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Electrochemical behavior of gallic acid interaction with DNA and detection of damage to DNA

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ABSTRACT

Electrochemical behavior of gallic acid (GA) and interaction with calf thymus DNA were explored with cyclic voltammetry and differential pulse voltammetry (DPV) in acetate buffer solution using a glassy carbon electrode (GCE) and a DNA modified GCE (DNA/GCE), respectively. A pair of redox peaks of GA appeared in the range of $-0.05 \sim +0.55$ V. The anodic peak potential (E_{pa}) was at +0.329 V and the cathodic peak potential (E_{pc}) was at +0.211 V. The oxidation peak potential of GA was dependent on pH of solution. With adding DNA into GA solution, the peak current value of GA decreased gradually, and the peak potential shifted positively. The electrochemical parameters (diffusion coefficient D, electron transfer coefficient α, and standard rate constant k_s) of free GA and binding compounds were obtained. The peak current of GA increased with the time of DNA/GCE being immersed in the GA solution. The results showed that GA could interact with DNA molecule by intercalation mode. The GA could mediate the double stranded DNA (ds-DNA) in situ damage, which was directly detected according to the anodic signal of the DNA purine bases with DPV.

1. Introduction

Gallic acid (GA; 3, 4, 5-trihydroxybenzoic acid), an important constituent of traditional Chinese medicine and black tea, is widely distributed in fruits and plants [1]. GA and its esters have a diverse range in industrial uses, such as additives in food and in cosmetics [2]. It is well known to show strong natural antioxidant [3] and has been pharmacologically active as an antiallergic, antimutagenic, anticarcinogenic, antiatherosclerotic, and antiinflammatory agent in a variety of in vivo and in vitro studies [4,5]. For example, it can selectively induce cell death via apoptosis in certain types of tumor cell lines (e.g. hepatocellular carcinoma, leukemia, and lung cancer cells) [6]. Besides, GA may also act as a prooxidant. Sakagami and Satoh [7] showed that GA acts as prooxidant in the induction of apoptotic cell death in human glioblastoma cells. It is also described as having a cytotoxic effect on isolated hepatocytes [8]. However, the detailed mechanism responsible for this effect of GA was not very understood.

The interaction between DNA and other molecules is important in life sciences and has attracted considerable interest, because it is related to the replication and transcription of DNA in vivo, mutation of genes and related variations of species in character, action mechanism of DNA-targeted drugs, etc. A variety of methods, such as gel electrophoresis [9], footprinting technique, X-ray crystallography [10], fluorescence [11], UV/visible spectroscopy [12], NMR [13] and so on, have been used to investigate this interaction. Recently, electrochemical method has also been applied to explore the interaction of DNA with redox-active molecules and represents a dynamically developing field [14–17]. This is mainly due to high sensitivity, relatively low cost, direct monitoring and simplicity [18]. Moreover, investigation of the electrochemical behavior of small molecule compounds by means of electrochemical techniques has the potential for providing valuable insights into the redox reaction of these molecules in living body.

In the present paper, we described a voltammetric study of the GA interaction with calf thymus DNA using GCE and DNA/GCE. The interaction of GA with DNA, produced a GA–DNA complex, which resulted in a decrease of peak current of GA, and a positive shift of the peak potential. The aim of this work was quantitative evaluation of the binding of GA with DNA in solution as well as the modified electrode surface and the damage to DNA. The research provided a convenient and sensitive method to explore the mechanism of electro-active compounds interacting with DNA and mediating DNA damage.

2. Experimental

2.1. Chemicals and Apparatus

Calf thymus DNA (sodium salt, type I) was purchased from Sigma and used without further purification. Stock solutions of

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DNA were prepared by dissolving an appropriate amount of the DNA in double-distilled water and were stored at 4 °C and used not more than 5 days. GA was received from Acrōs organics. The stock solution was prepared by directly dissolving it in ethanol-water (oxygen being removed) and diluted with buffer solution according to the demands. A 0.10 mol L^{-1} pH 4.5 acetate buffer solution was prepared using analytical grade reagents. Double-distilled water was used throughout.

All voltammetric measurements were carried out using a CHI440A electrochemical analyzer (Shanghai Chenhua Apparatus Corporation, China). The experiments were performed using a 5 ml one-compartment electrochemical cell with a three electrode system, which consisted of GCE or DNA/GCE as a working electrode, a platinum wire auxiliary electrode and a saturated calomel electrode (SCE) as reference electrode. All experiments were performed at room temperature.

2.2. Preparation of DNA/GCE

The DNA/GCE (Φ = 3 mm) was prepared as follows: a GCE was polished using a piece of 1500 diamond paper, and then polished to mirror smoothness with about 0.05 um alumina water slurry on silk. Afterward, it was washed with absolute alcohol and double-distilled water in an ultrasonic bath to remove the adsorbate on the electrode. Afterward, electrode was electrochemically pretreated (as described by Wang et al. [19]) and then dried in air. A 20.0 ul DNA solution (4.0 mg mL^{-1}) was dropped onto the surface of the clean GCE and solvent was evaporated at 4 °C for 6 h. In order to remove the excess of DNA on GCE, the DNA/GCE was immersed the blank acetate buffer solution for 15 min, and then gently rinsed with double-distilled water.

2.3. Determination of DNA damage

The deposit potential (E_d) was adjusted and started after DNA/GCE was immersed in the reagent solution (containing GA or not) for 15 min. After a given time, the circuit was opened. The DNA/GCE was taken out and transferred into the background electrolyte in an ordinary electrochemical cell and then DPV experiments were performed to observe the voltammetric curve changes of the DNA/GCE after GA was deposited.

3. Results and discussion

3.1. Electrochemical behavior of GA

The typical cyclic voltammogram of 7.30×10^{-5} mol L^{-1} GA in 0.10 mol L^{-1}, pH 4.5 acetate buffer solution is shown in Fig. 1a. A pair of redox peaks appeared using a bare GCE in the range of −0.05 to +0.55 V (vs. SCE). The anodic peak potential (E_{pa}) was at +0.329 V, which corresponds to the oxidation of phenolic hydroxyl group of GA, and the cathodic peak potential (E_{pc}) at +0.211 V with a scan rate of 100 mV s^{-1}. The oxidation peak current of GA was obviously much higher than the reduction peak current. The separation between the anodic and cathodic peak potential ($\Delta E_p = |E_{pc} - E_{pa}| = 118$ mV at 100 mV s^{-1}) and the current ratio of the anodic peak current to the cathodic one ($I_{pc}/I_{pa} \approx 0.19$) indicated that the electrochemical process of GA at a bare GCE was quasi-reversible.

The oxidation peak potential of the phenolic hydroxyl group of GA varied linearly with increasing pH value of solution, resulting in a slope which corresponds to 61 mV per pH unit (data not show). It could be inferred that the oxidation procedure of GA related to H^+ ions of solution and the same number of the protons and electrons participated in the oxidation process of GA. This result was similar

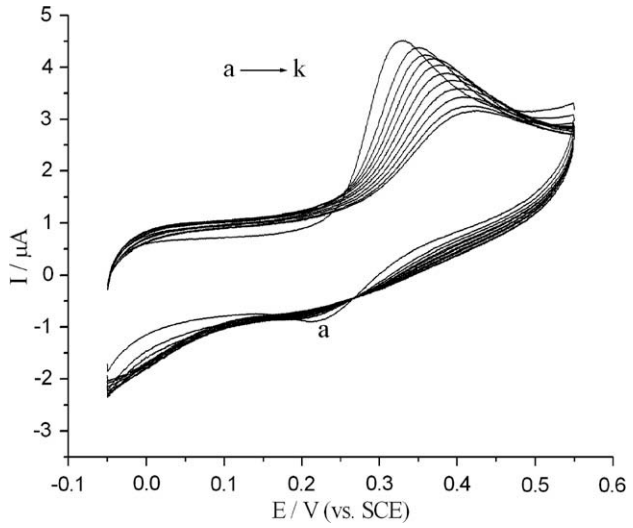


Fig. 1. Cyclic voltammograms of 7.30×10^{-5} mol L^{-1} GA at a bare GCE in the absence and presence of DNA: (a) 0; (b) 0.04; (c) 0.08; (d) 0.12; (e) 0.16; (f) 0.20; (g) 0.24; (h) 0.28; (i) 0.32; (j) 0.36; and (k) 0.40 (mg mL^{-1}) in 0.10 mol L^{-1}, pH 4.5 acetate buffer solution. Scan rate: 100 mV s^{-1}.

to previous work reported by Gunckel et al. [20]. Furthermore, it was observed that the oxidation peak current of GA (I_{pa}) varies linearly with v (scan rate) rather than $v^{1/2}$ (data not shown). The result indicated that the electrode process was controlled by adsorption step. According to the data obtained in cyclic voltammetry experiments and the following formula [21]:

$$I_p = n^2F^2A\Gamma v/4RT. \quad (1)$$

Because

$$Q = nFA\Gamma, \quad (2)$$

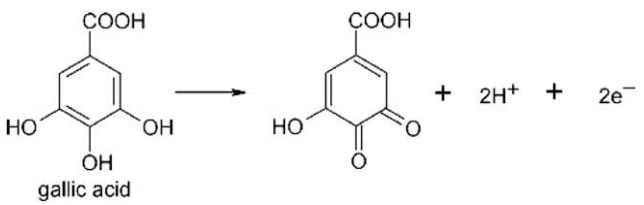
then

$$I_p = nQFv/4RT, \quad (3)$$

where R is the universal gas constant (8.314 J K^{-1} mol^{-1}), T is the Kelvin temperature, F is the Faraday constant (96,487 C mol^{-1}), n is the number of electrons transferred in reaction, A is the surface area of the working electrode, v is the scan rate, Γ is the surface concentration of reactant, Q is the coulomb in the process of redox reactant, and I_p is the peak current of reactant. The number of electrons transferred per molecule (n) was calculated to be 2. The most probable redox mechanism of GA at GCE was expressed in Scheme 1.

3.2. GA interaction with DNA in solution

The typical cyclic voltammetric curves of 7.30×10^{-5} mol L^{-1} GA in the absence (a) and presence of different amount of ds-DNA (b–k) in 0.10 mol L^{-1}, pH 4.5 acetate buffer solution at GCE was shown in Fig. 1. After recording a cyclic voltammetric curve of the GA in the presence of DNA, the surface of electrode was



Scheme 1. Chemical structure of GA and oxidation mechanism for it at GCE.