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Investigation of double stranded DNA damage induced by quercetin–copper(II) using piezoelectric quartz crystal impedance technique and potentiometric stripping analysis

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Abstract

DNA damage by quercetin–Cu^{2+} was monitored in real time by piezoelectric quartz crystal impedance (PQCI) technique. In the PQCI analysis, the frequency change was caused mainly by the changes in density–viscosity of DNA solution in the damage course. The influences of DNA, Cu^{2+}, and quercetin concentrations on the motional resistance change (ΔR_m) were investigated in detail. The results showed that quercetin exhibited pro-oxidative damage at lower concentrations while anti-oxidative protection at higher concentrations, and ΔR_m exhibited a linear relationship in the DNA concentration range from 200 to 1600 μg/ml. Potentiometric stripping analysis (PSA) was also used to observe the electrochemical behavior of damaged DNA. From PSA, a new peak at 0.84 V and a higher peak at 1.06 V were discovered, which suggested that more purines were exposed to the electrode surface during the damage course. In agarose-gel electrophoresis, catalase and biquinoline were found to effectively inhibit DNA damage, therefore, a possible damage mechanism was proposed.

Keywords: Quercetin; DNA damage; Piezoelectric quartz crystal impedance (PQCI); Potentiometric stripping analysis (PSA)

1. Introduction

Flavonoids are classified into several groups, such as flavones, flavonols and isoflavones, according to their structural differences [1]. Various flavonoids are produced by agricultural plants and are a traditional constituent of human diet and traditional herbal medicine and as such potentially safe drugs candidates [2]. The average daily diet of humans contains about 1 g of flavonoids [3]. Quercetin, for example, one of the most abundant natural flavonoids, exists in various vegetables and fruits with the average human daily intake estimated to be 16–25 mg/person [4]. Quercetin has various biological activities: the inhibition of cultured cell growth, inhibitory effects on glycolysis, macromolecules synthesis, and the activity of protein kinases and ATPases [5]. However, a few of mutagenic flavonoids caused oxidative damage to cellular and isolated DNA and had been tested for carcinogenicity including Vitamin A and its derivative [6], and *N*-acetylcysteine [7]. Among them, quercetin has been reported to be a rodent carcinogen and induce tumor development in rat liver, kidney, intestine and bladder [8,9].

Many techniques have been proposed to investigate the DNA damage [10,11]. Chromatographic or electrophoretic separation of hydrolyzed samples coupled to mass spectrometry provides detailed molecular information on DNA damage [10]. Autoradiograms have been widely and successfully used to explore the function of N-aceylcysteine [7], 4-aminobiphenyl [11] on ^{32}P-labled DNA fragments. However, chromatographic method is limited by its cost, and autoradiograms are limited by their harm to the human body. What's more, it is notable that DNA damage involves a large number of time-dependent dynamic phenomena, and

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conformational change may occur during the damage process. Most of the above techniques just can analyze the products after the damage reaction, but cannot in situ monitor the damage course and provide in real time the evidence of conformational effect. Therefore, it is necessary to seek for a convenient and harmless method to monitor the DNA damage.

Piezoelectric quartz crystal (PQC) sensor is a device highly sensitive to mass deposited on its surface and the physicochemical characteristics of the surrounding medium. Since it had been successfully operated in liquid in 1980s, the PQC sensor has been widely used in various fields, such as DNA or gene analysis [12], metal detection [13], microorganism assay [14], nucleic acid and enzyme determination [15] based on its high sensitivity and convenient operation. This method provides only one parameter, the series resonant frequency of the device. Piezoelectric quartz crystal impedance (PQCI) analysis is another kind of piezoelectric sensing technique. It can provide not only the resonant frequency (f_0), but also other parameters of the quartz crystal sensor, such as motional resistance (R_m), motional inductance (L_m), and motional capacitance (C_m) and static capacitance (C_0). All of these parameters have their different physical meanings and can be obtained by fitting the experimental data of the conductance and susceptance versus the frequency to the BVD model [16,17]. Therefore, PQCI can provide multi-dimensional information about the process that occurred at a solid–liquid interface and can be used to monitor changes in various properties of the system.

On the other hand, electrochemical methods, such as differential pulse voltammetry [18] and stripping voltammetry [19], have also been employed to observe electrochemical behavior of damaged DNA. The electrochemical behavior of adsorption of DNA and electrochemical oxidation of dsDNA had been studied on carbon electrodes and showed that all bases—G, A, C and T—can be oxidized. The occurrence of oxidative damage to dsDNA by hazardous compounds leads to the breaking of the hydrogen bonds and opening of the double helix. This caused the bases to come into contact with the electrode surface and enable electrochemical detection of the oxidative damage by monitoring the oxidation of the bases [18,19]. However, few reports on detecting DNA damage were found by using potentiometric-stripping analysis (PSA). The advantage of PSA is that the efficient background correction can be realized, so well-defined peaks over a relatively flat baseline can be easily obtained, and a little amount of substrate can respond on the electrode surface.

In this work, PQCI analysis, which can perform multi-formations including f_0, R_m, and C_0, was adopted to in situ monitor DNA damage by quercetin with the aid of Cu^{2+}. The influences of the variations in DNA, Cu^{2+} and quercetin concentrations on motional resistance change were investigated. Simultaneously, PSA, which can detect a little amount of substrate, was also used to observe the electrochemical behavior of damaged DNA.

2. Experimental

2.1. Chemicals

Double stranded DNA (dsDNA, from fish sperm) was bought from Amresco Company. Catalase (from bovine liver, EC 1.11.1.6, 2970 U/mg) and was obtained from Sigma. Cetyl trimethyl ammonium bromide (CTMAB) was purchased from Fluka. Biquinoline and quercetin were from Beijing Chemical Reagent Company. All other chemicals used in this work were of analytical grade. Double-distilled water was used throughout. Buffer solution used in this work was acetic acid-sodium acetate (pH 5.2).

2.2. Apparatus

AT-cut 9 MHz quartz crystals (12.5 mm in diameter), sandwiched between two gold electrodes (5 mm in diameter) were used. One of the electrodes was contacted with solution and used as the working electrode. The experimental setup for PQCI can be found anywhere in our earlier work [20]. The conductance (G) and susceptance (B) of the piezoelectric sensor were synchronously measured by a HP 4192A LF impedance analyzer (frequency range 5 MHz–13 MHz, USA). The setup also includes an IBM computer. A user program written in Visual Basic (VB) 5.0 is used to control the HP 4192A, to acquire and analyze the admittance data. The data-collecting time, scan times, resonant frequencies and equivalent parameters were displayed on the window and recorded in corresponding files during the experiments.

PSA experiments were performed with a CH Instrument Model 660-electrochemical analyzer (Shanghai Chenhua Apparatus Company, China) and a conventional three-electrode system. Graphite encapsulated in epoxy resin was used as working electrode, with an Ag/AgCl electrode as reference electrode and a platinum electrode as auxiliary electrode. All measurements were carried out in a 10-ml detection cell and the temperature was kept at 25 ± 0.2 °C. All potentials measured in this report were with respect to Ag/AgCl.

2.3. Procedure

For PQCI analysis, the crystal was stabilized in the solution containing DNA and Cu(II) for 30 min, then certain volume of various quercetin concentration was added. The variations of the equivalent parameters were real-time monitored. For electrochemical experiments, the carbon surface was hand-polished with fine emery paper, and washed with water before use. Then, the graphite electrode was accumulated from pH 5.2 acetate buffer containing

DNA for a desired time period at an appropriate potential, followed by oxidization with a constant current of 60 μA at an initial potential of 0 V. The derivative signal (dt/dE) was used as the analytical signal. Then, a certain volume of 1 mM $CuCl_2$ and 2 mM quercetin was successively injected. The chronopotentiongrams was correspondingly recorded. The electrophoresis was run on an agarose gel (1%) for 30 min (80 V) using 0.5×TBE (89 mM tris+89 mM boric acid+2 mM EDTA) as running buffer. Before electrophoresis, all samples were incubated for 0.5 h.

3. Results and discussion

3.1. PQCI analysis of DNA damage by quercetin-Cu^{2+} system

PQCI technique is based on the equivalent circuit model of a quartz crystal as shown in Fig. 1. R_m represents the loss in mechanical energy mainly dissipated to the surrounding medium and quartz interior. Its change (ΔR_m) reflects the change in the viscoelasticity of the film and in the viscosity and density of the contacting solution. It is known that when DNA is attacked by free radicals, various damages including strand cleavage and base and deoxyribose degradation will occur and macromolecular DNA will be turned into small fragments [21], which results in the density–viscosity variation of solution that are directly related to the PQCI responses.

Curves *a* and *b* in Fig. 2 are the PQCI analysis under the addition of quercetin into the solution containing DNA with Cu^{2+} or without Cu^{2+}, respectively. As can be seen from curves *b*, the values of Δf_0 decreased and ΔR_m increased. The reason may be that few DNA molecules could be adsorbed on the electrode [22], and quercetin can insert into the double helix of DNA [23], so the mass loading increased. However, in curves *a*, Δf_0 increased evidently and then gradually reached a relatively steady value, while ΔR_m decreased simultaneously. By comparing curves *a* with curves *b*, it is reasonable to think that the changes of the PQCI parameters mainly result from the changes in the properties of the DNA solution.

The earlier applications of PQC were based on Sauerbrey equation showing a linear relationship between the resonant

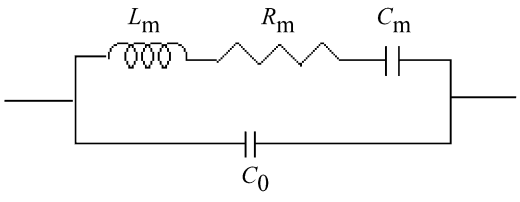

Fig. 1. The electrical equivalent circuit model for an AT-cut quartz crystal. C_0 represents the static capacitance, C_m represents the motional capacitance, R_m represents the motional resistance, L_m represents the motional inductance.

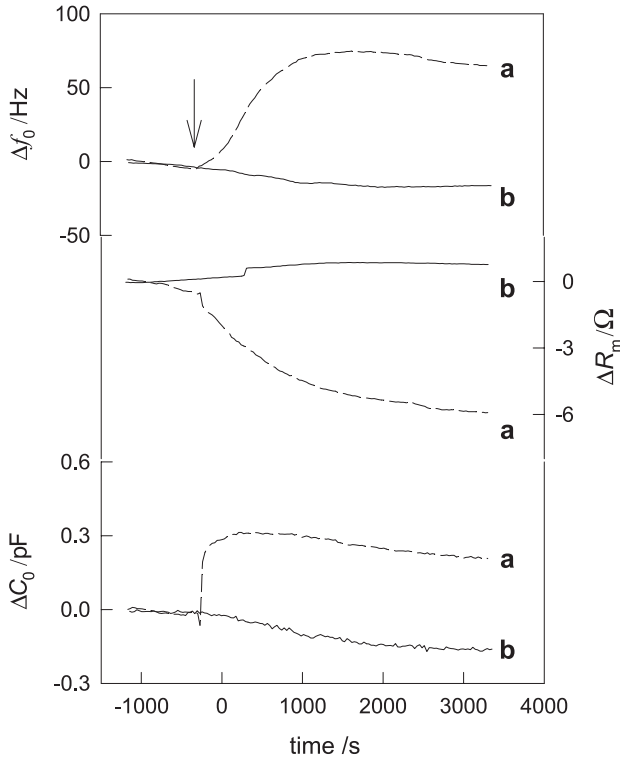

Fig. 2. The typical PQCI analysis of the addition of 25 μl 2 mM quercetin (the arrow) to the solution containing 1 mg/ml DNA and 50 μl 1 mM Cu^{2+} (curve a) or 1 mg/ml DNA alone (curve b).

frequency change (Δf_m) and the mass change. Indeed, the frequency change of PQC in viscous liquids relates not only to the mass layer, but also to the physico-chemical properties, e.g. the density and viscosity of the liquid. Studies by Martin et al. [24] indicated the effect of the variations in the density (ρ_L) and viscosity (η_L) of the liquid on the resonant frequency (Δf_L):

$$\Delta f_L = -\frac{f_0^{3/2}\Delta(\rho_L\eta_L)^{1/2}}{(\pi\rho_Q\alpha_Q)^{1/2}} \quad (1)$$

where ρ_Q and α_Q are the density (2648 kg m^{-3}) and the shear modulus (2.947×10^{10} N m^{-2}) of quartz, respectively; f_0 is the fundamental frequency of quartz. Thus, the total frequency changes (Δf_0) should be a sum of Δf_m and Δf_L. For a PQC with one side contacting with liquid, the solution viscosity–density effects on R_m can be deduced from Martin's model [24],

$$\Delta R_m = \frac{4f_0L_Q(\pi f)^{1/2}\Delta(\rho_L\eta_L)^{1/2}}{(\bar{c}_{66}\rho_Q)^{1/2}} \quad (2)$$

where L_Q is the inductantce (7.86 mH) of the PQC in air, $\bar{c}_{66}$ is lossy piezoelectrically stiffened elastic constant (2.957×10^{10} N m^{-2}), and f_0 can be used approximately in the calculation instead of f with an error below ca. 0.3%. For a viscous liquid, the relationship between Δf_0 and ΔR_m can

be obtained from Martin's equation and has been validated experimentally [24],

$$\Delta f_0 = \frac{\sqrt{\bar{c}_{66} f_0}}{4\pi L_Q \sqrt{f\,\alpha_Q}} \Delta R_m \approx -\frac{\Delta R_m}{4\pi L_Q} \quad (3)$$

For a 9 MHz crystal utilized in this work, the theoretical slope of Δf_0 and ΔR_m calculated from Eq. (3) is −10.1 Hz Ω^{-1}, i.e., the changes in solution density and viscosity equivalent to a ΔR_m of 1 Ω are able to cause a Δf_0 of 10.1 Hz.

From curves *a* in Fig. 2, the total Δf_0 and ΔR_m are 64.77 Hz and 5.91 Ω, respectively, so the ratio of Δf_0 and ΔR_m in this reaction process can be estimated as 10.96 Hz/Ω. The result is very close to the theoretical calculation, with a deviation of only 8.51%. According to the above deduce on the responses of the PQC sensor to the density–viscosity change, it is demonstrated that the changes of PQC in the present work are chiefly due to the variation in density–viscosity of the tested solution caused by the degradation of the DNA macromolecules. The deviation of the experimental value of Δf_0 and ΔR_m from the theoretical value is so small that ΔR_m can be used to reflect the information in the damage course.

On the other hand, the shape of the ΔR_m vs. time curve suggests an exponential upgrade. Using the nonlinear fitting program embedded in Sigmaplot 2.0, the frequency change can be represented as a first-order reaction kinetic equation as following:

$$\Delta R_m = \Delta R_{max}\left(1 - e^{-kt}\right) \quad (4)$$

where ΔR_{max} is the finite frequency change after a long period of time of reaction and k is the first-order rate constant (s^{-1}). The values of these parameters obtained are: k=3.73×10^{-3} s^{-1}, ΔR_{max}=−6.07 Ω and the relative sum of the residual square, q_r, was 8.91×10^{-3}. It can also be found that the motional resistance shift reaches a plateau after 15 min. Furthermore, according to the Eq. (2), ΔR_m due to the change in density–viscosity of the DNA solution can be obtained: for a 9-MHz AT-cut PQC used in this work, ΔR_m (Ω)=−1.7×10^3 $\Delta(\rho_L\eta_L)^{1/2}$ (g cm^{-2} s^{-1}); i.e., a 0.1% change in $(\rho_L\eta_L)^{1/2}$ will result in a 1.7-Ω shift in the PQC motional resistance.

At the same time, the single stranded DNA (ssDNA) was prepared from dsDNA by being heated in boiling water and then immediately cooled in ice water. Supposing dsDNA was fully denatured during this process, for 1 mg/ml DNA, the motional resistances of the dsDNA and ssDNA were measured, respectively, and ΔR_m was calculated as −20.15 Ω (defined as $\Delta R_{m,\,tot}$). Therefore, assuming the damage percentage as the ratio of ΔR_m in dsDNA damage course with $\Delta R_{m,\,tot}$, the damage degree can be estimated as 30.1%, that is to say, for 1 mg/ml DNA, about 30.1% dsDNA was damaged by quercetin–Cu(II).

C_0, originated from the two parallel plate metal electrodes on the quartz crystal surfaces, is predominately determined by the dielectric property of the quartz [25]. In a conductive liquid, C_0 is related to the capacity and structure of the electrical double layer at the charged interface. Its change (ΔC_0) will provide information on the capacity and structure of the interface. For curve *b*, considering the instability of DNA adsorption, the interaction of quercetin with DNA [23] can cause the descent of DNA from the electrode surface, so the capacity of interface decreased. However, for curve *a*, with the aid of Cu^{2+}, the bulk DNA solution was changed by quercetin, so more purins and pyridines were exposed to the solution and changed the interface state, which caused an increase in capacitance [20].

3.2. Influence of Cu^{2+}, quercetin and DNA concentration on DNA damage

To investigate the damaging effect of Cu^{2+}on DNA, Cu^{2+} solutions of different concentrations were injected in the system containing fixed concentrations of DNA and quercetin (Fig. 3). It is shown that with the increase in Cu^{2+} concentration in the range between 5 and 30 μM, ΔR_m decreased; and ΔR_m did not decrease further when the Cu^{2+} concentration was over 30 μM. It indicated that a trace of Cu^{2+} could accelerate the damage course in the presence of quercetin. However, Cu^{2+} alone, even in the great amount, caused no or little damage to DNA. It has been reported that morin and naringenin, like quercetin can induce lipid peroxidation and DNA breaks in isolated rat liver nuclei, and metal ions appear to stimulate these reactions, probably because of their redox reactions with these polyphenolic flavonoids. Therefore, it is suggested that Cu^{2+} probably functions as a catalyst in the oxidative damage system.

Fig. 4 shows the influence of varying quercetin concentration on DNA damage in the system under fixed concentration of Cu^{2+} and DNA. It can be found that ΔR_m decreases when the quercetin concentration increases in the range from

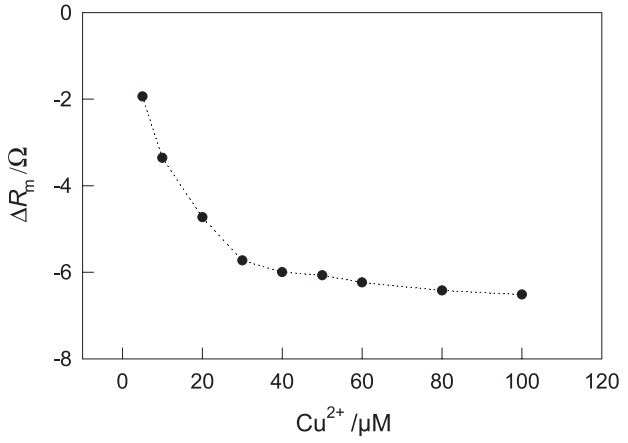

Fig. 3. Effect of Cu^{2+} concentration on end-point motional resistance change (ΔR_m) in the solution containing 1 mg/ml DNA and 50 μM quercetin.

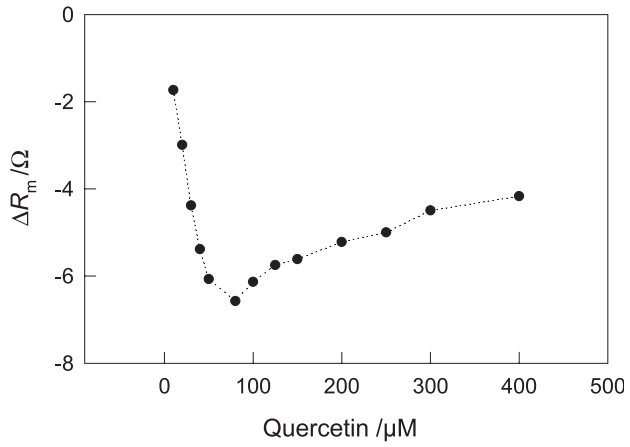

Fig. 4. The influence of quercetin concentration on the end-point motional resistance change (ΔR_m) in the solution containing 1 mg/ml DNA and 50 μM Cu^{2+}.

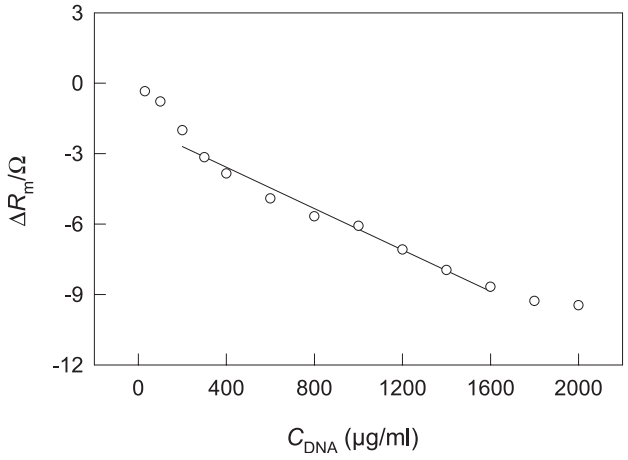

Fig. 5. The relationship of the end-point motional resistance change (ΔR_m) with DNA concentration. The solid line (—) is the calibration curve for ΔR_m vs. C_{DNA}.

10 to 75 μM. However, after the concentration is over 75 μM, a conflicting trend is observed, i.e., ΔR_m increases with the continuous increase in quercetin. In the PQCI analysis, ΔR_m is relative to the density–viscosity change of the DNA solution. The increase of ΔR_m from 10 to 75 μM suggests an increase in density–viscosity change of the DNA solution, which indicates that the DNA damage is gradually aggravated with the increase in quercetin concentration. However, the decrease of ΔR_m after 75 μM suggestes that the "excess" quercetin inhibits the damage reaction instead of enhancing it, which may mainly caused by the quenching of some radicals produced at the beginning. This is consistent with other flavonoids that have also been reported to show a pro-oxidation/anti-oxidation "threshold" [26,27]. Their polyphenolic molecular structures may be responsible for their autoxidation in the presence of oxygen and transition metals including iron or copper, so reactive oxygen species are generated, which is responsible for their pro-oxidant behavior. On the other hand, they can also react with oxygen free radicals produced by other compounds because of their radical-trapping properties, which would account for their antioxidant behavior. Thus, these molecules can act as both pro- and antioxidants, depending on the redox state of their biological environment [6,7]. If the concentration is high enough, quercetin can behave as antioxidants, considering it can adsorb the radical produced in the damage course and inhibits DNA damage.

Fig. 5 exhibits the effect of DNA concentration on the motional resistance response of PQC to DNA damage in the solution containing fixed concentration of Cu^{2+} and quercetin. ΔR_m decreased with the increase of DNA concentrations, and especially showed a linear relation to the concentration of DNA in the range of 200–1600 μg/ml. The regression equation is as following:

$$\Delta R_m = -1.81 - 4.40 \times 10^{-3} C_{DNA} (r = 97.7\%)$$

where C_{DNA} is DNA concentration (in μg/ml). Furthermore, from the curve, ΔR_m does not change much at DNA concentrations below 200 μg/ml or above 1600 μg/ml. The reason may be that for the lower concentration, the little viscosity–density change due to DNA damage could not be successfully detected by the PQC sensor; while for the higher concentration, the limited amounts of quercetin and Cu^{2+} could not cause further damage to the DNA, thus the curve behaves a reverse sigmoid shape.

3.3. PSA of DNA damage

From the above experiments, considering the effective signal in the PQCI analysis, larger amount of DNA (≥200 μg/ml) should be used. Thus, in the following course, PSA experiments, which can detect small amount of the analyte by accumulation, were performed to further investigate and validate DNA damage by quercetin–Cu^{2+}. Fig. 6 was the

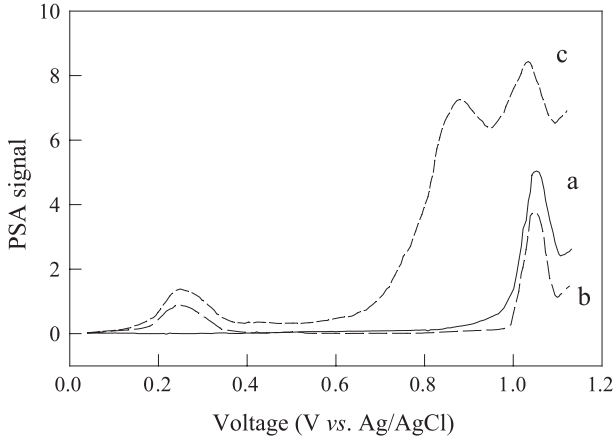

Fig. 6. The baseline corrected chronopotentiograms of 10 μg/ml dsDNA (curve a) at graphite electrode in pH 5.2 acetate buffer and the successive addition of 5 μl 1 mM Cu^{2+} (curve b) and 5 μl 1 mM quercetin (curve c). Immersion for 15 min, and then accumulation for 300 s at 0 V. Stripping conditions: initial potential, 0 V; ultimate potential, 1.2 V; constant current, 60 μA.

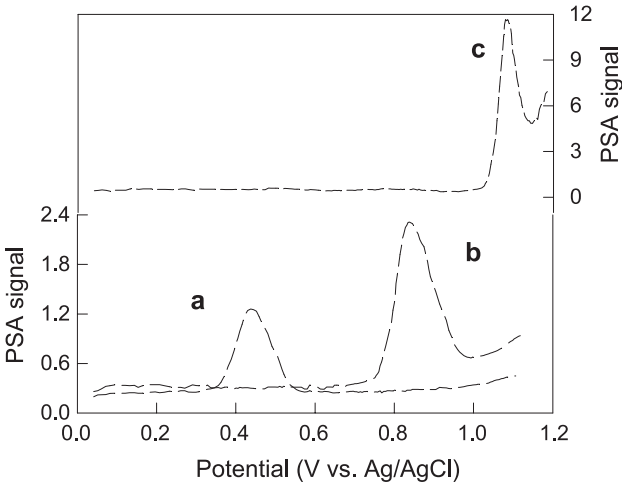

Fig. 7. The baseline corrected chronopotentiograms of 2 μM quercetin (a), guanine (b) and adenine (c). Immersion for 15 min, and then accumulation for 300 s at 0 V. Stripping: initial potential, 0 V; ultimate potential, 1.2 V; constant current, 60 μA.

baseline-corrected chronopotentiograms for the 10 μg/ml dsDNA in the background solution (*a*) and after the addition of 5 μl 1 mM $CuCl_2$ (*b*) and 1 mM quercetin (*c*), respectively. From curve *a*, a well-defined oxidation peak at 1.06 V can be discovered. In curve *b*, a new peak at about 0.28 V appeared due to Cu^{2+}, and the peak at 1.06 V was weakened, the reason of which may be that Cu(II) can bind with DNA at specific sites, but the association constant was weak (~10^4 M^{-1}) [28]. After the addition of quercetin, in curve *c*, the peak at the potential of 1.06 V is much higher, accompanied by the appearance of a new peak at about 0.84 V. However, the increase of the peak at 0.84 V was much higher that at 1.06 V, which may be responsible for the special structure of this DNA. In DNA electrochemistry, dsDNA can only give minimal voltammetric signals, but damaged DNA exposes more bases to the electrode and oxidative peaks can be relatively easily observed [18,19].

To investigate what these peaks belong to, the PSA experiments were further performed in the buffer solution only containing quercetin, guanine and adenine, respectively. Fig. 7 shows the base-line corrected chronopotentiograms for 2 μM quercetin (*a*), guanine (*b*) and adenine (*c*). The oxidative potentials for quercetin, guanine and adenine are at 0.42, 0.85 and 1.08 V, respectively, and the PSA signal for adenine is much higher than that of guanine at the same concentration. Similar results have been found by Oliveira-Brett using differential pulse voltammetry [29], and other authors using square wave voltammetry [30]. Therefore, The peak of curve *a* in Fig. 6 is due to the oxidation of adenine in dsDNA chain, however, no peak of guanine is found in curve *a*. In curve *c*, both the peaks of guanine and adenine are found and the adenine peak is higher than that in curve *a*. In the presence of Cu^{2+} and quercetin, the strand cleavage and base degradation of dsDNA take place, so more bases are exposed and can be detected at the electrode surface. The results can further prove that dsDNA is indeed damaged by quercetin with the aid of Cu^{2+}.

In this work, we have also found that cationic surfactant can affect DNA damage. Complex formation between cationic surfactants and DNA has been studied extensively in recent years. This is due to the increasing interest for using cationic liposomes as a possible way for in vivo gene transfer. To further investigate the DNA function in the damage cause, CTMAB, a cationic surfactant, was continually added to the detection cell containing 10 mg/l dsDNA, 5 μM quercetin and 5 μM Cu(II). The correspondence of PSA signal at 0.84 V with CTMAB concentration is shown in Fig. 8. It can be seen the PSA signal decreased with the increase of CTMAB concentration and the response is fitted to a two-exponential curve. The interaction of cationic lipids with DNA is known to induce condensation and subsequent precipitation of the condensate [31,32]. Therefore, surfactants can exchange with counterions condensed at the DNA chain by the electrostatic action of cationic head groups with the negatively charged phosphate sites, and then if the additional CTMAB was further added, the solution turned cloudy, which suggested that DNA-CTMAB precipitation was formed [33]. The complex of DNA-CTMAB may protect the DNA strands from further reaction, thus DNA damage was hindered.

3.4. Possible mechanism of DNA damage by quercetin with the aid of Cu(II)

From the above-mentioned results by PQCI analysis and PSA, and other previous reports [6,26,34], it can be deduced that the quercetin–Cu^{2+} system is a source of $HO^{\cdot}$ radical that can cause oxidative damage to DNA. To further verify such conclusion and explore the mechanism of DNA damage, electrophoresis analysis was also adopted and the results are shown in Fig. 9. From lane 2 and lane 3,

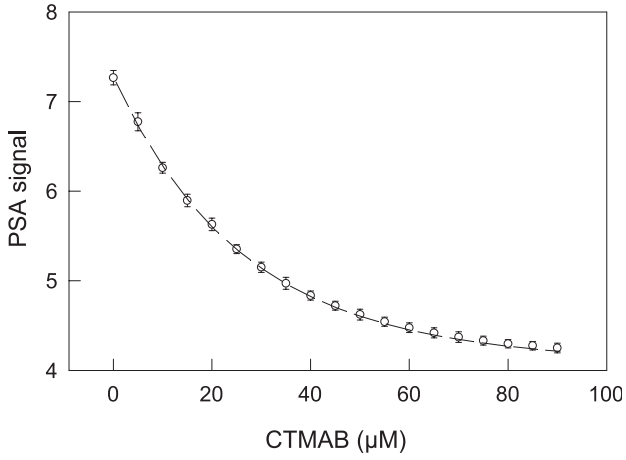

Fig. 8. The correspondence of PSA signal at 0.84 V with CTMAB concentration (*C*). The dashed line is the fitting curve: PSA signal= $4.03+2.84e^{(-C/24.92)}+0.37e^{(-C/87.67)}$ (q_r=1.03×10^{-5}).

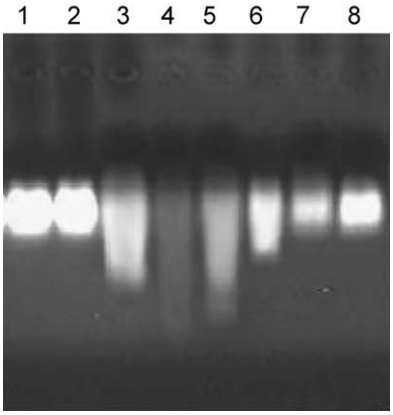

Fig. 9. Agarose-gel electrophoretic analysis for DNA (1 mg/ml) degradation. Lane 1, DNA alone; lane 2, DNA+50 μM quercetin; lane 3, DNA+50 μM Cu^{2+}+50 μM quercetin; lane 4, DNA+50 μM Cu^{2+}+50 μM quercetin+0.3% H_2O_2; lane 5, DNA+50 μM Cu^{2+}+80 μM quercetin; lane 6: DNA+50 μM Cu^{2+}+120 μM quercetin; lane 7, DNA+30 μM Cu^{2+}+50 μM quercetin+10 μM biquinoline; lane 8, DNA+50 μM Cu^{2+}+50 μM quercetin+10 U catalase.

quercetin caused no damage to dsDNA without Cu^{2+}; however, dsDNA damage was found in the presence of quercetin and Cu^{2+}. Compared lane 3, lane 5 and lane 6, different concentrations of quercetin will cause different phenomena. From lane 4, the addition of H_2O_2 nearly caused the absolute DNA degradation, and from lane 8, obvious inhibition of DNA damage was observed after being incubated with catalase, suggesting that H_2O_2 has a function on the damage course. Lane 7 shows the influence of biquinoline on DNA damage, and the result shows that DNA damage is greatly inhibited. It is known that biquinoline is an effective chelating agent for Cu(I), so the protective effect by biquinoline might result from the removal of Cu(I) due to the affinitive chelation of biquinoline with Cu(I), which imply that Cu(I) is an potential factor for DNA damage. According to earlier reports [3,6,7,11,24], a possible mechanism may be as following: firstly, semiquinone radical can be formed by the reaction of metal with quercetin; then, the radicals and metal ion react with oxygen to generate O_2^-, which is dismutated to H_2O_2. H_2O_2 can pass though the cellular membrane and induce OH formation by further reaction with metal ion, which can cause DNA damage.

Therefore, quercetin can not only function as antioxidant by scavenging free radicals, but also be used as pro-oxidant in the presence of Cu^{2+} to cause potential carcinogenicity. However, in healthy human body, it is not necessary to be worried since the physiological environment can adjust automatically. Firstly, the existence of efficient antioxidant enzyme such as catalase and superoxide dismutase can scavenge the free radicals; secondly, at normal physiological pH value, the concentration of Cu^{2+} is very slow since most Cu^{2+} is stably bound to some biomacromoleules due to its high affinity for organic ligands [35]. But for some pathologic disorder instances, a high intake of quercetin is proposed since Cu^{2+} can dissociate from its complexes with the changes of pH values [35].

4. Conclusion

DNA damage was monitored with quercetin in the presence of Cu^{2+} by using PQCI and PSA. In PQCI analysis, the frequency change was mainly caused by the changes in density–viscosity of DNA solution in the damage course. The influences of DNA, Cu^{2+}, and quercetin concentrations on the motional resistance change were investigated, and the results showed that quercetin exhibited pro-oxidative damage at lower concentrations while showed anti-oxidative protection at higher concentrations, which was in good agreement with other authors. In PSA, a new peak at 0.84 V and a higher peak at 1.06 V were discovered, which suggested that more purines were exposed to the electrode surface during the damage course. By monitoring the inhibition of catalase and biquinoline on DNA damage and the similar results by other authors, a possible mechanism was performed.

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