

Electrochemical Studies of Camptothecin and Its Interaction with Human Serum Albumin

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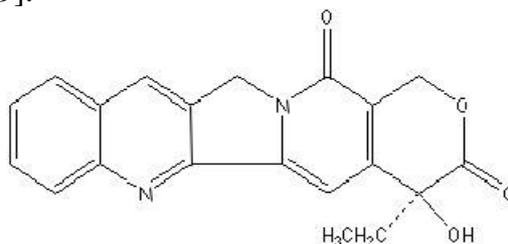
Abstract: Camptothecin, an anticancer component from *Camptotheca acuminata*, may interact with human serum albumin (HSA) at the subdomain IIA (site I), and then convert to its inactive form(carboxylate form). In this paper, the detailed electrochemical behaviors of camptothecin at a pyrolytic graphite electrode is presented. The interaction between camptothecin and HSA is also studied by electrochemical technique. By comparing with bovine serum albumin (BSA), which is highly homologous to HSA, we prove that camptothecin can specifically bind to HSA. Meanwhile, the inhibitory influence of sodium salicylate to this binding is also discussed.

Keywords: Camptothecin, human serum albumin, cyclic voltammetry

1. Introduction

Camptothecin (Scheme 1), 4-ethyl-4-hydroxy-(S)-1-H-Pyrano [3',4':6,7] indolizino [1,2-6] quinolino-3,14 (4H,12H)-dione, the active compound of *Camptotheca acuminata* [1], is a well-known antitumor drug for gastric cancer [2], breast cancer [3] and lung cancer [4]. Its primary cellular target is topoisomerase I, and the antitumor activity is attributed to the inhibition of topoisomerase I [5]. As a topoisomerase I "poison", camptothecin can bind both DNA and topoisomerase I to form a stable ternary complex [6, 7], which prevents DNA synthesis and induces the death of cells. Meanwhile, it

has also exhibited some other biological activities, such as anti-HIV [8] and against parasitic *trypanosomes* and *Leishmania* [9].



Scheme 1. Chemical structure of camptothecin

There are two forms of camptothecin (the carboxylate form and the lactone form), which exist with different conditions, however, only the lactone one plays the key role in forming the ternary complex [10]. On the other hand, human serum albumin(HSA), the most abundant protein in the serum, can bind to some drug molecules at two binding sites known as site I (subdomain IIA) and site II (subdomain IIIA) [11]. It is reported that in the presence of HSA, camptothecin may interact with the site I of HSA, leading to the conversion of camptothecin from active form to inactive form [12], which evidently reduces the antitumor activities of camptothecin. Thus, the study of the interaction between camptothecin and HSA is highly important for the clinical application of camptothecin. It is also crucial for maintaining the activity of camptothecin to find an effective way of inhibiting this interaction.

In this work, we have used electrochemical method to elucidate the interaction between camptothecin and HSA. By comparing with bovine serum albumin (BSA), we have also proved the existence of the specific binding of camptothecin to HSA. Moreover, the inhibitory influence of sodium salicylate to this specific binding is also discussed.

2. Results and Discussion

In order to use electrochemical technique to study the interaction between camptothecin and HSA, we have first studied the electrochemistry of the molecule. Up to now, no voltammetric study of this species has been reported, although the behavior of camptothecin at a hanging mercury drop electrode has been presented [13]. We have found that camptothecin is electroactive at a PG electrode in Britton-Robinson buffer, with the pH range of 2.0 to 7.0. As shown in Fig. 1, with 4.0×10^{-6} M camptothecin in a pH 4.0, 0.1 M Britton-Robinson buffer solution, a well-defined cathodic peak appears at -0.75 V (vs. SCE) at the PG electrode. Meanwhile, a slight anodic peak is also observed. However, compared with the cathodic peak, the anodic peak is unstable and disappears after several cycles of scanning. In contrast, no voltammetric peak can be obtained at the PG electrode in the absence of camptothecin. Furthermore, the anodic peak rises with the increasing concentration of camptothecin correspondingly. So, the cathodic peak should be attributed to the reduction of camptothecin at the PG electrode.

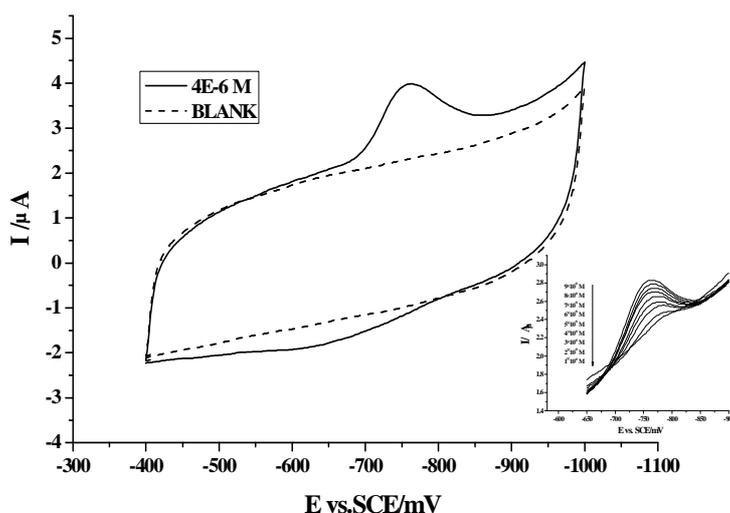


Figure 1. Cyclic voltammogram of camptothecin obtained at a PG electrode in 0.1 M Britton-Robinson buffer solution with pH 4.0 (The first scan). Scan rate: 100 mV s^{-1} . Accumulation time: 60 s. Inset is the cyclic voltammograms of camptothecin solutions with different concentrations.

The effect of scan rate on the voltammetric behavior of camptothecin has been examined (Fig. 2). The cathodic peak current is found to increase linearly with the scan rate from 100 mV s^{-1} to 700 mV s^{-1} . The linear regression equation is: $y = -0.01814 + 0.01377x$, $r = 0.999$, indicating that the reduction process on the electrode surface is mainly adsorption controlled [14, 15].

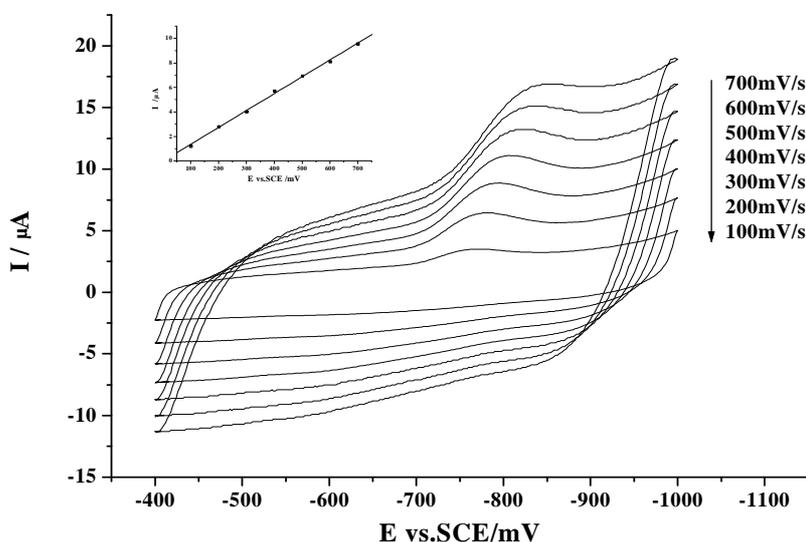


Figure 2. Cyclic voltammograms of camptothecin at scan rates varying from 100 to 700 mV s^{-1} (from inner to outer), and plot of the cathodic peak current against the scan rate (inset). Others same as in Figure 1.

The pH value plays an important role in the electrochemical behaviors of camptothecin. Although well-defined voltammograms can be obtained in the pH range from 2.0 to 7.0, with the increase of pH value, the cathodic peak shifts negatively. The cathodic peak potentials are found to be linearly proportional to the pH value, with a slope ($\Delta E/\Delta \text{pH}$) value of -71 mV pH^{-1} , which suggests a process

involving equivalent protons and electrons (Fig. 3A) [16]. The cathodic peak current will also change with the pH value. As is shown in Fig. 3B, the peak current is the highest at pH 5.0, which is the best for detection purpose. However, it is reported that HSA will preferably bind drugs at pH 4.0 [17]. So, for the studies of the interaction between camptothecin and HSA, we have selected a pH 4.0 buffer solution.

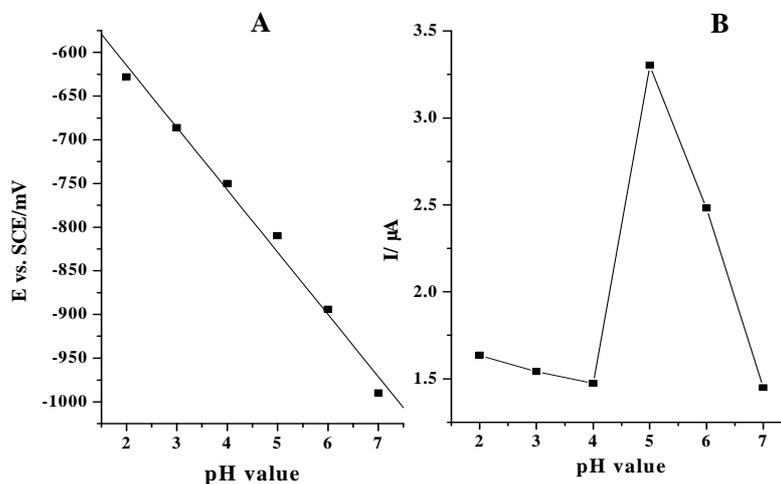


Figure 3. Plot of the cathodic (A) peak potential; (B) peak current against pH value. Others same as in Figure 1.

Since the electrochemical behavior of camptothecin on the electrode surface is adsorption-controlled, it is necessary to investigate the influence of accumulation time. As is shown in Fig. 4, the cathodic peak current increases significantly within the first 60 s, and then slowly and nearly unchanged later on. Thus, the first 60 seconds are chosen as the optimum time period of accumulation.

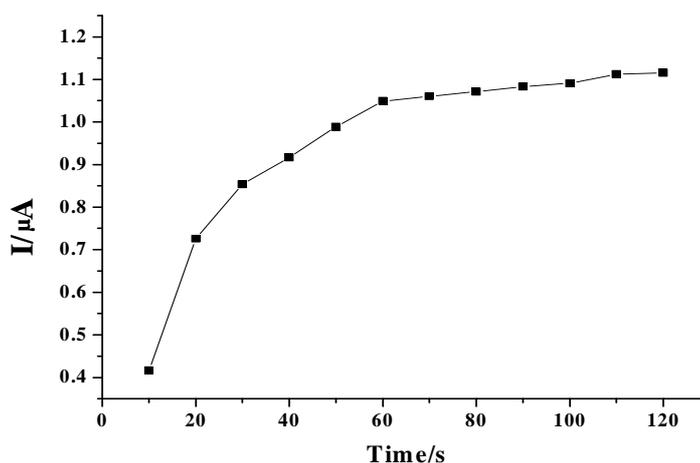


Figure 4. Dependence of the cathodic peak current on the accumulation time. Others same as in Figure 1.

Further studies reveal that the cathodic peak current increases with the continuous addition of camptothecin and a linear calibration plot ($y = 0.44833 + 0.19886x$, $r = 0.999$) can be obtained in the concentration range of 1×10^{-6} M - 1×10^{-5} M (Fig. 5), which suggests that the voltammetric response of

camptothecin at a PG electrode can be used for the concentration measurements. Although there are several analytical techniques including UV-spectrometry, fluorescence, infrared spectroscopy, and HPLC [18-20] currently available for determining camptothecin, it is still necessary to develop more methods for improving the accuracies of camptothecin measurement [13]. We have thus examined the analytical parameters. The limit of detection (LOD) of this electrochemical method is estimated to be 2×10^{-7} M, defined as three times the signal-to-noise ratio, which can be satisfactory compared with the previous report of 8×10^{-7} M [13]. The relative standard deviation (RSD) of five successive determinations at the concentration of 4×10^{-6} M is 5.6%.

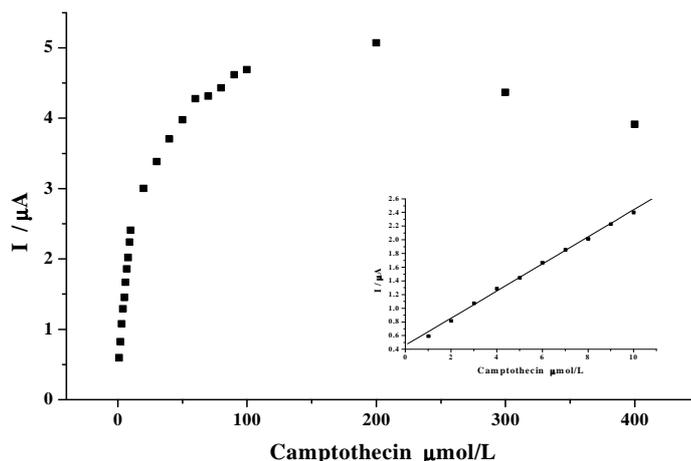


Figure 5. Dependence of the cathodic peak current on the camptothecin concentration. Others same as in Figure 1.

We have then studied the interaction between camptothecin and HSA with electrochemical technique. As shown in Fig. 6, with the addition of HSA into a 0.1 M Britton-Robinson buffer containing 4×10^{-6} M camptothecin, the peak current of camptothecin decreases distinctly, and no appearance of new redox peaks and no shift of the peak potential is observed.

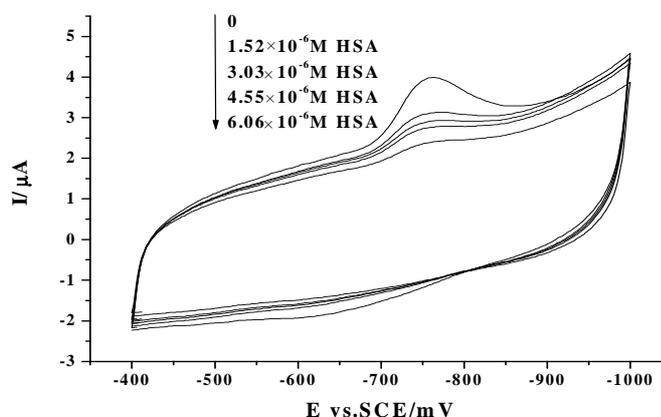


Figure 6. Cyclic voltammograms of 4×10^{-6} M camptothecin in the absence and presence of HSA with different concentrations. Others same as in Figure 1.

There are two possible mechanisms for explanation of this peak decrease. One is that HSA interacts with camptothecin to form a non-electrochemical complex, which blocks the electron transfer between

camptothecin and electrode. The other is the competitive adsorption of HSA at the PG electrode surface. Since the electrochemical behavior of camptothecin is an adsorptive process, the competitive adsorption of HSA may also reduce the adsorption of camptothecin and cause the decrease of the peak current of camptothecin.

We assume that it is the interaction between HSA and camptothecin and the formation of the non-electrochemical complex that makes the decrease of peak current of camptothecin. In order to confirm the specific interaction between HSA and camptothecin and the formation of the non-electrochemical complex, we have employed BSA as a contrast. HSA and BSA are highly homologous proteins [22], but camptothecin can not bind BSA [12]. If no interaction exists and no complex forms between HSA and camptothecin, the decrease of the peak current of camptothecin caused by HSA and BSA should be almost the same. However, as is shown in Fig. 7, the decrease caused by BSA is obviously less than HSA. Therefore, the result has not only revealed the existence of the specific binding between camptothecin and HSA, but also suggested that the competitive adsorption of HSA is not the main reason of the decrease of camptothecin peak current.

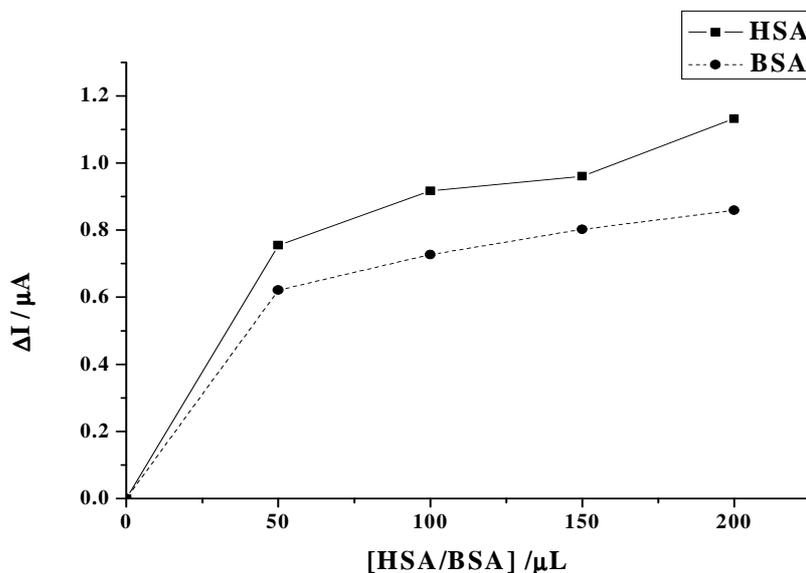


Figure 7. Plot of the decrease of the camptothecin peak current versus the addition of different volume of HSA/BSA in the solutions. HSA/BSA concentration: 10 mg/ml (1.52×10^{-4} M). Others same as in Figure 1.

Camptothecin has been known to have good antitumor activity. However, we have not used this agent as the treatment for cancers, and we believe that the specific interaction between the molecule and HSA is a major barrier to be overcome. Effort should be made to inhibit the binding of camptothecin to HSA and increase the amount of active camptothecin. It has been known that salicylate can specifically bind the site I of HSA with high affinity [23], so salicylate sodium has been used in this work as a competitive molecule to bind to HSA, which may inhibit the binding of camptothecin with HSA and increase the level of the active camptothecin. As shown in Fig. 8, compared with HSA alone, the decrease of the camptothecin peak current caused by the mixed solution of HSA and salicylate sodium is much less, which indicates that more free camptothecin molecules exist in the presence of salicylate sodium. The reason is that the binding of salicylate to HSA may

prevent the interaction between camptothecin and HSA. As a result, the free and active camptothecin molecules will increase accordingly.

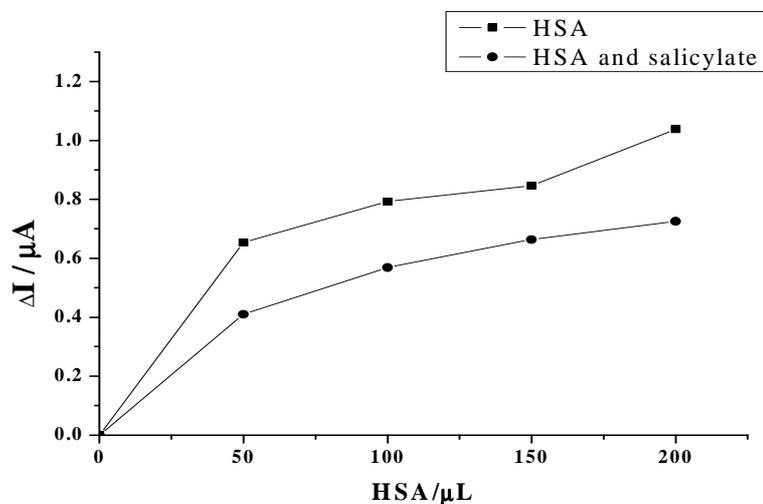


Figure 8. Plot of the decrease of the camptothecin peak current versus the addition of different volume of mixture of HSA and salicylate sodium (The volume ratio of HSA to salicylate is 1:1). The concentration of salicylate sodium: 1.0×10^{-6} M. Others same as in Figure 7.

3. Experimental section

3.1 Reagents

Camptothecin was purchased from Sigma. Stock solutions were prepared by dissolving known amounts of camptothecin in 10 mL of dimethyl sulphoxide. Salicylate sodium was purchased from Shanghai Shiyi Chemicals Regent Co., Ltd. Stock solutions were prepared by dissolving known amounts of salicylate sodium in 10 mL of methanol. HSA (~99%, molecular mass 69 kDa) and BSA (~99%, molecular mass 66 kDa), were purchased from Sigma and used without further purification. Stock solutions were prepared by dissolving known amounts of HSA/BSA in 10 mL of double-distilled water. Other chemicals were of analytical grade. Double-distilled water, which was purified with a Milli-Q purification system (Branstead, Boston, MA, U.S.A.) to a specific resistance of $>18\text{M}\Omega$ cm was used in all experiments, and all solutions were stored in the refrigerator at 4°C .

3.2 Preparation of working electrode

The pyrolytic graphite (PG) rod was purchased from Shanghai Carbon Co. (Shanghai, P.R. China). The substrate PG electrode was prepared by inserting a PG rod in a glass tube and fixing it with epoxy resin. Electrical contact was made by attaching a copper wire to the rod with the help of Wood's alloy (a fusible bismuth-based alloy). For pretreatment, the PG electrode was first polished using rough and fine sand papers. Its surface was then polished to mirror smoothness with alumina (particle size of approx. $0.05\mu\text{m}$)/water slurry on silk. Finally, the electrode was thoroughly washed by ultrasonically in both double-distilled water and ethanol for approx. 5 min.

3.3 Apparatus

Cyclic voltammetry (CV) was performed with a PARC 263A potentiostat/galvanostat (EG&G; Princeton, NJ, USA), using a three-electrode configuration at 25 ± 0.5 °C. The reference electrode was a saturated calomel electrode (SCE) and the counter electrode was a platinum electrode. Potentials are reported with respect to SCE unless specially stated. The substrate electrode was a PG electrode ($A = 6.28 \text{ mm}^2$). All test solutions were thoroughly deoxygenated by bubbling high-purity nitrogen through the solution for at least 10 min. A stream of nitrogen was blown gently across the surface of the solution in order to maintain the solution anaerobic throughout all the experiments.

Acknowledgements

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