Int. J. Electrochem. Sci., 1(**2006**)130-138

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

Electrochemical Studies of the Interaction of Quercetin with DNA

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Received: 30 April 2006 / *Accepted:* 10 June 2006 / *Published:* 15 June 2006

The interaction of DNA with quercetin was studied using scanning electrochemical microscopy and electrochemical impedance spectroscopy at glass carbon electrode. Information such as the surface coverage of the film and apparent rate constant of the redox active probe in solution was obtained. It is deduced that quercetin interacting with DNA forms a kind of electrochemical inactive supramolecular complex. It has been pointed out that the electron transfer on the electrode surface was hindered□therefore the normalized current decreased. The estimation of the electrode coverage and hindrance provided further evidences for information of the interaction of quercetin with DNA. The kinetics constant was obtained from electrochemical impedance spectroscopy.

Keywords: Quercetin; DNA; Scanning electrochemical microscopy; Electrochemical Impedance Spectroscopy.

1. INTRODUCTION

Quercetin, one of the most abundant natural flavonoids, presents in daily food with the average human daily intake estimated to be 16mg/person [1-3]. Quercetin is of interest because of its pharmacological function. Quercetin can protect human DNA from oxidative attack in vitro [4].

In recent years, there is a growing interest in the electrochemical investigations of interactions between anticancer drugs and other DNA targeted molecules. Hendrickson etc reported 3',4'-adjacent hydroxyl groups of quercetin interaction with DNA, and they drew the conclusion that it is a two-electron and two-proton reaction [5]. As far as quercetin was concerned, it can intercalate into the double-stranded DNA, inducing DNA strand scission [6-7]. Active part of quercetin interacts with DNA, so the product is an electrochemically inactive supramolecular complex [4]. Oliveora-Brett and Diculescu suggested that quercetin binds to the double-stranded DNA where it can undergo oxidation

and showed the interaction mechanism between quercetin and DNA [8]. Jingwan Kang etc reported the information of quercetin interaction with DNA about intrinsic binding constant, binding numbers of bound speciesper DNA and interaction mode [9].

To further understand the interaction of quercetin with DNA, we studied the film on the glass carbon electrode (GCE), the product of quercetin and DNA. The film was probed in scanning electrochemical microscopy (SECM) and electrochemical impedance spectroscopy (EIS) experiments. As far as we are aware, the study represents the first example of investigation on interaction of quercetin with DNA by SECM and EIS. SECM is developed by Bard and his group, which is a very valuable technique for the study of biological system [10-11] and EIS is a sensitive technique, which monitors response of the electrochemical and surface properties of the resulting modified electrode to the application of a periodic small amplitude AC signal [12]. Analysis of the system response contains information about the interface and the charge transfer occurring at modified electrode/solution interfaces as well as their variation in time. We hope the present work could provide valuable information about the interaction of quercetin with DNA, the surface coverage of the film and the apparent rate constant of the redox active probe in solution.

2. EXPERIMENTAL PART

2.1. Reagents

Quercetin was purchased from Institute of Chemical Physics, Chinese Academy of Sciences (Lanzhou). DNA was purchased from Huamei Chemical Co. (PR China) and used as received. DNA was dissolved in doubly distilled water. Ratios of UV absorbance of DNA at 260 and 280 nm, A_{260}/A_{280}, of 1.8–1.9, indicate that the DNA was sufficiently free of protein. Potassium ferricyanide was purchased from Beijing Chemical Co. (China). The measurements were performed in the presence of a 1.0 mmol/l $K_3[Fe(CN)_6]$ as a redox probe (containing 0.1 mol/l KCl, pH 7.0). All stock solutions were stored at 4 ^{0}C and solutions were prepared using analytical grade reagents and purified water. All experiments were done at room temperature (25±1 ^{0}C).

2.2. Instrumentation

The SECM detections were taken on a CHI 900 electrochemical workstation (CH Instrument Co. Ltd, Austin, USA) at room temperature. The SECM tip is a 25 μm diameter Pt UME. Before each experiment, the tip was polished with 0.05 μm alumina and rinsed with pure water, and the solutions were purged with pure nitrogen for 5 min. The main quantitative operation was obtained from the feedback mode.

The EIS experiments were carried out on a VMP2 (Princeton Applied Research, USA) at room temperature and performed in the frequency range from 0.1 to 100K Hz at the formal potential of 220 mV, using alternating voltage of 10 mV.

All experiments were carried out with a three-electrode cell, the modified GCE, a platinum wire, and a Ag/AgCl (saturated KCl) as the working, counter and reference electrodes, respectively.

2.3. Preparation of DNA Modified Electrode

A 2 mm diameter GCE was polished with alumina powder (0.3 and 0.05 μm) and then cleaned ultrasonically in water and acetone respectively for 5 min. The pretreated glass carbon electrode was modified by transferring a drop of 1 μg/μl DNA solution onto its surface, followed by airing overnight. Then, it was soaked in double distilled water for more than 4 h to remove unabsorbed DNA. Thus, a DNA modified GCE was obtained.

3. RESULTS AND DISCUSSION

The DNA modified GCE was immerged quercetin solution for a space. Then, the GCE was rinsed with absolute ethanol and pure water, respectively, to remove quercetin was not reacted. In this way, the quercetin had interacted with DNA without any contribution from the diffusion process from quercetin in solution once the phenomenon of experiment has been changed.

3.1 Interaction of quercetin with DNA by SECM

The modified GCE was acted as substrate. The feedback detection mode to image interaction of DNA with quercetin on microcosmic domains is illustrated schematically in Fig. 1. Microcosmic domains containing immobilized DNA or the product of quercetin and DNA were bathed in a 1.0 mmol/l $K_3[Fe(CN)_6]$ solution. The interaction was imaged through monitoring the normalized current change. All values of tip current (i_T) was divided by the steady state current $(i_{T,\infty})$ to show as the normalized current. All images were obtained at approximately 10μm away from the electrode surface. The results had been normalized in order to compare with different experiments.

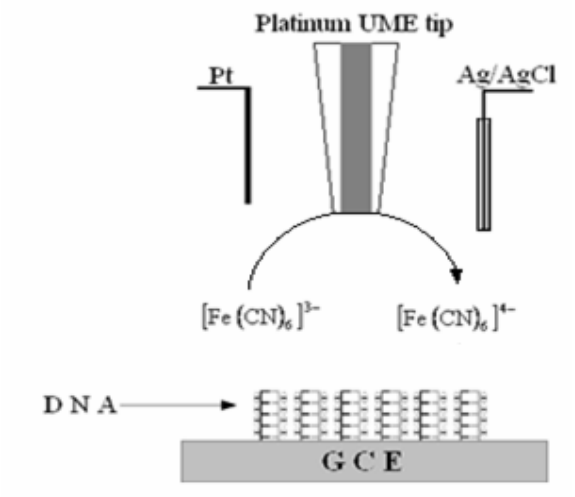

Figure 1. Schematic of the SECM feedback imaging principle. The schematic is (not to scale).

The changes occurring in the DNA during the interaction with quercetin were followed by SECM, as shown in Fig. 2, Fig. 3 and Fig. 4. Fig. 2 shows SECM obtained at the DNA modified GCE. Fig. 3 is the image of SECM obtained with the DNA modified GCE immersed into the quercetin for a space.

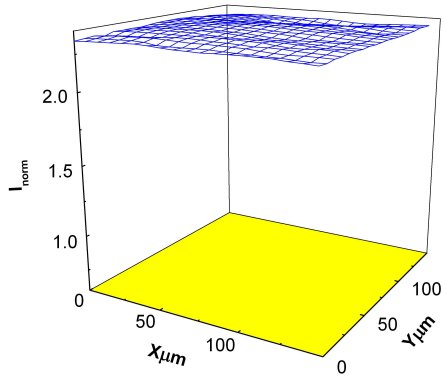

Figure 2. SECM image of DNA modified electrode in the presence of 1.0 mmol/l $K_3[Fe(CN)_6]$ with 0.1 mol/l KCl as the supporting electrolyte.

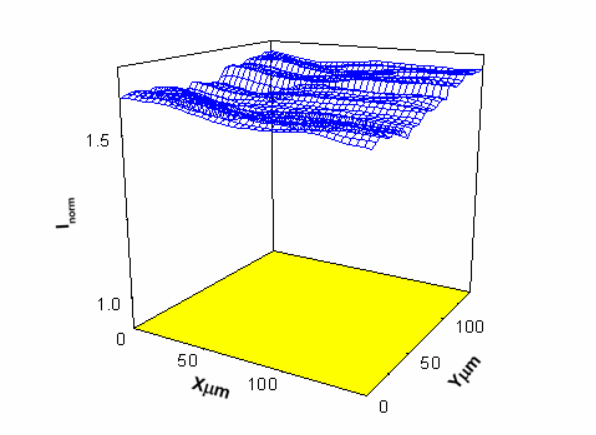

Figure 3. SECM images of the interaction of quercetin with DNA in the presence of 1.0 mmol/l $K_3[Fe(CN)_6]$ with 0.1 mol/l KCl as the supporting electrolyte.

The result obtained after DNA interacting with quercetin is in Fig.4. By comparing these images, it is clear that the images of DNA and the product of quercetin and DNA (Fig. 4) are very similar: the topographies are both regular and flat. As it is known that DNA is electrochemical inactive in this condition the image of DNA is flat (Fig. 2). It is deduced that quercetin, being shorter, probably had experienced a smaller steric hindrance during their intercalation DNA and consequently can be packed more densely. The arrangements of molecules were stability and the balance was built finally. It is deduced that quercetin interacting with DNA forms a kind of electrochemical inactive supramolecular complex. Our observations are consistent with that quercetin intercalating DNA masks the

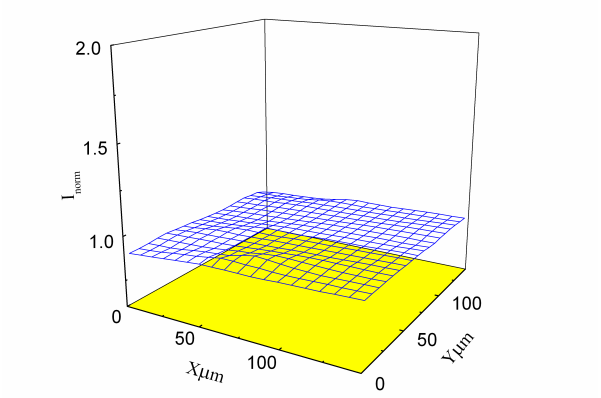

Figure 4. SECM image of the product of interaction of quercetin with DNA in the presence of 1.0 mmol/l $K_3[Fe(CN)_6]$ with 0.1 mol/l KCl as the supporting electrolyte.

electroactive site of quercetin, which reported by Zhiwei Zhu and co-workers [4]. It has been pointed out that the transfer of electron on the electrode surface was counteracted, therefore the normalized current decrease from approximately 2.50 (Fig. 2) to 1.65 (Fig. 3), then to 0.90 (Fig. 4).

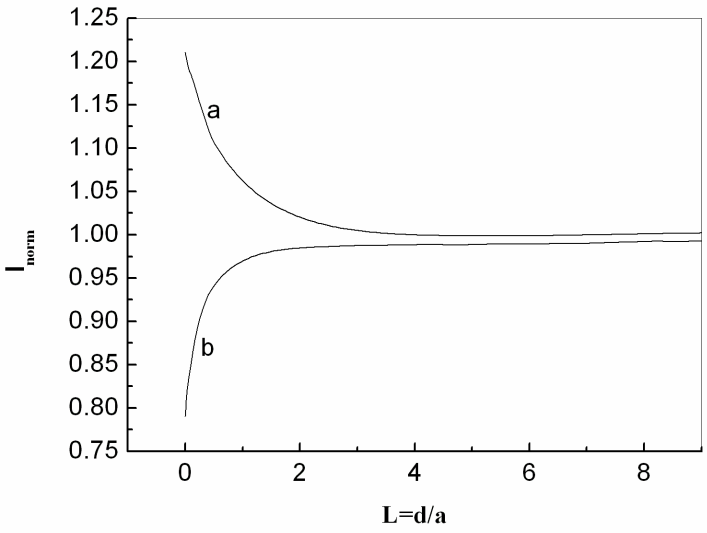

Figure 5. The approach curve of (a) the DNA modified electrode and (b) the product of interaction of quercetin with DNA in the presence of 1.0 mmol/l $K_3[Fe(CN)_6]$ with 0.1 mol/l KCl as the supporting electrolyte.

Fig. 5a and Fig. 5b are illustrating representative SECM approach curves as typically obtained in 1.0 mmol/l $K_3[Fe(CN)_6]$ solution while moving the UME tip toward the modified GCE surface, DNA and the product of quercetin and DNA, respectively. The approach curves describe the different feedback current of the DNA and the product of quercetin and DNA, respectively. In this case, the different feedback currents are due only to the quercetin incorporated into DNA film without any contribution

when quercetin interact with DNA. It is found that the approach curve is positive feedback at the DNA modified GCE (Fig. 5a), whereas is negative feedback at the product of quercetin and DNA modified GCE (Fig. 5b). Such an abrupt transition from positive to negative feedback behavior, which was consistent with the results of images, can be attributed to the electron transfer having been counteracted successfully after quercetin reaction with DNA.

3.2 Interaction of DNA with quercetin by EIS

Electrochemical impedance spectroscopy (EIS) has been applied successfully to investigate thin film which allows charge transfer between a redox active probe in the solution and the electrode surface, as they can provide valuable information about the surface coverage of the film and apparent rate constant of the redox active probe in solution. According to Randles' equivalent circuit [13] two frequency regions can be distinguished to understand the change in faradic impedance due to the presence of electroactive species at the electrode/film/electrolyte interface. We focused our attention on the interesting part of the spectrum at higher frequencies where the electrode reaction is purely kinetically controlled, and the heterogeneous charge transfer resistance R_{ct} is expected to increase due to the inhibition of the electron transfer by the quercetin and DNA complex film present on the electrode surface [14].

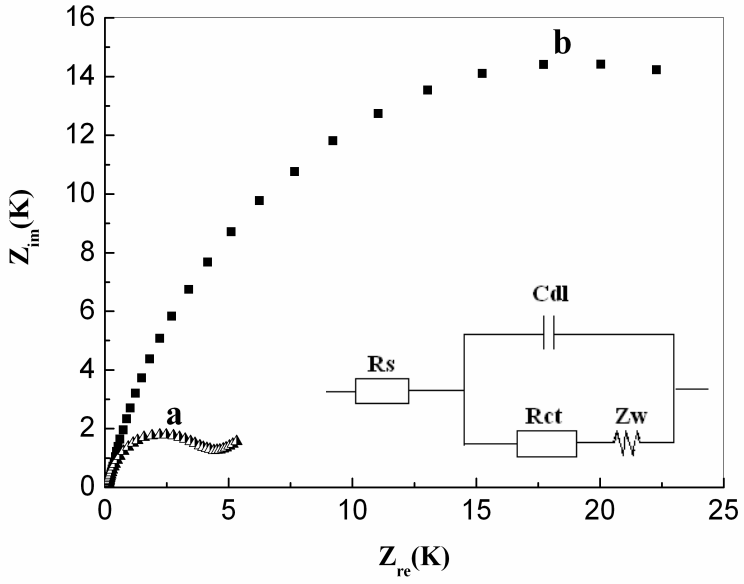

Figure 6. EIS characteristic of (a) the DNA modified GCE; (b) the product of quercetin and DNA modified GCE in the presence of 1.0 mmol/l $K_3[Fe(CN)_6]$ with 0.1 mol/l KCl as the supporting electrolyte. The electrode potential was 0.22 V vs. Ag/AgCl/KCl; the frequency range was 0.1 Hz to 100 KHz. Inset is the Randles circuit.

Fig. 6 shows the results of AC impedance spectroscopy on DNA modified GCE and the product of quercetin and DNA modified GCE in the presence of 1.0 mmol/l $K_3Fe(CN)_6$. To give more detailed information about the electrical properties of the GCE/DNA/solution, the Randles circuit was chosen to fit the obtained impedance data [15-17]. It was assumed that the resistance to charge transfer (R_{ct}) and the diffusion impedance (Z_W) were both in parallel to the interfacial capacity (C_{dl}). This parallel combination of R_{ct} and C_{dl} gave rise to a semicircle in the complex plane plot of Z_{im} against Z_{re}. A comparison of the complex impedance plots of DNA modified GCE and the product of quercetin and DNA modified GCE in Fig. 6 shows the effect of quercetin intercalating DNA on the AC response of DNA modified GCE. From this increase in the charge transfer resistance, one can calculate the approximate coverage of the GCE (θ) [15], assuming that the current is due to the defects within the film:

$$\theta = 1 - \left[R_{ct} / R'_{ct} \right] \qquad (1)$$

where R_{ct} indicates the charge transfer resistance of the electrode covered by DNA and R'_{ct} the corresponding term of the electrode covered by the product of DNA interact with quercetin. The charge transfer resistance with the product of quercetin and DNA modified GCE was found to be greater than that measured on the DNA modified GCE due to inhibition of electron transfer rate. For example, from the analysis of the spectra shown in Fig. 6a. and b. the charge transfer resistances of 4.5KΩ cm^2 and 89.4KΩ cm^2, respectively. The surface coverage (θ) was determined by impedance analysis, making use of the electroactive probe ions $K_3Fe(CN)_6$. These ions tend to penetrate through the defects of the film, therefore impedance measurements yield information about the uncovered surface fraction which can be derived from Eq. (1). One can calculate that the surface coverage of DNA modified GCE and product of quercetin and DNA modified GCE are 84.0 and 94.9%, respectively. These data indicated that quercetin intercalated DNA on the GCE surface and formed a tunable kinetic barrier counteracting the transfer of electron on the electrode surface, which was consistent with SECM results.

The rate constant of the $K_3Fe(CN)_6/K_4Fe(CN)_6$ couple can be calculated on the DNA modified GCE and the product of quercetin and DNA modified GCE from the impedance plot, since the film behaves as a microelectrode assembly leading to an expected decrease in the rate constant. Under equilibrium, the charge transfer rate constant (K_{ct}) was given by following equations:

$$K_f = \frac{i_0}{nFA[S]} \qquad (2)$$

$$i_0 = \frac{RT}{nFR_{ct}} \qquad (3)$$

$$\therefore K_f = \frac{RT}{n^2F^2A[S]} \times \frac{1}{R_{ct}} \qquad (4)$$

Where, R is the gas constant, T is the temperature, n is the number of electrons transferred in the probe reaction, F is the Faraday constant, $[S]$ is the concentration of ferricyanide, A is the geometric area (cm^2). The K_{ct} was inversely proportional to R_{ct}.

Using *R*ct, the charge transfer rate constant for the $K_3Fe(CN)_6/K_4Fe(CN)_6$ couple was obtained to be 8.93×10^{-5} cm s^{-1} (from Eq.(4)). It is found that the transfer of electron on the electrode surface was still existing because the coverage of the film for it was not full. In this case, it is reasonable to believe that the $K_3Fe(CN)_6/K_4Fe(CN)_6$ couple still can reach the GCE surface when the film become more and more packed.

4. CONCLUSIONS

In this paper we studied the electrochemical behavior of quercetin interaction with DNA by SECM and EIS. The interaction has different electrochemical information. Quercetin can intercalate DNA and consequently can be packed more densely. Quercetin interacting with DNA forms a kind of electrochemical inactive supramolecular complex. The arrangements of molecules were stability and the balance was built finally. The transfer of electron on the electrode surface was counteracted□therefore the normalized current decreased and the charge transfer resistances increased. Using R_{ct}, the charge transfer rate constant for the $K_3Fe(CN)_6/K_4Fe(CN)_6$ couple was obtained. The results provide new insight into rational drugs design and would lead us to further understanding the information of interaction between anticancer drugs and DNA.

ACKNOLEDGEMENTS

This work was supported by the Key Laboratory of Ploymer Materials of Gansu Province and the Natural Science Foundation of China (No. 20275031, 20335030), The Teaching and Research Award Program for Outstanding Young Teachers in Higher Education Institutions of MOE P.R.C., The State Key Laboratory of Electroanalytical Chemistry (SKLEAC), Changchun Institute of Applied Chemistry, Chinese Academy of Sciences.

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