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Electrochemical Experimental Investigation on Behavior and Mode of the Interaction Between DNA and (S)-2-(5-Fluorouracil-1-Acetyl) Amido -1,4 -Succinic Acid

Wu-yi Liu[1, *], *Ke-jun Zhang*[2]

[1] Department of Biology Science, Fuyang Normal College, Fuyang City 236041, China
[2] Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao City 266071, China
[*]E-mail: lwycau@yahoo.com.cn

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The cyclic voltammetry and spectroscopy have been proved to be powerful electrochemical means for the elucidation of interaction betweens Deoxyribonucleic acid (DNA) and chemical anti-carcinogens. With the aim to elucidate the interaction between DNA and anti-carcinogenic 5-fluorouracil derivatives using the instruments and techniques above, the electrochemical behavior of (S)-2-(5-Fluorouracil-1-Acetyl) amido -1,4 -succinic acid (5FU-ASP) and the electrostatic interaction of 5FU-ASP with DNA using $Fe(CN)_6^{3-/4-}$as electroactive indicator of dominance were investigated in this study. The binding equilibrium constant of interaction is estimated to be $1.1\times10^3\times M^{-1}$ and DNA modified gold electrodes were prepared by the dry adsorptive method. Based on the electrochemical and ultraviolet–visible spectrum data, the interaction mode of DNA with 5FU-ASP was further explored, and the results serve as good reference for studies on 5FU-ASP targeting DNA strands or base pairs in the natural environment of living cells in vivo.

Keywords: DNA, gold electrode, anti-carcinogen, 5FU-ASP, cvclic voltammetry

1. INTRODUCTION

It is well known that Deoxyribonucleic acid (DNA) plays important roles in life, because it bears heritage information and instructs the biological synthesis of proteins through replication and transcription of genetic information in living cells. However, DNA molecules are prone to be damaged and yield strand cleavages under linkage reactions, which affects its higher order structure and gene regulation and thus leading to various pathological changes in living organisms. Meanwhile, studies of the structure, binding specificity, mechanism and dynamics of the interactions of small molecules with

double helical DNA have attracted continuous interest in chemistry and biology, because the interactions may influence replication and transcription of DNA in vivo, or cause gene mutations that may be the origin of some diseases. These studies are also helpful in understanding the reaction mechanisms of some antitumor and antivirus drugs, as well as protein–DNA interaction modes.

In recent years, studies on the properties of anti-carcinogenic medicines and their interaction with DNA are significantly important in developing new cancer therapy treatments or novel anti-carcinogens. Among these studies, 5-Fluorouracil (5-FU) has been increasingly employed alone or in combination with various cytotoxic drugs and hormones in the treatment of several tumours, such as breast, colorectal and gastric cancers [1-6]. However, because of its poor tumor selectivity and high incidence of toxicity in the bone marrow, gastrointestinal tract, central nervous system and skin, many derivatives of 5-FU have been developed to improve the topical delivery and reduce side effects [7-10]. Aspartic acid (Asp) plays important roles in life status of human beings and other organisms; they function as hormone, growth promoter, inhibitor, neurotransmitter, immunomodulating agents as well as antibiotics, driving considerable pharmacological interest in design and application of novel drugs [11, 12]. To extend our interest in searching for new aspartic acid derivatives of 5-FU with higher bioactivity and take advantage of the concept of bioisosterism, (S)-2-(5-Fluorouracil-1-Acetyl）amido -1,4 -succinic acid (5FU-ASP) (Fig.1), which is a derivative of 5-FU containing Asp dipeptide group, has been designed and synthesized.

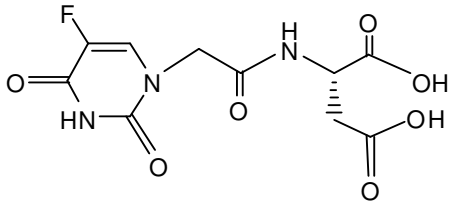

Figure 1. The chemical structure of (S)-2-(5-Fluorouracil-1-Acetyl）amido -1,4 -succinic acid

Moreover, the analysis of 5FU-ASP is crucial to the health of human bodies and animals. Recently, DNA-modified electrodes are of great interest to electrochemists [12-26]. In particular, electrochemistry of nucleic acids (NAs) is booming due to the development of electrochemical transducer-based devices for detection of nucleotide sequences and DNA damages [27-43]. This technique is also applied in veterinary and forensic medicine, diagnosing, and environmental testing for its low-cost, fast response, simple design, small dimensions and low power requirements. The present study reports a surface-based micro-method to investigate the interaction of DNA with 5FU-ASP using DNA-modified Au electrodes prepared by adsorption. The method overcomes the limitations of other methods [44-50] in little sensitivity and large requirement for DNA samples. The experiments show that the modified surfaces exhibit more useful electrochemical properties than unmodified electrode, and DNA-modified electrodes are suitable for the quantitative determination of neomycin. Meanwhile, the interactive mechanism of the antibiotics with DNA is studied by the

interaction of 5FU-ASP with DNA in solution. The result suggests that 5FU-ASP intercalates as a whole unit into base pairs of DNA to form a complex.

2. EXPERIMENTAL

2.1. Chemicals and instrumentation

Electrochemical measurements were carried out with a model AUTOLAB PGSTAT30 electrochemical workstation (Metrohm AG) controlled by a personal computer. A conventional three-electrode system was used in the measurements at room temperature (25°C), with a bare or modified gold electrode (d = 2 mm) as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and Pt plate as the counter electrode. All potentials given were referred to the SCE. Ultraviolet–visible absorbance spectra were conducted with a double beam UV-2501PC spectrophotometer from Tianmei Scientific Equipment Corporation of Shanghai, China. Unless specially stated, the electrolyte solutions were thoroughly degassed with N_2 and kept under a N_2 blanket.

Calf thymus DNA (CT DNA obtained from Sino-American Biotechnical Corporation) was used as received. Solutions of DNA ($\approx 10^{-4}$ M in nucleotide phosphate NP) in 5.0 mM pH=6.80 Tris–HCl buffer solution containing 5.0 mM NaCl were purified to reach a high purity (A_{260}/A_{280} was larger than 1.8, where A represents the absorbance), indicating that the DNA could be used [51]. Stock solutions were stored at 4 ^{o}C and used within three days. The concentration was determined by UV absorbency at 260 nm in 1:100 diluted solutions. The extinction coefficient, ε260, was taken as 6600 M^{-1} cm^{-1}, 5.0×10^{-3} M $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) mixture containing 0.1M KCl was used as a redox probe in the electrochemical measurements.

5FU-ASP and DNA were dissolved in 5.0 mM pH=6.80 Tris–HCl buffer solution containing 5.0 mM NaCl, which is used as the supporting electrolyte. Other chemicals were at least of analytical reagent grades. The buffer solution refers to 5.0 mM Tris–HCl buffer solution with pH 6.80 containing 5mM NaCl supporting electrolyte. Ultra-pure water (18.22 MΩ cm^{-1}) was used for the preparation of all solutions.

2.2. Preparation of DNA-modified gold electrodes

The gold electrodes were first polished carefully with 1.0, 0.3 and 0.05μm alumina slurry and then cleaned ultrasonically in acetone, ethanol and water for 10 min, respectively. The real electrode area was estimated from cyclic voltammogram (CV) by integrating the cathodic peak for the reduction of the oxide layer in 0.5 M H_2SO_4. The freshly polished electrodes were scanned over the potential range of 0.0 to +1.5 V (vs. SCE) in 0.5 M H_2SO_4 until a constant voltammogram was obtained. Then, they were polarized at 0 V for 3 min. Finally, the electrodes were rinsed with water and modified immediately by transferring a droplet of 20 μL of 0.5 mM DNA solution onto the surface, followed by air-drying overnight. The electrodes were then soaked in sterile water for at least 4 h before being

rinsed with water to remove any unadsorbed DNA. Thus, the DNA-modified gold electrode obtained is denoted as Au/ds-DNA later.

3. RESULTS AND DISCUSSION

3.1. Electrochemical characterization of DNA-modified electrode

Cyclic voltammetry of electroactive species $Fe(CN)_6^{3-/4-}$ has been used widely to test the kinetic of the interface barrier. The extent of kinetic hindrance to the electrontransfer process increases with increasing thickness and decreasing defect density of the barrier [28].

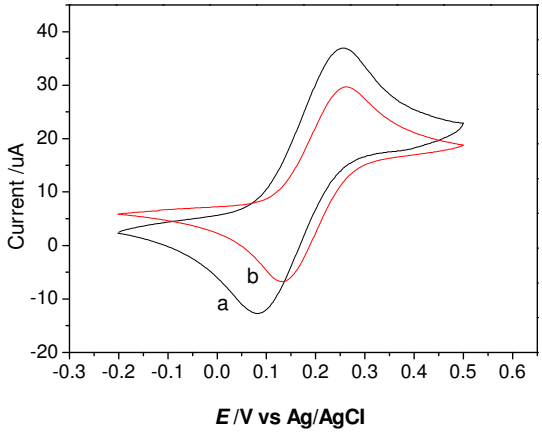

Figure 2. Cyclic voltammograms of differently modified electrodes at the scanning rate of 0.1 $V \cdot S^{-1}$ The supporting electrolyte is 5 $mmol \cdot L^{-1}$ pH 6.8 Tris-HC1 buffer containing 5 $mmol \cdot L^{-1}$ $K_2Fe(CN)_6$ and 5 $mmol \cdot L^{-1}$ KCl. (a) bare gold electrode; (b) Au/ds-DNA.

Fig. 2 shows the CV (cyclic voltammogram) responses of 5.0 mM $Fe(CN)_6^{3-/4-}$ at bare Au and DNA/Au, respectively. $Fe(CN)_6^{3-/4-}$ produces a couple of well-defined redox waves at bare Au (Fig. 2) with a peak-to-peak separation (ΔEp) of 94 mV at 100 mV/s. After the electrode was modified with DNA, an obvious decrease in redox peak current was observed (Fig. 2), indicating that the DNA acts as the inert electron and mass transfer blocking layer and thus hinders the diffusion of ferricyanide toward the electrode surface. This demonstrates that DNA has been successfully assembled on the Au surface. The cyclic voltammograms of the same DNA electrode remain stable after 20 scans in the Tris-HCl buffer solution, suggesting the electrochemical stability of the DNA-coated film.

3.2. Interaction of dsDNA with 5FU-ASP

In order to investigate the interaction of DNA with 5FU-ASP, the DNA-modified electrode was scanned in buffer solution containing 5.0 mM $Fe(CN)_6^{3-/4-}$ probe molecule, then 5FU-ASP was

added into the test solution. The experiments showed that the peak current of probe molecule decreased as 5FU-ASP added into the test solution. The more 5FU-ASP was added, the more the peak current of probe molecule decreased. Thus, the concentration of 5FU-ASP was adjusted to 4.3×10^{-4}M, while the peak current decreased with respect to the original peak current, as showed in Fig. 3.

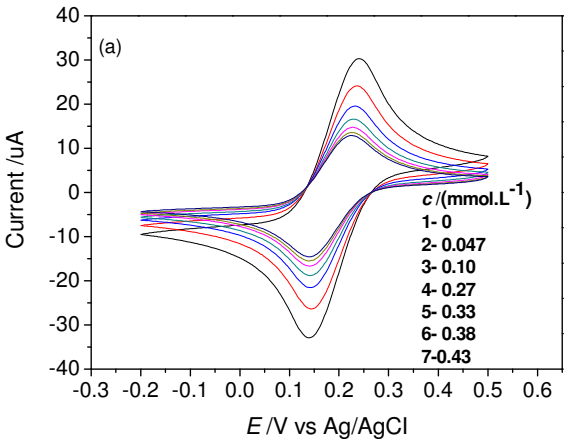

Figure 3. Cyclic voltammograms of ds-DNA modified electrode at different concentration of 5FU-ASP

The phenomenon confirmed that the 5FU-ASP molecule interact with ds-DNA under three models of DNA with the targeting molecules [44]. Whether it was Au/ds-DNA electrode or not, the peak potential shifting in a negative direction confirms the dominance of intercalative interaction between 5FU-ASP and ds-DNA, with the 5FU-ASP concentration increasing.

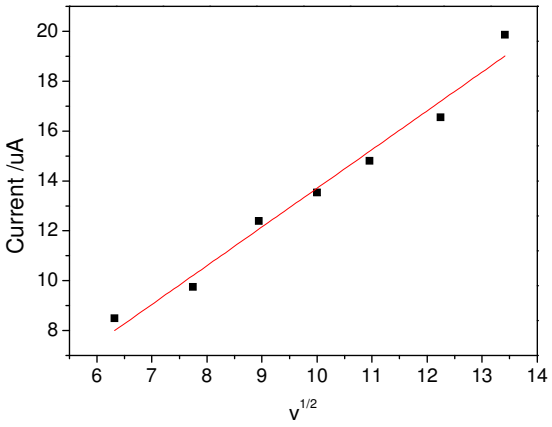

Figure 4. The relationship between anodic peak current i_{pa} and the root of scanning rates for DNA modified electrode

The influence of scan rates on CV peaks was also examined. With the increasing scan rates, the anodic peak potentials shifted in gradually positive direction and the cathodic peak potentials shifted in negative direction. Meanwhile, the potential separation also increased, which means that the electrode reaction was a quasi-reversible reaction in dynamics. The peak currents were directly proportional to the root of scanning rates in the range of 40-200 mV s^{-1}, as shown in Fig. 4. These results indicate that the electrode reactions are adsorption-controlled processes [50].

3.3. Binding equilibrium constant

Cyclic voltammetric experiments showed that DNA-modified electrode has a couple of stable voltammetric peaks from probe molecules when it was scanned in the probe solution. Further analyses showed when 5FU-ASP was added into the solution, the peak current had an obvious decrease. Moreover, the higher the amount of 5FU-ASP was added, the lower the peak current was. The curves are shown in Fig. 3. When the concentration of 5FU-ASP reached 0.060M, the peak current no longer decreased. Fig. 3 shows the relationship between the decrease of the peak current and the concentration of 5FU-ASP added. From Fig.3, we can further obtain a curve of $1/\Delta I_p \sim 1/c$ (Fig. 5). It is apparent that a good linear relationship (R = 0.9934) exists between the reciprocal of the current drop and that of the 5FU-ASP concentration.

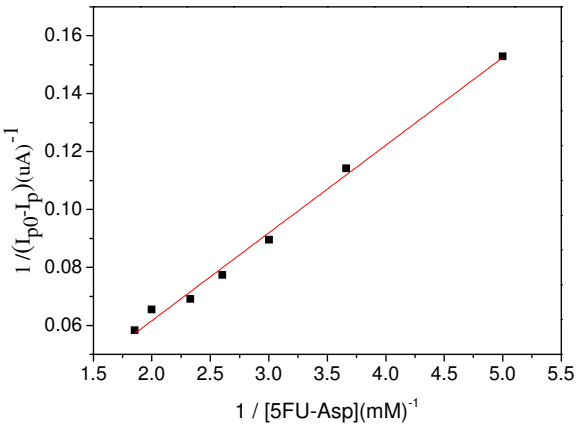

Figure 5. The relationship between $1/(I_{p0}-I_p)$ and 1/[5FU-ASP]

This was in good agreement with the Langmuir equation [19]. That is $1/\Delta I_p=\Delta I_{p,max}+1/(\Delta I_{p,max}kc)$, where k is equilibrium constant of the interaction between 5FU-ASP and DNA, and C is the concentration of 5FU-ASP, while ΔI_p is the current drop value and $\Delta I_{p,max}$ stands for the maximum value of ΔI_p. According to the Langmuir equation and the slope of the curve in Fig. 5, the binding equilibrium constant was estimated as 1.1×10^3 M^{-1}.

3.4 UV–vis spectra studies

Ultraviolet–visible absorbance spectra of 5FU-ASP, dsDNA and their mixture were measured, respectively (Fig. 6). 5FU-ASP has an absorption peak at 269nm, and DNA has an absorption peak at 260 nm which is from nucleotide base pairs on both the DNA strands.

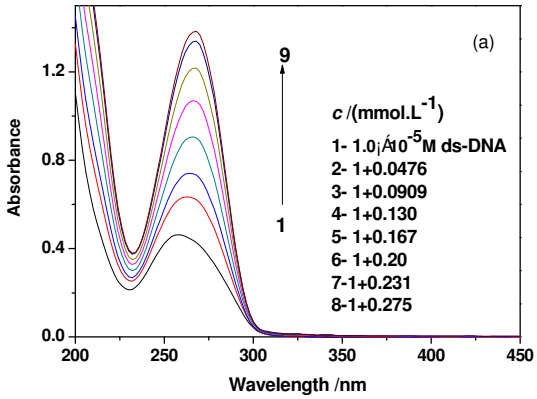

Figure 6. Ultraviolet – visible absorbance spectra of DNA with different concentration of 5FU-ASP

However, after DNA and 5FU-ASP were mixed, the spectrum was changed to spectra, and the absorption peak moved to 266 nm, indicating the strong interaction between DNA and rutin. It can be found that the absorption spectrum is significantly different from the sum of corresponding absorption spectrum of DNA or 5FU-ASP alone. In the experiments, the concentration of DNA was always kept at 1.0×10^{-5} M, while the concentrations of 5FU-ASP were increasing. Then these solutions of mixtures were kept at 4°C for 1h for full completion of binding between DNA and 5FU-ASP. These results showed that the value of absorbance increased as the concentration of 5FU-ASP increased, and a bathochromic shift of 4 nm was observed (Fig. 6). It may be attributed to the formation of new π-conjugated system. Bathochromic shift and hypochromic effect are also suggested due to a strong interaction between the electronic states of intercalative chromophores and that of DNA bases [52-56].

4. CONCLUSIONS

In this study, the electrochemical behavior of anticancer drug 5FU-ASP and its interaction with DNA were investigated by the electrochemical and spectroscopic methods. All the experimental results indicate that the principal interaction mode of 5FU-ASP with DNA is a kind of cooperative intercalative interaction. The interaction can be quantified in terms of the Hill model for cooperative interactions. The results demonstrate that the method of electrochemistry combined with spectrophotometry is available and provides significant promise to study the mechanism of DNA interaction with targeting compounds at both the macrocosmic aspect and molecular levels.

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