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Bioelectrochemistry 72 (2008) 169–173
Bioelectrochemistry
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The electron transfer reactivity of kaempferol and its interaction with amino acid residues

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Received 1 February 2007; received in revised form 14 September 2007; accepted 6 February 2008
Available online 14 February 2008

Abstract

In this work, the electron transfer reactivity of kaempferol was studied and the interaction in vivo between kaempferol and protein was simulated. Dimethylsulfoxide (DMSO) as an aprotic solvent was employed to simulate the specific environment. Various residues of amino acids were used to study the effect of the amino acids in the active site of protein on the electron transfer reactivity of kaempferol. Experimental results revealed that the redox activity of kaempferol was different in aprotic medium DMSO from that in water, and a new redox process was further found. Of all the residues tested, nitrogenous nucleophile, for example, imidazole, was observed to be able to facilitate the electron transfer of kaempferol, and the mechanism was also proposed. This work might provide a simple model to study the electron transfer reactivity of some small active organic molecules, especially medicines, in specific environment, which might approach a more accurate understanding of the activity of some medicines in vivo.

Keywords: Kaempferol; Imidazole; Amino acid residues; Simulation; Electron transfer

1. Introduction

Flavonoids are a large group of natural polyphenolic products that are ubiquitous in vegetables and fruits, and are components in human diet. They are famous as potent antioxidants [1]. Besides, flavonoids possess anticancer, antiviral, anti-inflammatory and anticoagulant activities [2]. As a typical kind of flavonoids, kaempferol (3,4′,5,7-tetrahydroxyflavone, chemical structure shown in Fig. 1) is also reported to exhibit tyrosinase inhibitory activity [3], protein tyrosinase kinase inhibitory activity [4] and alkaline phosphatase stimulating activity, etc [5]. As is known, these activities often begin with interaction between the small molecules here kaempferol and various proteins especially enzymes. And thereby, downstream signal transduction can be induced. So, studies on the interaction between protein and the small active organic molecules, especially the medicinal herbs, have attracted more and more attention. And, various methods have been employed [6].

An electrochemical method has been proven to be a powerful method to study the electron transfer process and electrochemical reaction mechanism [7]. In fact, flavonoids have also been studied by using electrochemical methods. For example, the electrochemical behavior of kaempferol has been studied by Jørgensen and Kusu in acetonitrile and neutral buffer solution, respectively [8,9]. However, these studies focus on the electrochemistry of flavonoids. To our knowledge, the electrochemical behavior of flavonoids in a simulated environment where they will interact with proteins has not been reported.

In this work, we simulate the environment in vivo where interaction between kaempferol and protein occur to study the electron transfer reactivity of kaempferol. Dimethylsulfoxide (DMSO) as an aprotic solvent is employed to simulate the specific environment for the active region of protein. Various residues of amino acids have been used to study the amino acids' effect in the protein's active site on the electron transfer reactivity of

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doi:10.1016/j.bioelechem.2008.02.001

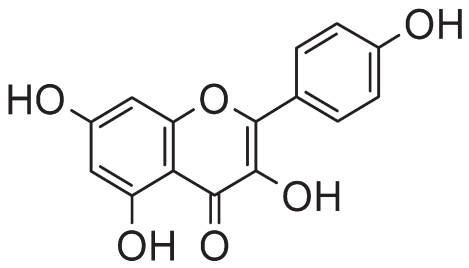


Fig. 1. Chemical structure of kaempferol.

kaempferol. For comparison, similar experiments have also been carried out in protic solvent water.

2. Experimental

2.1. Reagents and apparatus

Kaempferol (98%) was obtained from DELTA Information Centre For Natural Organic Compounds (Anhui, China), and was used as received. Imidazole 99%, isopropylamine, arginine, glycine, acetamide, *n*-tetrabutylammonium hexafluorophosphate (n-Bu_4NPF_6) 98% and DMSO containing less than 0.1% water were Aldrich chemicals. All other reagents were of analytical grade. Double distilled water was purified with a Milli-Q purification system (Branstead, USA) to a specific resistance of >18 MΩ cm.

Electrochemical experiments were performed on a VMP Potentiostat (PerkinElmer, USA) with a three-electrode system. In a one-compartment (volume 10 ml) glass cell, a pyrolytic graphite (PG) electrode as the working electrode, a saturated calomel reference electrode (SCE) and a platinum wire auxiliary electrode were used for the measurements. All the following potentials reported in this paper were versus SCE. UV–vis spectroscopy was performed using a UV-2550 spectrophotometer (Shimadzu, Japan).

2.2. Electrochemical measurements

The PG electrode was first polished with rough and fine sand papers. Then its surface was polished to mirror smoothness with alumina powder (particle size of about 0.05 μm)/water slurry on silk. Finally, the electrode was thoroughly ultrasonically washed first with ethanol and then with double distilled water both for 3 min, respectively. The polished PG electrode was tested with cyclic voltammetric scanning in 0.1 M NaCl solution in a range from −200 mV to 800 mV. If a reproducible voltammetric wave could be obtained, the electrode was ready for further use. Otherwise, the electrode should be pretreated again.

DMSO containing 50 mM n-Bu_4NPF_6 and double distilled water containing 0.1 M NaCl were used as aprotic and protic eletrolyte, respectively. The test solution was firstly bubbled thoroughly with high purity nitrogen for 5 min. Then a stream of nitrogen was blown gently across the surface of the solution in order to maintain the solution anaerobic throughout the experiment. Cyclic voltammetry was employed to monitor and record the electrochemical signals related to kaempferol. All experiments were performed at room temperature of 25±2 °C. Each experiment was replicated for at least three times.

2.3. UV–vis spectroanalysis

Spectrophotometric analysis of kaempferol alone or with an amino acid single residue in DMSO or water solutions were also conducted. 100 μl of DMSO or water containing 0.05 mM

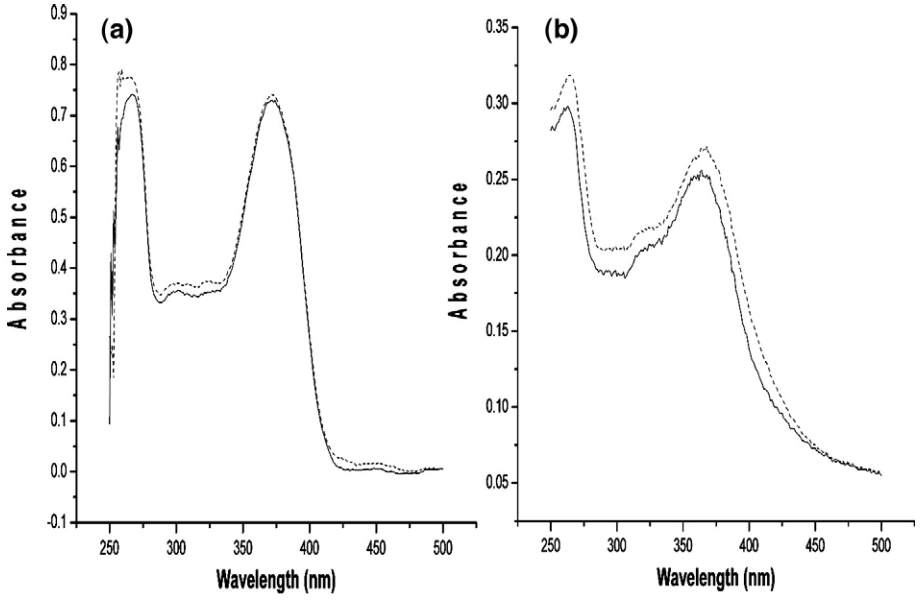


Fig. 2. UV–vis spectra of 0.05 mM kaempferol in (a) DMSO or (b) water along with (dash line) or without (solid line) 0.2 mM imidazole.

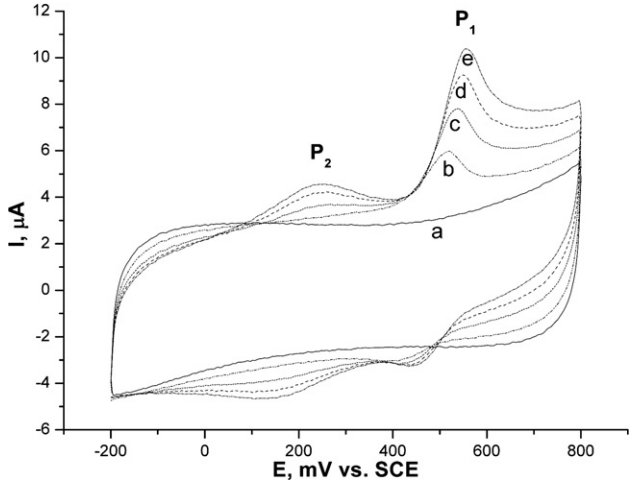


Fig. 3. Cyclic voltammograms of different concentrations of kaempferol in DMSO containing 50 mM n-Bu_4NPF_6. (a) 0 mM, (b) 0.1 mM, (c) 0.2 mM, (d) 0.3 mM, (e) 0.4 mM. Scan rate: 100 mVs^{-1}. Accumulation time: 30 s.

kaempferol and an amino acid single residue was first bubbled thoroughly with high purity nitrogen for 5 min. The sample was then immediately analyzed with the spectrophotometer.

3. Results and discussion

3.1. UV–vis absorption spectra of kaempferol

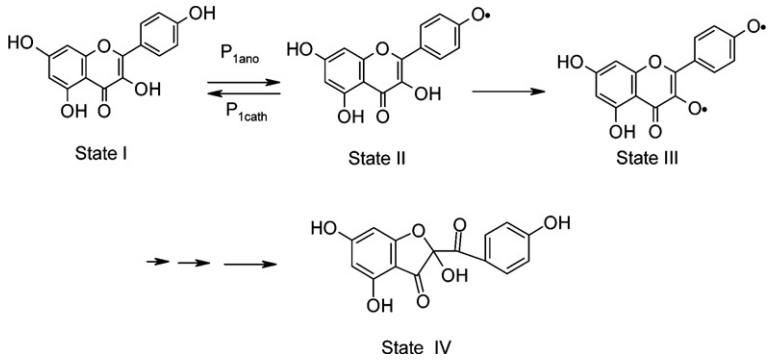
UV–vis spectroscopy was employed to study whether there are any structural changes of kaempferol in aprotic and protic solvents in the presence of some residues of amino acids. Results show that UV–vis absorption spectra of kaempferol remain approximately unchanged with different kinds of amino acid residues (Fig. 2 shows the case for imidazole). We conclude that there is no significant effect of residues of amino acids on the ring structure of kaempferol. Certainly, this does not necessarily exclude the possibility that the residues affect kaempferol through some weak chemical bonds, which is revealed by the following electrochemical experiments.

3.2. Electron transfer reactivity of kaempferol in DMSO

As is shown in Fig. 3, in the low concentration range, kaempferol shows only one redox peak couple (P_1), which are located at 520 mV (P_{1anod}) and 450 mV (P_{1cath}), respectively. When increasing the concentration of kaempferol, we can observe that another redox peak couple (P_2) appears. The new anodic and cathodic peaks are located at about 250 mV (P_{2anod}) and 160 mV (P_{2cath}), respectively. According to a previous report [8,9], the P_1 couple can be regarded as the response of 4′-hydroxyl group on the B-ring (Scheme 1). However, the P_2 couple has not been reported yet. Interestingly, when we set the potential scan range from 0mV to 500mV to only observe P_2, to our surprise, P_2 disappears. Only when P_{1anod} is observed, P_2 can be obtained (Fig. 4). So, we can conclude that the appearance of P_2 is dependent on P_1 and the first oxidation state of kaempferol (state II in Scheme 1) plays an important role in the following redox process. Considering that further oxidation beyond state II needs a higher potential [10,11], P_2 cannot be the redox peaks associated with the state II/III transition. With reference to the other studies on the other medicinal herbs [12,13], we propose that it should be the phenoxyl radical of state II that attacks the original species in state I, which produces a dimer that can be subsequently oxidized at a potential less positive than that of kaempferol oxidation.

3.3. Effect of imidazole on kaempferol

In order to exclude other unnecessary influence, we use a relatively low concentration of kamepferol to study the effect of various residues of amino acids on kamepferol. Imidazole, which is one of the most important residues in the active site of protein, is initially employed. The cyclic voltammograms (CVs) for 0.15 mM kamepferol in DMSO with imidazole are shown in Fig. 5. With the increase of the concentration of imidazole, the current of the original peaks of kaempferol decreases. Simultaneously, a new redox peak couple appears and keeps increasing with the imidazole concentration. After addition of enough imidazole, the concentration of which will be 0.54 mM, we observe that the original redox peaks of



Scheme 1. The electrode reaction mechanism of kaempferol.

kaempferol completely disappear. The new redox peak couple is located at a potential about 150 mV, which is less positive than the original one.

The effect of imidazole on the electrochemical behavior of kaempferol may be explained in the following two step pathway:

$$QH \rightarrow Q^{\bullet} + H^{+}e^{-} \quad (1)$$

$$Q^{\bullet} + RNH \rightarrow QNR + H^{+} + e^{-} \quad (2)$$

where QH is kaempferol, $Q^{\bullet}$ is the phenoxyl radical (state II in Scheme 1) and RNH represents imidazole as a nucleophile. By oxidation of QH, $Q^{\bullet}$ produced at the electrode surface are likely to be attacked by imidazole, which serves to decrease the Gibbs energy for the oxidation of QH in Eq. (1). In other words, imidazole accelerates the electron transfer of kaempferol. For comparison, if water containing 0.1 M NaCl is used as the protic solvent, imidazole cannot accelerate the electron transfer of kaempferol, since H_2O instead of $Q^{\bullet}$ becomes the substrate of the nucleophile imidazole.

3.4. Effect of nitrogenous residues on kaempferol

Isopropylamine, arginine, acetamide and glycine, are further employed to study the effect of nitrogenous residues of amino acids on the electron transfer reactivity of kaempferol in DMSO. Isopropylamine, arginine, and glycine, except acetamide, can give a similar effect on kaempferol just like imidazole in aprotic solution (data not shown). However, in protic solution, to our surprise, accelerated redox process appears also in the case of isopropylamine and arginine. The reason might be that the mechanism of the accelerated redox process in aprotic and protic solutions are different. In protic solution, isopropylamine and arginine are all protonated, that is to say, H_2O instead of $Q^{\cdot}$ is the substrate of them. As amines, isopropylamine and arginine can react with the H^{+} which is a production from the oxidation of kaempferol, thus decrease the Gibbs energy for the oxidation of kaempferol. For the glycine case, because glycine is neutral in water, it cannot neutralize the H^{+}, thus cannot decrease the Gibbs energy for the oxidation of kaempferol. Consequently no accelerated redox peaks can be observed.

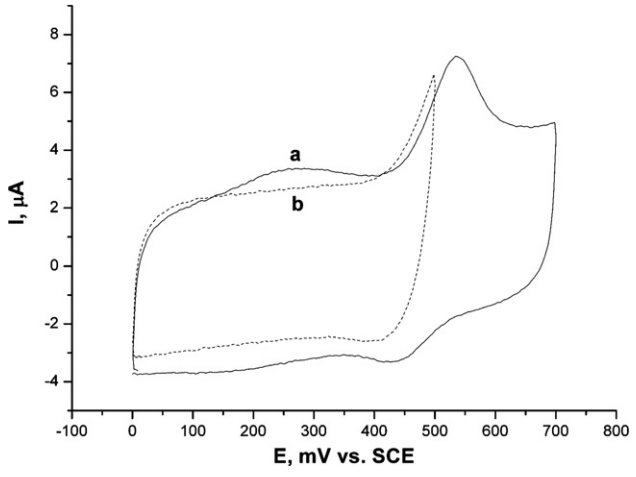


Fig. 4. Cyclic voltammograms of 0.2 mM kaempferol. Scan range: (a) 0 – 700 mV, (b) 0 – 500 mV. Others same as in Fig. 3.

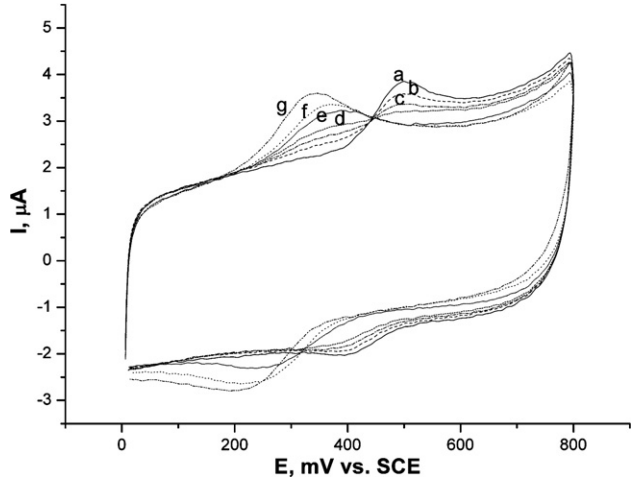


Fig. 5. Cyclic voltammograms of 0.15 mM kaempferol with different concentrations of imidazole. (a) 0 μM, (b) 20 μM, (c) 40 μM, (d) 60 μM, (e) 130 μM, (f) 200 μM, (g) 540 μM. Others same as in Fig. 3.

Acetamide, which is a residue of glutamine or asparagine, is found to have no effect on the voltammetric behaviour of kaempferol in both the aprotic and protic solutions. Though acetamide has an amino group, its nucleophilicity is greatly depressed by the acyl group, and cannot work as a nucleophile to attack kaempferol. So we can conclude that nucleophilicity of the residues of amino acids is important in accelerating the electron transfer reactivity of kaempferol.

3.5. Effect of other residues of amino acids on kaempferol

The effect of ethanethiol, ethanol, phenol and acetic acid, which are the residues of Cys, Ser, Thr, Tyr, Asp and Glu, was also examined. In all of these cases, no effect on the voltammetric behaviour of kaempferol was found. These results are in accordance with the principle we have proposed above.

4. Conclusions

This study has revealed that the redox reactivity of kaempferol in aprotic medium is different from that in water. In the aprotic medium DMSO, two redox peak couples can be observed for the high concentrations of kaempferol, and the appearance of P_2 is dependent on P_1. The first oxidation state of kaempferol plays an important role in the following redox process. Of all the residues tested, nitrogenous nucleophile such as imidazole, isopropylamine, arginine and glycine have been observed to be able to facilitate the electron transfer of kaempferol in aprotic solution. In protic solution, accelerated redox process appears only for the case of isopropylamine and arginine. The mechanism of the accelerated redox process in aprotic solution is proposed to be different from that in protic solution. This work might provide a simple model to study the electron transfer reactivity of some

small active organic molecules, especially medicines, in specific environment, which might approach a more accurate understanding of the activity of some medicines in vivo.

Acknowledgements

This work is supported by the National Natural Science Foundation of China (Grant No. 90406005, 20575028) and the Program for New Century Excellent Talents in University (NCET-04-0452), the Chinese Ministry of Education.

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