In situ Spectroelectrochemical Study of Quercetin Oxidation and Complexation with Metal Ions in Acidic Solutions

Guang-Ri Xu,[†,‡] Mo Youn In,[†] Yong Yuan,[†] Jae-Joon Lee,[§] and Sunghyun Kim[†,*]

[†]*Department of Bioscience and Biotechnology, Konkuk University, Seoul 143-701, Korea. *E-mail: skim100@konkuk.ac.kr*
[‡]*Henan Institute of Science and Technology, Xinxiang 453003, P. R. China*
[§]*Department of Applied Chemistry, Konkuk University, Chungju 380-701, Korea*
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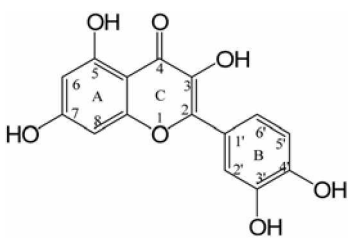
Polyphenols having a benzo-γ-pyrone structure constitute a family known as flavonoids. Flavonoids are widely distributed among higher plants. By virtue of astringent taste, they are believed to serve as defensive molecules, protecting plant tissues from herbivorous attack.[1] Depending on a multiplicity of phenolic groups, flavonoids are classified into several groups, such as flavones, flavonols (quercetin, kaempferol), flavanones (naringenin), and flavanes (catechin), displaying functional diversities and complexities.[2] Flavonoids have been best known as antioxidants and radical scavengers. While this beneficial effect mainly comes from their ability to accept free radicals, complexation properties with metal ions have also been recognized to contribute to the total biological activity.

As one of the most common flavonoids, quercetin (Scheme 1) has been a subject of thorough study. Electrochemically it undergoes a series of oxidation reactions, in which the two -OH groups in ring B are readily oxidized.[3-5] It has recently been reported that polyphenol oxidase (PPO) and mushroom tyrosinase can oxidize quercetin to corresponding four tautomeric forms of quercetin-quinone.[6-8] More interesting is the fact that the biological activities are influenced by the presence of metal ions. Hydroxy and oxo groups present in a quercetin structure have ability to form complexes with various metal ions. As the 3-hydroxy group (3-OH) and 4-oxo group have more acidic protons, they are the first sites to be involved in the complexation processes.[9] Another complexation can occur with 3'- and 4'-OH groups. Many metal ions such as Fe^{3+}, Cu^{2+}, Zn^{2-}, and lanthanide ions have been known to have antitumer activities,[10-12] scavenge peroxy and superoxide radicals when formed complexes with quercetin. These complexes also exhibit highly sensitive molecular fluorescence properties and were used in analytical methods for the detection of trace metal ions.[13,14] Structural and spectroscopic studies have also been made. Cornard[12] showed that Al^{3-} successively forms two complexes with stoichiometry (Al^{3-}:Q = 1:2 and 2:1). Lexa *et al.*[15] showed that one-electron oxidation product of quercetin could be stabilized through Zn^{2-}-Q_{ox} complex formation (Q_{ox} is oxidized quercetin). Interestingly, Tang *et al.*[16] proposed 1:3 structure where one rare earth metal ion is coordinated with three quercetin molecules.



Scheme 1. Structure of quercetin.

In this work, we have attempted spectroelectrochemical study for the oxidation and coordination aspects of quercetin in the presence of some metal ions. We put emphasis on the interaction between quercetin and Fe^{3+}, as most study concerns with dioxygen reduction by Fe^{2-} in biological systems and an inhibiting role of quercetin in the reactive oxygen species formation. We also studied the effect of hydrogen peroxide, one of the reactive oxygen species, on the quercetin-Fe^{3-} complex.

Experimental

All the chemicals were of analytical reagent grade. Metal

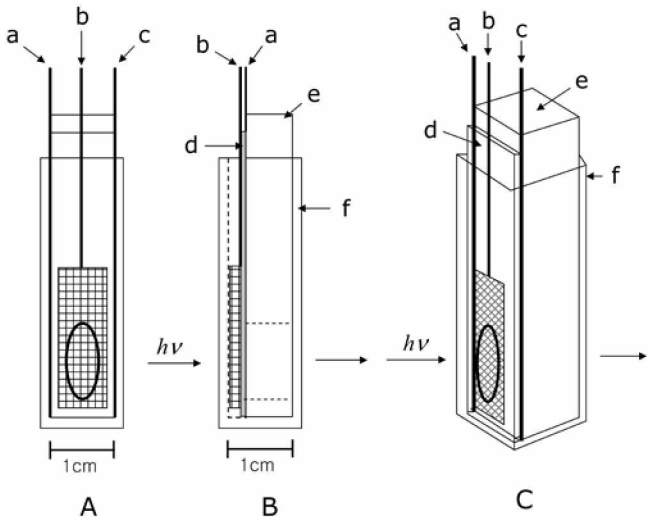


Figure 1. Schematic diagram of in situ spectroelectrochemical cell. Ag/AgCl reference electrode (a), Au mesh working electrode (b), Pt counter electrode (c), thin quartz plate (d), Teflon block (e), and quartz cuvette (f). A: front view, B: side view, C: 3-D view.

ion solutions were made from corresponding nitrate or sulfate salts using 18 MΩ·cm deionized water as before.[17,18] Becaused of limited solubility, quercetin (Aldrich. Milwaukee, WI. USA) was dissolved in ethanol before adding to the metal ion solutions.

The schematic diagram of the in situ cell is shown in Figure 1. A standard quartz cuvette of 1 cm path length was used. For the thin layer configuration, a Teflon block with a hole for the light path was inserted and a thin quartz plate and a gold mesh were placed between the Teflon block and the cuvette wall. The thin gap was filled with solution by capillary action. A Pt counter electrode and a Ag/AgCl reference electrode were inserted at each side of the Au working electrode. All UV/vis spectra were recorded with a spectrometer (Scinco. Model S-2100, Korea) at room temperature. Potential was controlled by a potentiostat (Autolab PGSTAT30. ECO CHEM, Netherlands).

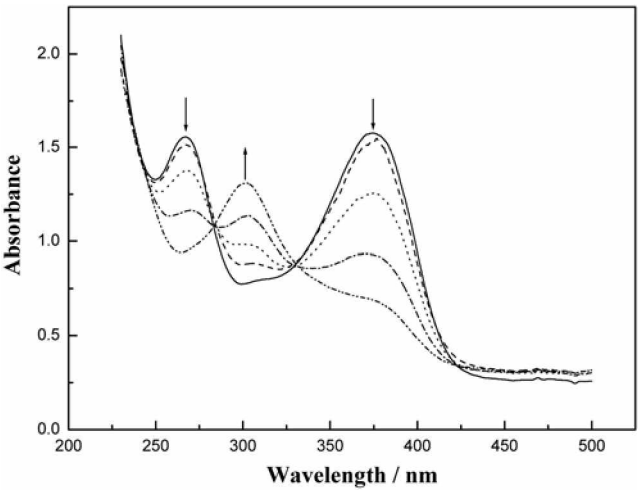


Figure 2. Spectral changes of quercetin collected at 0, 5, 10, 15, and 20 min after applying 0.5 V to the gold electrode at pH 2. Changes are indicated by arrows. [Q] = 2.5×10^{-5} M.

Results and Discussion

Figure 2 shows the progression of spectral change of quercetin when the oxidation potential of 0.5 V was applied. Full oxidation took rather long time because of high solution resistance due to the thin layer configuration. The appearance of two isosbestic points at 284 and 331 nm confirms that only two quercetin species are present. Quercetin has two characteristic peaks at 267 and 375 nm that are due to $\pi \rightarrow \pi^*$ transitions at rings A and B, respectively. Upon oxidation, these peaks begin to decrease and another peak at 301 nm appears. This has been recognized as quercetin quinone (QQ). QQ can take four tautomeric forms that are responsible for the absorption at 301 nm (Fig. 3). It is not certain at the moment which form of QQ is a main contributor.

Complexation phenomenon has been studied for several metal ions. Spectral changes were hardly made at strongly acidic solutions. For example, Fe^{2+} species did not form a complex with quercetin below pH 4. This is because all the -OH groups are protonated, hindering the metal complexation. A characteristic complexation peak was observed at 425-450 nm region. Figure 4 shows a series of spectral changes for Cu^{2-}-Q (panel A) as a function of mol ratio. Job's plots for Cu^{2+}-Q and Fe^{3+}-Q are shown in panels B and C, respectively. It was found that 2:1 (both Cu^{2+}:Q and Fe^{3-}:Q) complex was formed in which 3-OH and 4-oxo groups and 3',4'-OH groups are coordination sites. The same result was obtained for Fe^{2+}, and Pb^{2-}. However, Ag^{-}, Cd^{2+}, and Tl^{3+} ions did not form a complex.

Fe^{3+} has oxidation power even in acidic conditions (Fig. 5). In the pH range of 2 and 4, a new absorption peak at 301 nm appeared. Comparing with spectra in Figure 2, this peak was identified as that of quercetin quinone. As in the case of electrochemical oxidation of quercetin, two isosbestic points 285 and 332 nm were found, suggesting that only one oxidation product was formed. The oxidation process was rather slow, taking a few minutes for full oxidation. In strongly acidic solutions below pH 2, however, oxidation hardly took place. Above pH 5, a new peak began to appear

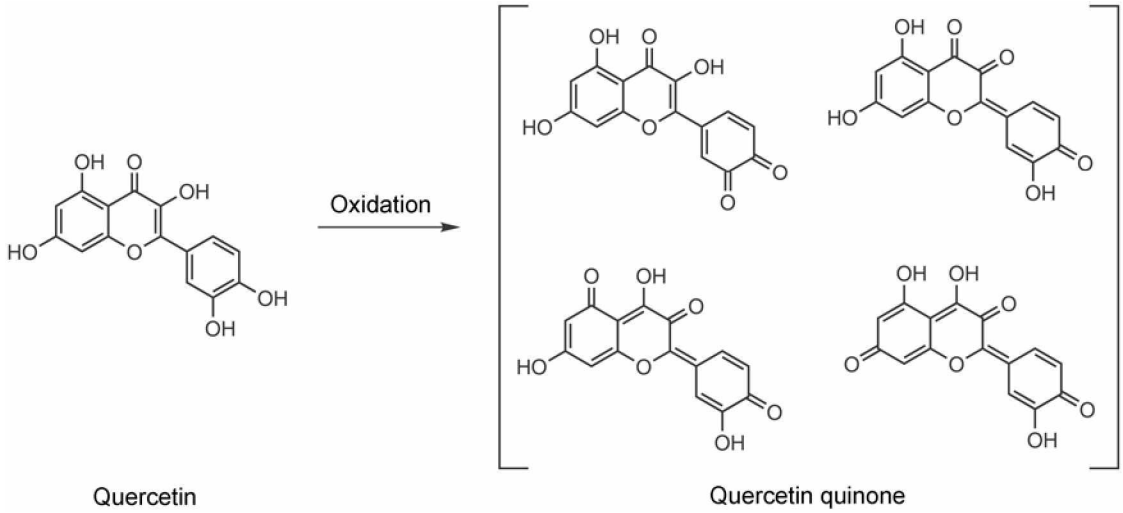


Figure 3. Structures of four-possible tautomeric forms of quercetin quinone, oxidation products of quercetin.

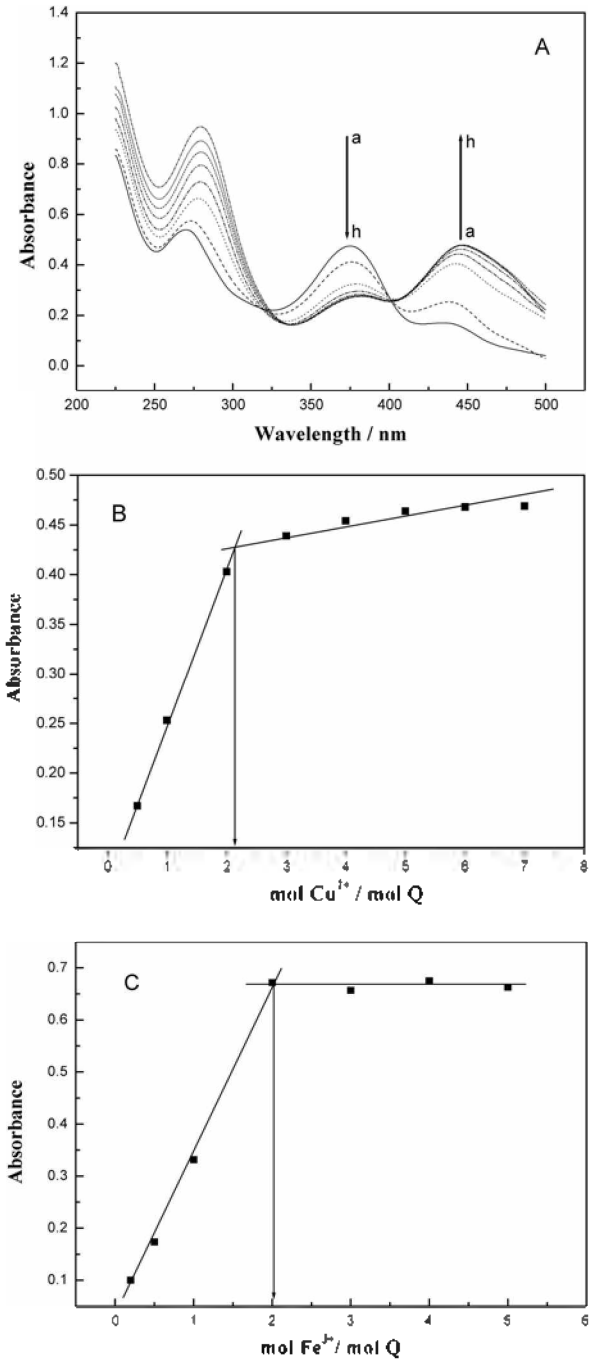


Figure 4. Spectral changes of quercetin upon Cu^{2-} addition (panel A) and corresponding Job's plot made for absorbances at 442 nm (panel B). Mol ratios of Cu^{2-} and quercetin for curves (a) to (h) are 0.5, 1, 2, 3, 4, 5, 6, and 7, respectively. Panel C is the Job's plot at 428 nm for the quercetin-Fe^{3+} complex formation in ethanol. [Q] = 2.5×10^{-5} M.

at *ca.* 425 nm. By analogy with Fe^{2-}-Q complex, this peak was assigned as the one due to the Fe^{3+}-Q complex formation. When complexation takes place, the quercetin quinone formation is suppressed. Note that the minimum pH for Fe^{3-}-Q complexation is lower than that of Fe^{2+}. This is because highly charged metal ions usually form a stronger complex with ligands than less charged ones, easily displacing protons

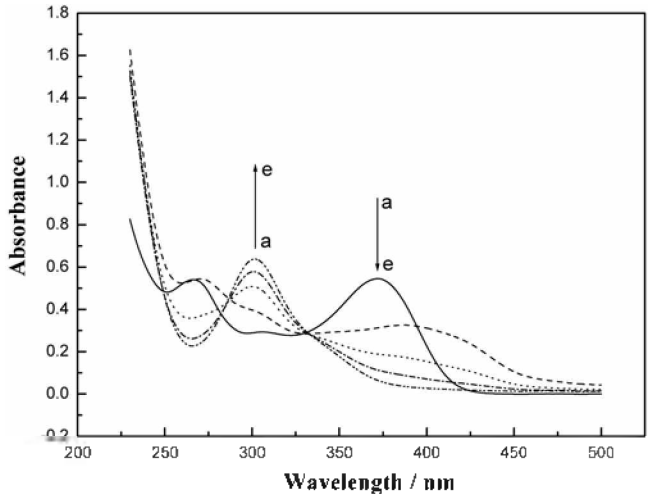


Figure 5. Spectral changes of quercetin upon Fe^{3-} addition at pH 2. Curve a is the quercetin spectrum. Curves (b) to (e) are collected immediately, 1, 3, and 20 min after addition of Fe^{3-} solution, respectively. [Q] = 2.5×10^{-5} M, [Fe^{3-}] = 5.0×10^{-5} M.

for the ligation.

Spectral changes of quercetin in the presence of Fe^{2-} species was monitored while Fe^{2-} was electrochemically oxidized to Fe^{3+} (data not shown). The electrolyte contained 0.5 mM quercetin and 1.0 mM $FeSO_4$ at pH 2.0. 0.5 V *vs* Ag/AgCl was applied to oxidize Fe^{2-} to Fe^{3-} and spectral change was recorded with time. The change was almost identical with when Fe^{3-} species was directly injected into quercetin solution as in Figure 5. Peaks at 267 and 375 nm became smaller and a new peak at *ca.* 302 nm appeared, which was identified with quercetin quinone. Two isosbestic points at 284 and 331 nm were also observed. A broad peak appearing over 400 nm that eventually disappeared indicates that Fe^{3+}-quercetin complex forms before quercetin is oxidized to quercetin quinone. However, electrochemical regeneration of Fe^{2+} species did not affect spectrum, implying quercetin quinone is stable and not affected by ferrous species.

Quercetin oxidation by hydrogen peroxide and the effect of Fe^{3+} were also investigated. In plant, this process is carried out by peroxidase. Peroxidase catalyzes one-electron oxidation of quercetin, leading to incorporation of oxygen into the quercetin structure, followed by enzymatic degradation, producing many reaction products.[19,20] Without Fe^{3+}, quercetin oxidation was very slow even in strongly acidic solutions. At pH 2, it took about 20 h for the complete oxidation. The resulting spectrum is exactly same as that of quercetin quinone (Fig. 6). However, at higher pHs than 5, essentially no quercetin oxidation was observed. In the presence of Fe^{3-}, quercetin oxidation was accelerated. After several minutes, the peak at 302 nm reached a maximum value, indicating quercetin quinone was produced. Peculiarly this peak began to decrease as time elapsed. After 20 h, absorbance was less than a half maximum value, implying degradation of quercetin quinone. The exact nature of degradation production is not certain at the moment.

In this study, we have shown that quercetin forms 2:1

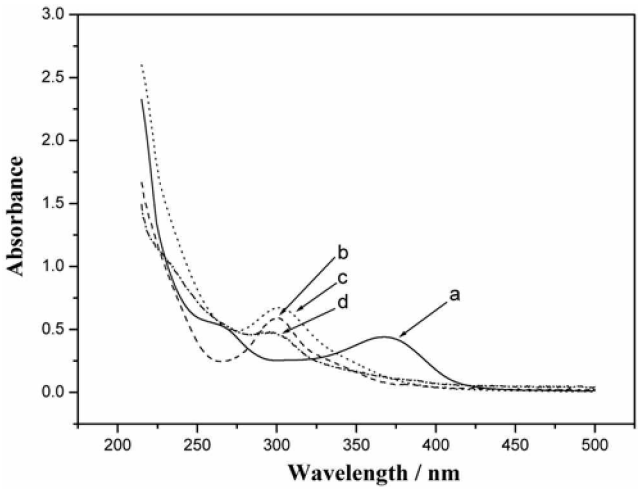


Figure 6. Quercetin oxidation by hydrogen peroxide at pH 2 without (a and b) and with (c and d) Fe^{3+}. Curves a and c, and curve b and d were collected 5 min and 20 h after the H_2O_2 addition. [Q] = 2.5×10^{-5} M, [Fe^{3+}] = 5.0×10^{-5} M, [H_2O_2] = 8.8 mM.

complexes (M:Q = 2:1) with Fe^{2+}, Fe^{3+}, Cu^{2+}, and Pb^{2-}. Fe^{3+} can oxidize quercetin to quercetin quinone in the pH region of 2 to 4, whose characteristic absorption peak appears at ca. 300 nm. Above pH 5, Fe^{3-} forms a complex with quercetin with an absorption peak at 425 nm. It is also possible to electrochemically produce quercetin quinone. In strongly acidic solutions, none of studied ions can form a complex with quercetin. The slow quercetin oxidation by hydrogen peroxide is accelerated in the presence of Fe^{3-}.

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