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Interaction of echinomycin with guanine: electrochemistry and spectroscopy studies

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Abstract

The interaction of antitumor antibiotic, echinomycin (Echi) with guanine (Gua) was thoroughly investigated by adsorptive transfer stripping cyclic voltammetry, ultraviolet and visible adsorption spectra (UV/Vis) and Fourier-transform infrared spectroscopy (FTIR). Electrochemistry provided a simple tool for verifying the occurrence of interaction between Echi and Gua. Echi could be accumulated from the solution and give well-defined electrochemical signals in 0.1 M phosphate buffer solution (pH 7.0) only when Gua was present on the surface of the electrochemically pretreated glass carbon electrode (GCE), suggesting a strong binding of Echi to Gua. All the acquired spectral data showed that a new adduct between Echi and Gua was formed, and two pairs of adjacent intermolecular hydrogen bonds between the Ala backbone atoms in Echi and Gua (Ala-NH to Gua-N3 and Gua-NH_2 to Ala-CO) played a dominating role in the interaction. Electrochemistry coupled with spectroscopy techniques could provide a relatively easy way to obtain useful insights into the molecular mechanism of drug–DNA interactions, which should be important in the development of new anticancer drugs with specific base recognition.

Keywords: Echinomycin; Guanine; Interaction; Adsorptive transfer stripping cyclic voltammetry; UV/Vis; FTIR

1. Introduction

Chemotherapy is an effective part of the program for combating cancers. Many anticancer drugs are known to exert their biological activities by interacting with DNA [1]. Therefore, the investigation of drug–DNA interaction is an important aspect of biological studies in drug discovery and pharmaceutical development processes. Many drugs bind to DNA mainly through several types [2]: intercalation, groove-binding, covalent linkage, DNA cleaving, nucleoside analog incorporation and so on. These interactions can result in structural change of both DNA and drug molecule to accommodate complex formation. In the last few years, many technological advances have made us develop new analytical tools to study the interaction of anticancer drugs with DNA. These results have provided useful insights into DNA conformation and drug–DNA interactions. In particular, it has been found that specific atomic sites of DNA bases are often targets for drug binding [3].

Due to its antimicirobial and antitumor activity, quinoxaline antibiotics have been developed as potential candidates for anticancer drugs. Among them, echinomycin (Echi) was originally discovered as an antibiotic presenting in culture filtrates of *Streptomycin echinatus* [4]. The structure of Echi is shown in Fig. 1 [5]. It consists of an octapeptide ring, which is cross-bridged by thioacteted linker and two quinoxaline intercalating units [6,7]. It is generally agreed that its antitumor and other biological activities result from its capacity to powerfully inhibit DNA-directed RNA synthesis by a peculiar mechanism of bifunctional intercalation [8]. The interaction of Echi with DNA has been extensively studied over the past years using various techniques, such as Footprinting [9–11], NMR [12–14] and X-ray [15,16] crystallographic studies. Circular dichroism spectra [17] were used to study Echi–DNA complex to gain information about the structure of Echi in the DNA-bound state. The kinetics experimental results of stopped-flow methods [18] showed that both chromophores of Echi intercalated simultaneously other than sequentially to DNA. Recently, elec-

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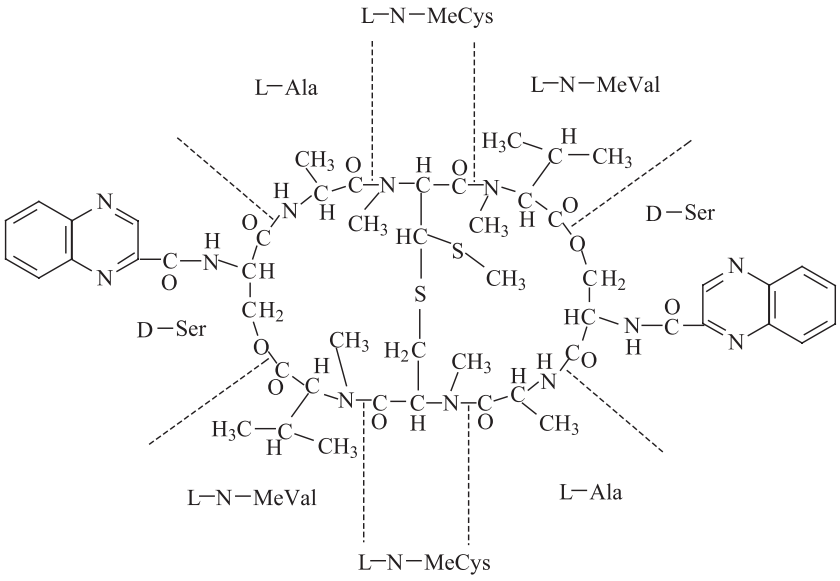

Fig. 1. Structure of ehcinomycin. Abbreviations: MeVal, methylvaline; Ser, serine; Ala, alanine; Cys, cysteine; L-N-MeCys, N-methyl-L-cysteine.

trochemical methods were carried out for studying the interaction of Echi with DNA [19–21]. Reports demonstrated that Echi bond preferentially to CpG steps [9] and depsipeptide linker of Echi molecular faced the minor groove region of the two base pairs presenting between the chromophores [22]. Clearly, the tight binding of Echi to DNA was derived not only from intercalation of the chromophores but also from interactions with functional substituents on the peptide ring [23]. There are a number of reactive sites on the surface of the DNA double helix and guanine (Gua) sitting in the minor groove is particularly susceptible to drug action. The binding specificity of many drugs to DNA often involves the recognition of Gua base in the minor groove through the hydrogen bonding [3].

To our knowledge, there are no reports on detailed investigation on the interaction of Echi with Gua. Herein, the aim of this work was to gain information about the mechanism of interaction of Echi with Gua. Electrochemical methods and ultraviolet and visible adsorption spectra (UV/Vis) and Fourier-transform infrared spectroscopy (FTIR) spectroscopy were employed to study the association of Echi with Gua and evaluate the relative importance of hydrogen bonding, which particularly contributed to the stability of Echi–Gua complex. These results should be helpful for understanding the drug–DNA interaction at the molecular level and be useful for the design of new drugs binding selectively to predetermined sequences.

2. Experimental

2.1. Materials

Echi and Gua (Sigma, USA) were used without further purification. Stock solution of Echi was prepared by dissolving the drug in dimethyl sulfoxide. The supporting electrolyte was 0.1 M phosphate-buffered solution (PBS) with pH 7.0 for measurements while pH 5.0 PBS for the electrochemically pretreated glass carbon electrode (GCE). Stock solution of 1.0 mM Gua was made in diluted NaOH solution because Gua was weakly soluble in water. All reagents were analytical grade and aqueous solutions were prepared using doubly distilled water.

2.2. Apparatus

Cyclic voltammetry studies were carried out by CHI 800 (Shanghai, China). A three-electrode system was used. The working electrode was glassy carbon electrode (GCE) with 3 mm diameter. Ag/AgCl-saturated KCl served as the reference electrode and platinum wire as the counter electrode. pH values were measured with pH meter Orion 420A.

UV/Vis absorbance spectra were obtained by Cary 500 UV/Vis–NIR spectrophotometer (Varian, USA) equipped with quartz micro-colorimetric vessel.

Fourier-transform infrared spectra were obtained by 520 Fourier-transform infrared spectrometer (Nicolet, USA) equipped with a homemade cell using CaF_2 windows for the solution spectra measurements and Ominic E.S.P. software was used to data analysis. Spectra were obtained for samples containing 100 mM Gua in PBS, in the presence or absence of Echi in the measuring cell.

2.3. Pretreatment of the glassy carbon electrode

The GCE was polished with 0.3 and 0.05 μm alumina powders, respectively, then washed with distilled water under the aid of ultrasonication, and oxidized at +1.80 V for 300 s in 0.1 M PBS (pH 5.0). After that, the electrode was scanned between +0.3 and +1.25 V until a steady-

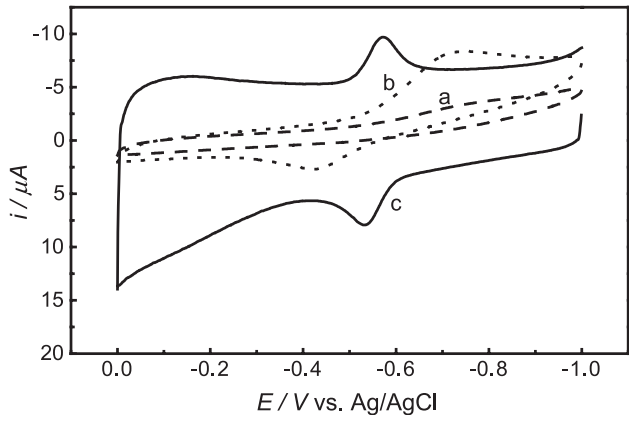

Fig. 2. Cyclic voltammograms of 0.1 M PBS (pH 7.0) in the absence (a) and presence of 10 μM Echi at the unpretreated GCE (b) and the pretreated GCE (c). Scan rate: 100 mV s^{-1}.

state current–voltage curve was obtained [24]. A thin blue film could be observed on the activated electrode surface after this treatment. According to the results of Kepley and Bard [25], the electrochemical activation of GCE could form a new phase, which contained a significant amount of microcrystallinity and graphite oxide. The characteristic of the new phase was very porous and hydrated, and it appeared that the pretreatment affected primarily the accumulation process and not the charge transfer process.

2.4. Adsorption of guanine at the pretreated electrode

The accumulation of Gua on the electrochemically pretreated GCE surface could be identified by the following procedures. The electrochemically pretreated GCE was immersed into PBS (pH 7.0) containing 0.12 mM Gua at the given constant potential of +0.3 V 300 s. In order to gain the electrochemical response of Gua immobilized on the surface of pretreated GCE, the electrode was washed and cyclic voltammetry was carried out in PBS (pH 7.0) free of Gua between +0.3 and +1.2 V. The observed oxidation peak of Gua suggested that Gua was able to strongly adsorb on the surface of electrochemically pretreated GCE.

2.5. Immobilization of Echi on the Gua-modified electrode

After Gua accumulation, the Gua-modified electrode was washed by water and immersed into the Echi solution for physical adsorption 15 min without stirring. Subsequently, the electrode was removed from the solution, thoroughly washed and transferred into PBS (pH 7.0). Cyclic voltammetric experiments were carried out in deaerated PBS (pH 7.0) at room temperature in the potential range from −1.0 to 0 V.

3. Results and discussion

3.1. Verifying the occurrence of interaction of Echi with Gua by electrochemistry

3.1.1. Electrochemical behavior of Echi at the electrochemically pretreated GCE

To obtain the mechanism of interaction of Echi with Gua in more detail, the electrochemical response of the free drug was studied. The behavior of quinoxaline antibiotics at dropping mercury electrode showed that qui-

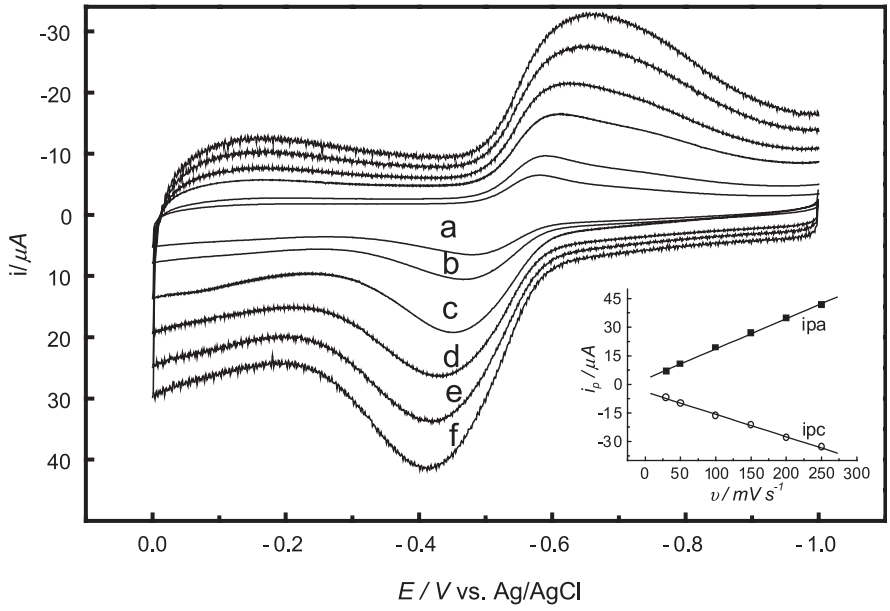

Fig. 3. Cyclic voltammograms of 0.1 M PBS (pH 7.0) containing 10 μM Echi at the pretreated GCE at different scan rate: (a) 30; (b) 50; (c) 100; (d) 150; (e) 200 and (f) 250 mV s^{-1}. Inset: the relationship between the peak currents and the scan rate.

noxaline ring was polarographically reducible [26]. But the electrochemical behavior of Echi at GCE was unknown. Recently, electrochemical pretreatment of GCE has been widely used to improve the electrode performance [27,28]. Fig. 2 showed the cyclic voltammograms of 10 μM Echi at the unpretreated and pretreated GCE, respectively. At the pretreated GCE, Echi gave well-defined redox peaks between −0.4 and −0.6 V (vs. Ag/AgCl) in PBS (pH 7.0), while the response at the unpretreated electrode was very poor. So the sensitivity for the electrochemical response of Echi was improved greatly by modifying the electrode with a simple electrochemical oxidation. It was reasonable that the background current of GCE increased markedly after the pretreatment. Taking into consideration the structure of Echi, the redox peaks resulted from the quinoxaline of Echi.

The dependence of the voltammetric response of Echi at the pretreated GCE on the scan rate was also examined. The peak current varied with scan rate as shown in Fig. 3. Both $i_{p,c}$ and $i_{p,a}$ were linear dependence on the scan rate in the range 30–250 mV s^{-1} (inset of Fig. 3), suggesting that the redox electrode process was controlled by adsorption [29]. Moreover, with increasing scan rate, oxidation peak potential positively shifted and reduction peak potential of Echi negatively shifted but with a little change in the peak shapes, demonstrating that the electrode process was quasi-reversible.

3.1.2. Adsorption of guanine on the surface of pretreated GCE

Many electrochemical approaches have been performed for analysis or quantification of DNA. However, among the constituents of DNA, only purine bases underwent reduction and/or oxidation at electrode surfaces [30,31]. Fig. 4 showed the cyclic voltammograms of 20 μM Gua at the unpretreated and pretreated GCE in PBS (pH 7.0). Compared with the unpretreated GCE, Gua gave well-defined oxidation peaks in the anodic sweep at about

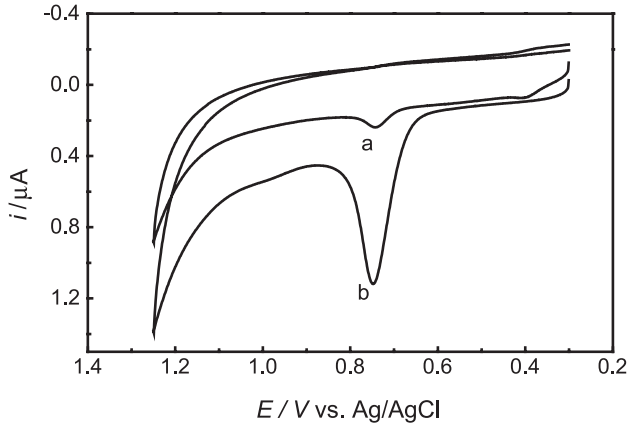

Fig. 4. Cyclic voltammograms of 0.1 M PBS (pH 7.0) containing 20 μM guanine at the unpretreated (a) and pretreated GCE (b). Scan rate: 50 mV s^{-1}.

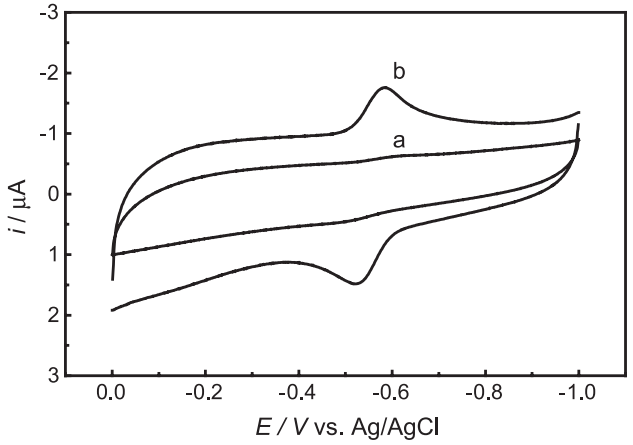

Fig. 5. Cyclic voltammograms of Echi at the Gua-unmodified (a) and Gua-modified GCE (b). The curves were obtained by dipping the Gua-modified GCE into 10 μM Echi for 15 min, and then washing and transferring into 0.1 M PBS (pH 7.0).

+0.75 V at the pretreated GCE and its peak current increased significantly about fivefold. The electrochemical oxidation of Gua on the pretreated GCE was an entirely irreversible process because of no reduction peak observed in the cathodic sweep. The oxidation of Gua at solid electrodes was expected to follow a two-step mechanism involving the total loss of four electrons and four protons [32]. The stability of Gua immobilized on pretreated GCE was studied. After Gua accumulation, the electrode was washed and cyclic voltammetry was done many scans in PBS (pH 7.0) free of Gua between −1.0 and 0 V, which was the suitable potential range for the detection of Echi. Then, the electrode was transferred into renewed PBS (pH 7.0) and the oxidation peak of Gua at positive potentials was still observed. These results illuminated that Gua was strongly adsorbed on the surface of the pretreated GCE and no desorption occurred in the redox potential of Echi. This point was very favorable for investigating the interaction of Echi with Gua on the electrode surface.

3.1.3. Interaction of Echi with surface-adsorbed guanine

The electrochemical evidence of Echi–Gua interaction was obtained by three steps: Gua immobilization, interaction of Echi with Gua and its adsorptive transfer cyclic voltammetry measurements. The above-mentioned experimental data suggested that Gua was strongly absorbed on the electrochemically pretreated GCE surface. The Gua-modified electrode was placed in solution of 10 μM Echi and incubated 15 min. After washing with distilled water thoroughly, the electrode was then transferred into 0.1 M deaerated PBS (pH 7.0) and cyclic voltammograms were recorded in the potential range from −1.0 to 0 V. For comparison, another control experiment was done in the same conditions, only without Gua immobilized at the same pretreated GCE surface. For the Gua-modified electrode, a redox couple appeared at about −0.5 V

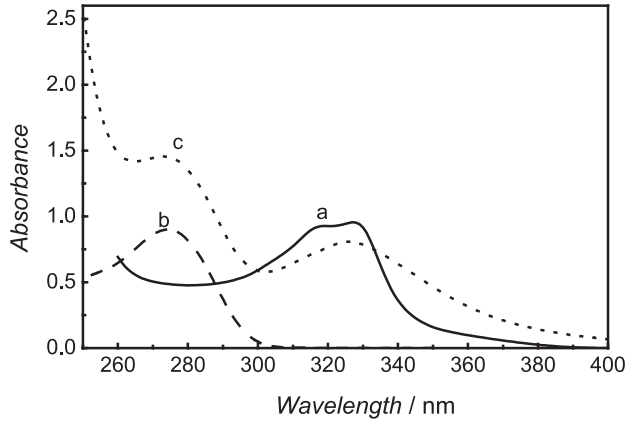

Fig. 6. UV/Vis absorption spectra of 0.1 M PBS (pH 7.0) containing 0.06 mM Echi (a), containing 0.1 mM guanine (b) and a mixture of 0.1 mM guanine and 0.06 mM Echi (c).

corresponding to peaks of Echi as shown in Fig. 5. However, under the same conditions, no obvious signal of Echi at the Gua-unmodified electrode was observed, which illustrated Echi bond to the bare electrode surface very weakly. In contrast, the Gua-modified electrode showed sufficiently larger signals, reflecting that Echi had strong affinity to Gua so as to present well redox peaks on the pretreated electrode surface in blank PBS. Such high affinity was attributed to the interaction of Echi with the surface-confined Gua. Therefore, by simple electrochemical experiments, we figured out that there was strong interaction between Echi and Gua. Considering the molecular structure of Echi and Gua, we deemed that inter-molecular hydrogen bonding existed between them, which played a crucial role in the Echi–Gua association. This conclusion was also confirmed by the following spectroscopic experiments.

3.2. Further detailed information about the interaction studied by UV/Vis and FTIR spectroscopy

3.2.1. UV/Vis spectroscopic probing of Echi–Gua association

Spectroscopic techniques are very useful tools to gain important information on biological science. Spectroscopic investigation of the interaction of Echi with Gua may be helpful for clarifying the mechanism of action. The interaction of Echi with Gua in PBS (pH 7.0) could be further confirmed by UV/Vis spectra. The variation of adsorption spectroscopy resulted from such interaction was presented in Fig. 6. For the spectrum of free Echi, a broad band was observed from 317 to 327 nm, which could be assigned to slightly different energy levels of quinoxaline chromophore of Echi (Curve a). Peak maximum of free Gua occurred at 274 nm resulted from electronic transition of purine ring (Curve b). However, in the presence of Gua, the maximum absorption peak of Echi shifted to longer wavelengths and the absorption intensity decreased (Curve c). Such variations should be attributed to the interaction of Echi with Gua. According to these observations, Echi exhibited hypochromic effect and bathochromic shift in the absorption spectra upon addition of Gua. It seems that the intermolecular hydrogen bonds formed between Echi and Gua resulted in a decrease in the bond order [33] and led to the low energy electronic transition. In addition, the steric hindrance might be arisen by the interaction of Gua with the relatively bulky Echi molecule, which also resulted in the overlap of electron clouds between the large π-bond of quinoxaline ring of Echi and purine ring of Gua molecule.

3.2.2. FTIR spectroscopic probing of Echi–Gua binding

FTIR spectroscopy is an essential tool in the detailed investigation of DNA conformation and the mechanism of

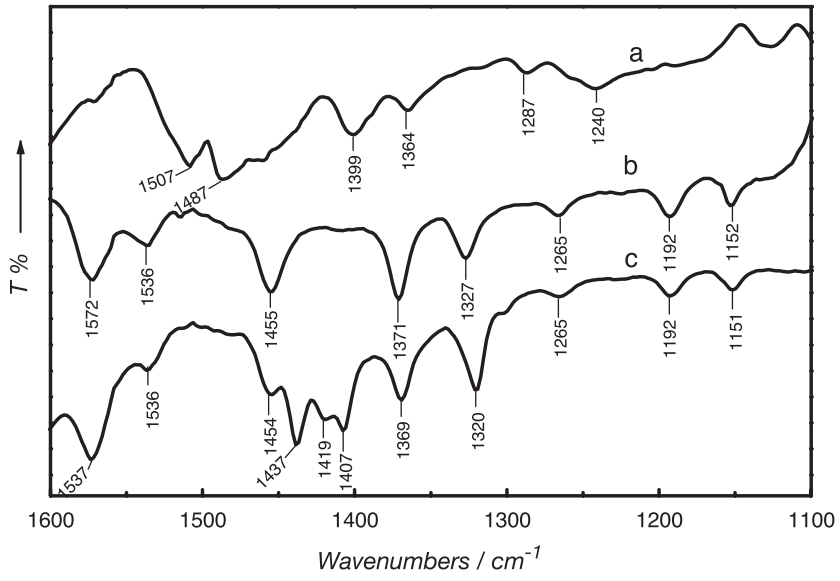

Fig. 7. FTIR spectra of 0.1 M PBS (pH 7.0) containing 0.5 mM Echi (a), containing 100 mM Gua (b) and a mixture of 100 mM Gua and 0.5 mM Echi (c).

Table 1
Assignments of the strong bands of Gua in solution

Band positions (cm^{-1})	Assignments
1573	$\nu_{C=N}$, $\nu_{C=C}$
1536	ν_{N-H}
1455	ν_{NH_2}
1371, 1327	ν_{C-N}, δ_{N-H}, δ_{C-H}
1265	τ_{N-H}
1192, 1152	δ_{C-H}, δ_{C-C}

its interaction with drugs [34,35]. The FTIR spectral characterizations of Gua and the influence caused by adding Echi were studied in PBS (pH 7.0), as shown in Fig. 7. Strong interference arisen from water molecules allowed us to examine only the region from 1600 to 1100 cm^{-1}. Because of the complicated structure of Echi, it was difficult to reflect the refined bands in FTIR spectrum of Echi (curve a), owing to the overlap of many bands. However, the spectrum of Gua showed a series of legible bands (curve b). So, the FTIR spectral information about the occurrence of interaction could be easily gained by comparing the spectrum of Gua with that of Echi–Gua complex. These bands shown in the spectrum of Gua should be assigned based on the literature [36–39]. The band at 1573 cm^{-1} was ascribed to the stretching vibration mode of C=N and C=C [37] and the 1536 cm^{-1} was contributed to the N—H stretching vibration in purine ring of Gua; the band at 1455 cm^{-1} was assigned to the NH_2 stretching and motions from the ring atoms [37]; the bands at 1371 and 1327 cm^{-1} were ascribed to the stretching vibration mode of C–N and the bending vibration mode of C—H; the band at 1265 cm^{-1} was assigned to the distorted vibration mode of N—H; the bands at 1192 and 1152 cm^{-1} were ascribed to the bending vibration mode of C—H and C—C. Assignment of the stronger bands was shown in Table 1. Curve c referred to the spectrum of Echi–Gua complex. Comparing with Curve b, we could find that there were several changes. For example, the decreased intensity of the vibrations at 1455 and 1371 cm^{-1} and new bands in the region from 1437 to 1407 cm^{-1} appeared. The new bands were assigned to the bending vibration mode of $NH_2 \cdots O$ and $CN \cdots H$. The changes in the FTIR spectra resulted only from the associate interaction between Echi and Gua. The facts that the NH_2 and C–N stretching frequency decreased and the NH_2 bending vibrations frequency increased proved two pairs of adjacent intermolecular hydrogen bonds formed. Echi could bind to both the N3 atoms and the NH_2 groups of Gua through the NH and CO of alanines (Ala-NH to Gua-N3 and Gua-NH_2 to Ala-CO).

4. Conclusions

In this work, electrochemical and spectroscopic methods were applied to investigate the interaction of Echi with Gua. The mechanism of Echi interaction with Gua was briefly discussed. By using the adsorptive transfer stripping cyclic voltammetry, it was very easy to confirm Echi–Gua associate binding. The changes in the observed adsorption and vibration spectra showed that a complex of Echi–Gua formed due to the hydrogen bond interactions between Ala-NH and Ala-CO in Echi to Gua-N3 and Gua-NH_2. The interactions were strong enough to endure washing and exposing to air convinced by electrochemistry. Electrochemical combined with spectroscopic techniques would provide a relatively easy way to better understand the underlying mechanism of Echi–Gua interaction not only from the macrocosmic aspect but also at the molecular level. It will be helpful for the development of new drugs of binding specificity by recognition of bases on the DNA double helix.

Acknowledgements

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