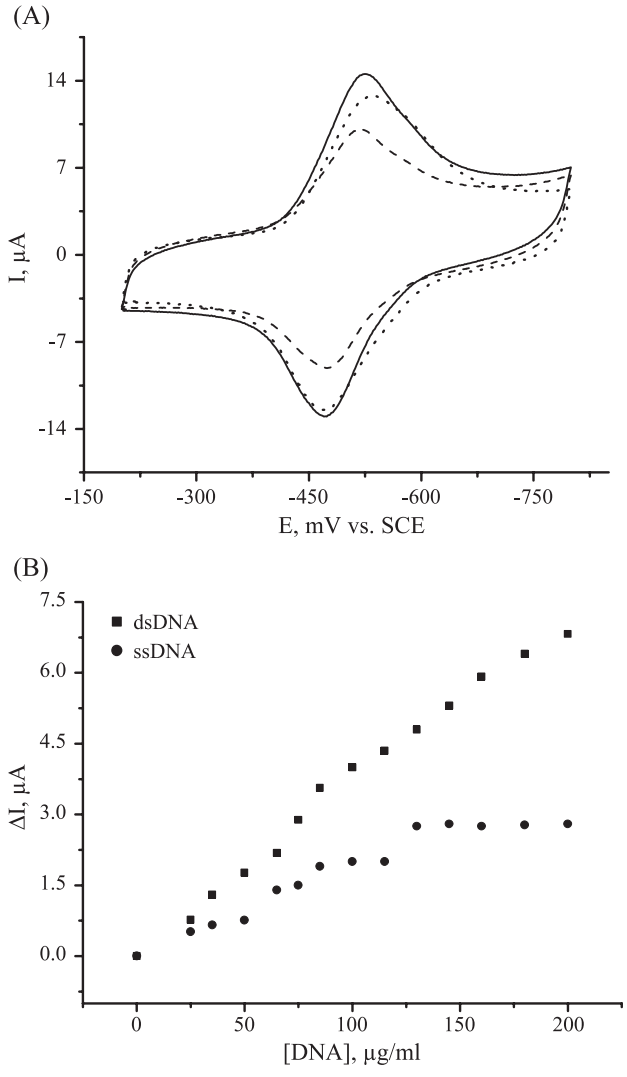


Fig. 6. Effects of dsDNA and ssDNA on the electrochemistry of danthron. (A) CV curves for 4.0 μM danthron in the absence (solid line) and presence of 200.0 μg/ml dsDNA/ssDNA (dash line/dot line) in 0.2 M NaAc–HAc buffers with pH 5.0. (B) Plot of the decrease of the peak currents in the presence of DNA (ΔI) versus the concentration of either dsDNA (squares) or ssDNA (dots). Other conditions are the same as in Fig. 1.

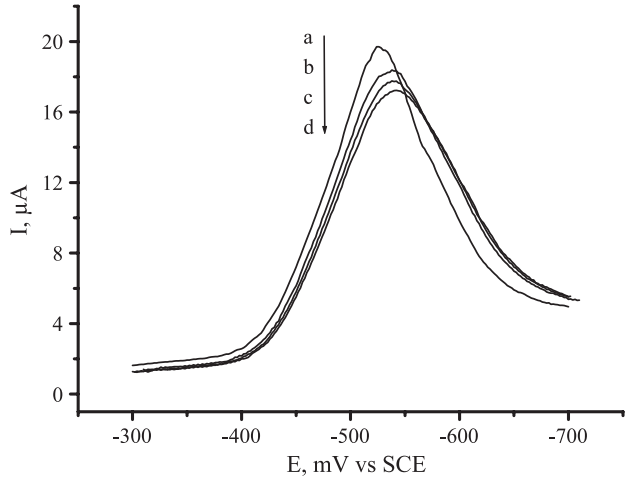


Fig. 7. Cathodic peaks of 6.0 μM danthron in the absence (a) and presence of (b) 100.0 μg/ml; (c) 150.0 μg/ml; (d) 200.0 μg/ml ssDNA in 0.2 M NaAc–HAc buffers with pH 5.0. Other conditions are the same as in Fig. 1.

possible intercalation of danthron (Fig. 5). As shown in UV–visible spectra, in the ultraviolet range, danthron exhibits a maximal absorbance peak wavelength (λ_m) at 253 nm and a secondary one at 283.6 nm. There is no apparent change on the maximal absorption peak of danthron upon the addition of dsDNA. However, a notable decrease of the secondary absorbance peak at 283.6 nm has been observed, which proves the existence of binding between danthron and DNA [20]. Comparatively, the UV–Vis absorption spectrum is much distinct with the addition of ssDNA. The peak value at 283.6 nm diminishes a little. The different change of absorbance peak with the addition of dsDNA and ssDNA, respectively, indicates the variant interaction modes between danthron with dsDNA and with ssDNA.

Meanwhile, experimental results reveal that the electrochemical behavior of danthron is different with the addition of dsDNA and ssDNA. As demonstrated in Fig. 6a, the peak currents of danthron diminish in both cases. However, the peak currents decrease more sharply with the addition of dsDNA than with ssDNA, because denatured ssDNA has much ruleless structure that has the weaker potential on the intercalation with dathron (Fig. 6b). Further studies have also demonstrated the discrimination between ssDNA and dsDNA (Fig. 7). According to the previous studies of Bard and Millian [21–23], the negative shift of apparent standard potential should be attributed to electrostatic interaction. Therefore, there exists electrostatic interaction between ssDNA and danthron, and danthron can exhibit nice discrimination between dsDNA and ssDNA.

4. Conclusion

In this work, the electrochemical property of danthron and the DNA–dathron interaction is investigated. Danthron and DNA can form a nonelectrochemical complex that induces the decrease of the electrochemical response, due to the danthron binding to DNA by intercalation with its planar structure insertion between adjacent base pairs of DNA duplex strand. It provides the possible mechanism to the carcinogenic potential of danthron, which should be utilized cautiously. Moreover, danthron exhibits a nice ability to discriminate dsDNA from ssDNA, making it a candidate for "electrochemical" indicator in detecting DNA hybridization events.

Acknowledgements

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