http://dx.doi.org/10.5012/bkcs.2011.32.9.3443

Spectroscopic Studies on the Oxidation of Catechin in Aqueous Solution

Ki-Min Bark,[†] Ji-Eun Yeom, Jeong-Im Yang, Ik-Jun Yang, Chul-Ho Park,[‡] and Hyoung-Ryun Park[*]

Department of Chemistry, Chonnam National University, Gwangju 500-757, Korea. [*]E-mail: hrpark@chonnam.ac.kr
[†]Department of Chemical Education and Research Institute of Life Science, Gyeongsang National University, Chinju 660-701, Korea
[‡]Department of Cosmetic Science, Nambu University, Gwangju 506-706, Korea
Received June 24, 2011, Accepted August 1, 2011

The spectroscopic behavior of catechin (5,7,3',4'-tetrahydroxyflavan-3-ol), has been studied in the presence and the absence of air using UV-vis absorption spectrophotometry and fluorescence spectroscopy. The UV-vis absorption spectrum of catechin shows a very sharp and strong absorption maximum peak at 275 nm in deaerated water. New absorption maximum peaks appeared in aerated water, as well as in basic aqueous solution, caused by the oxidation of catechin. The absorbances in the UV-vis absorption spectrum of catechin decreased when the solution was left in the dark for a long time. The fluorescence emission spectrum of catechin after a long time period differs markedly from that in freshly prepared solution; the fluorescence maxia shifted as time passes after adding catechin to the solutions. When the deaerated basic catechin solutions were left in the dark for a long time, their fluorescence quantum yields were found to be nearly zero. This suggests that the oxidized catechin molecules were seen to have slowly undergone successive chemical reactions in basic buffer solution.

Key Words : Catechin, Oxidation, UV-vis spectrum, Fluorescence spectroscopy, Aqueous solution

Introduction

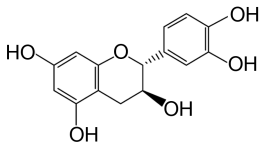
In recent decades the beneficial effects of plant polyphenols have received a great deal of attention due to their many biological activities, *i.e.*, anticarcinogenic, antiatherosclerotic, antimicrobial, and antioxidant properties.[1-8] Polyphenolic substances are commonly used to refer to the related family of flavonoids and the subgroup flavanols. They are contained in green tea, red wine and numerous fruits and vegetables.[9-11] Catechin (5,7,3',4'-tetrahydroxyflavan-3-ol), the major constituent of green tea, is one of the flavanol compounds. This substance is a polyphenolic compound in which there are 5 hydroxyl groups. The antioxidant action of catechins originates from favorable one-electron donation properties.[12-14] The particular importance of catechins is their ability to repair vitamin E, which is a characteristic unique to catechins. In addition, these tea phenols are very efficient scavengers of biologically damaging oxy radicals, such as the superoxide radical and singlet oxygen.[12,13] The reason for this biological activity is reported as being due to their ability to act as free radical acceptors.[14,15] Catechin dissolved in water displays a yellow color, and the intensity of the color changes as time passes after adding the catechin to water. It seems that there are some interactions between catechin and water. Generally hydroxyl groups are ionized to form hydronium ion in water. If the molecular structure of catechin is changed in water, a new chemical species can be produced and the spectroscopic properties of the solution are also varied as a result. In addition, it may affect on the antioxidant ability of catechin. It is, therefore, of interest to examine the spectroscopic behavior of catechin in aqueous solution. Although a large number of studies have examined the physiological functions of catechin, few papers have dealt with the phenomenon.[10,16,17] Torreggiani *et al.* reported the catechol moiety on the B-ring in catechin is oxidized in aqueous solution.[10]

In this paper, the spectroscopic properties of catechin were investigated using UV-vis spectrophotometry and fluorescence spectroscopy in order to obtain further insight into the interaction between catechin and water, In addition, we examined the spectroscopic behavior of catechin not only in aerated and deaerated water but also at the various pH values in solution to clarify the oxidation process of catechin.

Experimental Section

Catechin was purchased from Sigma Chemical Co. (St. Louis, U.S.A.) and was used without further purification.

The other chemicals were reagent grade and used as received. The aqueous solutions were prepared using doubly distilled and deionized water, which was obtained by passing doubly distilled water through a Barnstead (U.S.A.) Nanopure II deionization system. To prepare the deaerated aqueous catechin, the freshly distilled water was first saturated by



Catechin

Scheme 1

bubbling with high purity argon (99.999%) for about 90 min and then adding an aliquot of catechin to the air-free distilled water. A low-concentration solution (about 1 mM) was used as the sample because of the low solubility of catechin in water. The pH of the solution was adjusted by adding $HClO_4$, NaOH or ammonium chloride buffer solution. The buffer solution was also prepared using air-free distilled water as mentioned above. $HClO_4$, NaOH and the ammonium chloride buffer solution did not absorb UV light longer than 240 nm. To perform qualitative analysis of the products after oxidation of catechin in water, 100 mL of the aerated aqueous solution was first left in the dark at room temperature for about 60 days. The solution was then dried using a freeze dryer. The sample prepared was then analyzed using NMR (Varian Gemini; $CDCl_3$, 300 MHz), GC/MS (GSMS-QP2010, Shimadzu, Japan, DB-5 capillary column) and LC/MS systems (Mariner MS-spectrometer equipped with ESI interface, capillary C_{18} reversed column).

The UV/vis absorption spectra of the solutions were then taken using a UV/vis spectrophotometer (Uvikon, model 943, Italy) at room temperature. The steady-state fluorescence emission spectra were obtained on a Varian Cary Eclipse spectrofluorometer with 5 nm slits at room temperature after elimination of the oxygen from the solution by means of bubbling with high purity argon (99.999%). To improve reproducibility, each sample was scanned 10 times and the average of these measurements was recorded as the emission spectrum. The fluorescence quantum yields (Φ) were measured using quinine sulfate ($\Phi = 0.546$) and naphthalene ($\Phi = 0.23$) as references.[18-20] The pH values of the solution were measured using a DMP400 pH meter with a glass combination electrode.

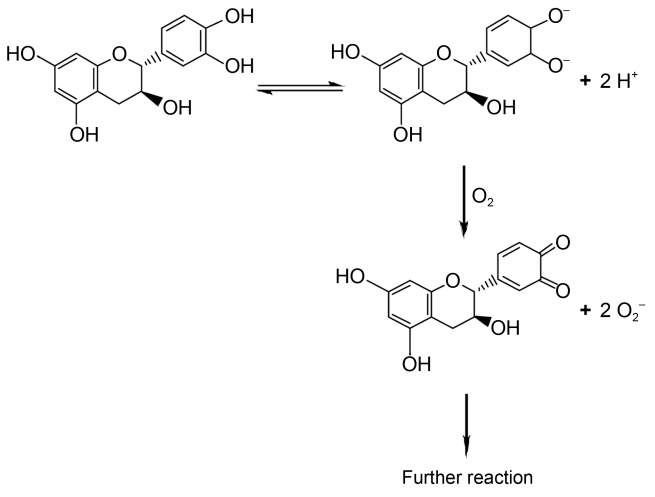
Results and Discussion

To prepare the aqueous catechin solution, an aliquot of catechin put into water and left it in the dark at room temperature. Catechin is slightly soluble in water and the aqueous solution then displayed a yellowish color as time passes about 2 days. The change of color implies that there are some interactions between catechin and water. Since the interaction may involve some chemical reactions, we tried to detect the reaction products by using of NMR, GC/MS and LC/MS systems in order to clarify the reason. No products were detected in the yellowish colored solution under our experimental conditions. However, the UV-vis absorption spectra of the solution changed as the time passes after adding the catechin to the water. This finding means that the results from the UV-vis spectrophotometer may be more sensitive than those obtained from NMR, GC/MS and LC/MS systems under our experimental conditions.

As shown in Figure 1. the UV-vis absorption spectrum of 91 μM catechin reveals a very sharp and strong absorption maximum peak at 275 nm in deaerated water. The absorption spectrum of 91 μM deaerated aqueous catechin solution did not change, even if the solution was left in the dark at room temperature for 5 days. However, its absorption spectrum did change, when the aerated aqueous catechin solution was stored for 2 days. In addition, the absorption maximum peak at 275 nm is shifted to longer wavelength, and new maximum peaks appeared at wavelengths of 340 nm, 430 nm and 480 nm. This indicates that the oxygen dissolved in the water affects the molecular structure of catechin. Both the change of color and the difference of the UV-vis absorption spectra of the catechin solution might be related to the oxidation of catechin. The oxidation of alcohols often takes place in organic chemistry. Catechin is a polyphenolic compounds. Phenols are ionized into the hydronium ion and the phenoxide ion in aqueous solution. They are easily converted into their salts by aqueous hydroxides.

$$ArOH \rightleftarrows H^+ + ArO^-$$
$$ArOH + OH^- \rightarrow ArO^- + H_2O$$

The phenoxide ions contain a benzene ring and therefore must have resonance structures. Since the phenoxide ions only carry a negative charge in a benzene ring, they can exist as an aromatic ketone by resonance.[21] Catechol is also oxidized by a similar oxidation mechanism.[10,22-26] Catechin contains the catechol moiety and it can therefore be converted into its oxidized form in aqueous solution. Quinones are oxidized derivatives of aromatic compounds and are often readily made from reactive aromatic compounds with an electron-donating substituent, for example phenols and catechols, which increase the nucleophilicity of the ring and contribute to the large redox potential needed to break aromaticity. The resonance structures for the phenoxide ion carry only a negative charge. The negative charge density is enhanced at the ortho and para positions.[21] As a result, oxidation might be more easily undergone in the case of *o*-phenols and *p*-phenols. To confirm this hypothesis, we studied the oxidation reaction of some other phenolic compounds such as phenol and β-naphthol in water. Neither a change of color nor a distinct difference in the UV-vis absorption spectra were observed under our experimental conditions. We can also suggest that the oxidation of catechin is mainly carried out in the catechol moiety, in agree-



Scheme 2

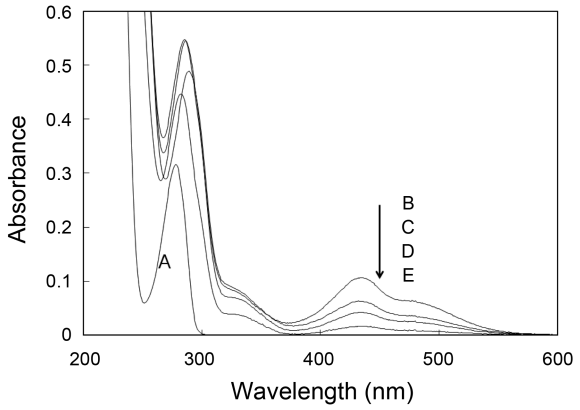


Figure 1. UV-vis absorption spectra of 91 μM catechin: (a) in deaerated water, (b) in deaerated buffer solution at pH = 10.52, (c) in deaerated buffer solution at pH = 10.01, (d) in deaerated buffer solution at pH = 8.81, and (e) in aerated water.

ment with the literature as presented in Scheme 2.[10]

The change of the UV-vis absorption spectrum of catechin is also observed in the deaerated basic solution, whereas this is not observed in the deaerated acidic solution. The UV-visible absorption properties of the deaerated basic catechin solution are quite similar to those of the aerated neutral catechin solution as presented in Figure 1. Its maximum peaks were measured at the same wavelength as for the aerated neutral catechin solution. This finding indicates that the same chemical reactions occur in the aqueous catechin solution in the presence of air or in basic solution. However, the yellowish color in basic solution appears much faster than in the aerated catechin solution. Besides, the appearance of the yellowish color depended strongly on the pH values in the solution. The higher the pH value of the solution, the faster the yellowish color appears. Moreover, the intense yellowish color in the aqueous catechin solution varies depending on the storage time of the solution. The yellowish color faded down when the solution was left in the dark for a long time. It might be mentioned that the other successive chemical reactions also occurred slowly in the solution.

In order to obtain further information on the oxidation process of catechin, the UV-vis absorption spectra of catechin were monitored with time in the deaerated ammonium chloride buffer solution. As shown in Figure 2, the absorption spectra of 91 μM catechin at pH = 8.81 buffer solution change with the storage time of the solution. The absorbances of the new peaks appeared at wavelengths of 340 nm, 430 nm, and 480 nm, increased at first after adding catechin to the deaerated pH = 8.81 buffer solution. However, the absorption maximum peak at 430 nm is shifted to 405 nm, and the absorbance at this peak gradually decreased after 19 hours. In addition, the UV-vis absorption spectra of catechin depended on the pH of the deaerated ammonium chloride buffer solution. The higher pH values of the solution increase the oxidation rate of catechin. As the pH of the solution was increased, its absorbance at 405 nm decreased whereas that at 480 nm increased, as presented in Figure 3.

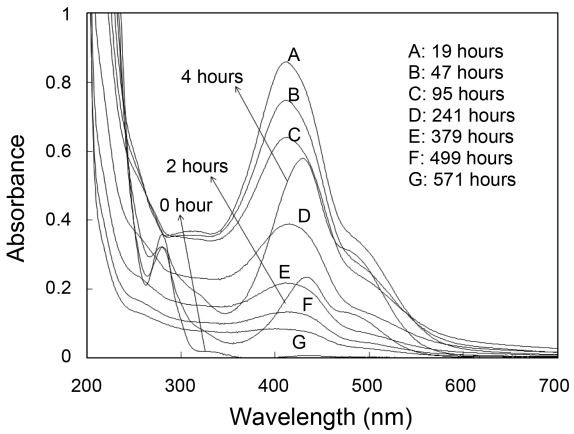


Figure 2. UV-vis absorption spectra of 91 μM catechin in a buffer solution at pH = 8.81 at various times.

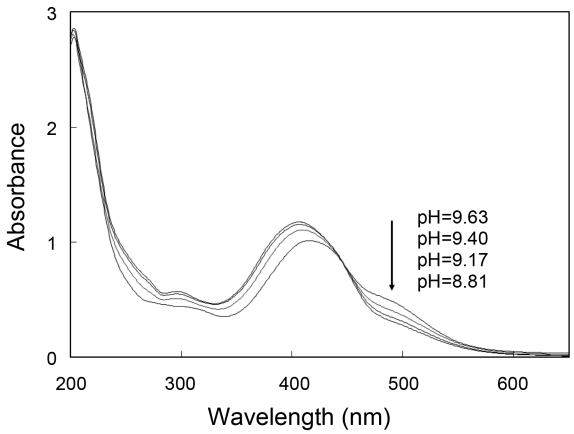


Figure 3. UV-vis absorption spectra of 109 μM catechin in basic buffer solutions 19 hours after the addition of catechin to the buffer solution.

Moreover, catechin has one isosbestic point at 422 nm in the deaerated basic buffer solution 19 hours after solution preparation. This isosbestic point disappeared when the catechin solutions were left for about 2 days, although this period is strongly dependent on their pH values. It might be mentioned that oxidized catechin molecules have reached their equilibrium state in basic buffer solution, and then another chemical reaction is undergone in succession.

The steady-state fluorescence emission spectra of 91 μM catechin were measured in deaerated basic buffer solution. Their emission spectra were obtained in the range of 290 nm to 390 nm with an emission maximum peak at 314 nm in the initial state. As time passes after adding catechin to the deaerated basic solutions, the fluorescence intensity decreased and a new emission peak appeared in the range of 390 nm to 590 nm with an emission maximum peak at 446 nm, as presented in Figure 4. In addition, the new maximum peaks gradually shift to a wavelength of 470 nm as time passes. These fluorescence spectral changes depended strongly not only on the storage time of the solution but on the pH value in the solution. Figure 5 shows the change in the fluorescence quantum yield for the emission spectrum in the range of 290 nm to 390 nm. Although the initial concentrations of

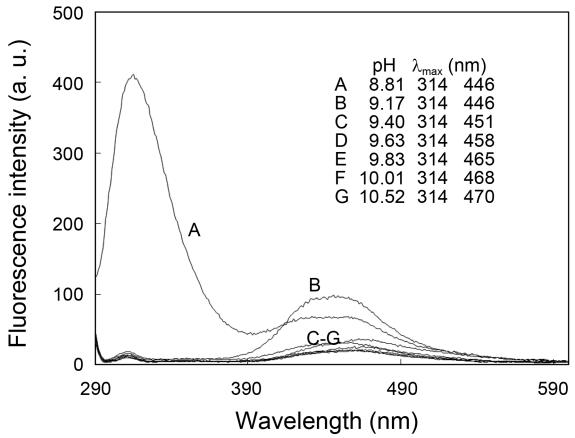


Figure 4. Fluorescence spectra (excitation at 280 nm) of 91 μM catechin in basic buffer solutions 4 hours after adding the catechin to the solution.

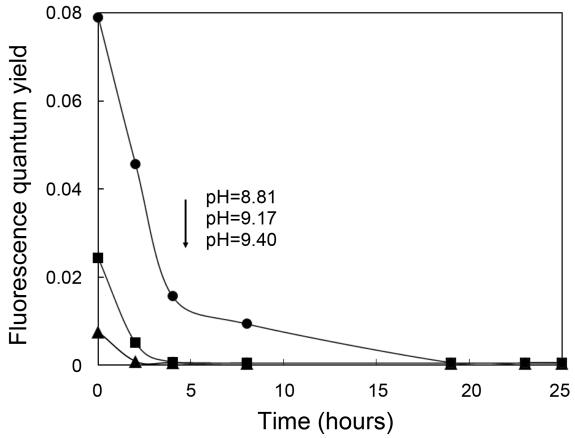


Figure 5. Fluorescence quantum yields of the 91 μM catechin as a function of the storage time at various pH values for the basic buffer solution.

catechin are the same, their fluorescence quantum yields for the emission spectrum in the range of 290 nm to 390 nm rapidly diminish depending on the pH values of the solution. The higher the pH value of the solution, the faster the spectral changes take place. This finding supports the conclusion that oxidation started with the dissociation of the -OH part of the catechin in aqueous solution. Moreover, the fact that the new maximum peaks are gradually shifted to a wavelength of 470 nm as the time passes is evidence that the oxidation of catechin is performed by a sequence of multi-step reactions in the deaerated basic buffer solution.

When the deaerated basic catechin solutions were left in the dark at room temperature for a long time, the fluorescence quantum yields of catechin for the emission spectrum were found to be almost zero. Substances that display significant fluorescence generally possess delocalized electrons that are formally present in conjugated double bonds.[27] Since catechin molecules contain delocalized electrons, the substances fluoresce. However, their fluorescence quantum yield decreases as time passes after solution preparation. This means that the delocalized electrons in the catechin molecules were converted into localized electrons. These results allow us to predict that the oxidation of catechin is carried out by a sequence of multi-step reactions in deaerated basic buffer solution. Unfortunately, we can not detect the oxidation products of catechin in these experimental results. Further studies will be performed to detect these oxidation products.

Conclusion

Catechin displayed a yellowish color in aerated water when the solution was left in the dark for 2 days. The UV-vis absorption spectra of the solution were changed as time passed following addition of catechin to water. The change in the UV-vis absorption spectra of catechin is also observed in the deaerated basic solution, whereas it is not observed in the deaerated acidic solution. In addition, the change is also observed in the case of deaerated basic catechin solution. The higher the pH value of the solution, the faster the yellowish color appears. This is related to the oxidation of catechin. The catechol moiety bound to the catechin molecule is first dissociated and then its oxidation takes place successively in aqueous solution. The yellowish color faded down when the solution was left in the dark for a long time, indicating that another chemical reaction is undergone in succession. The steady-state fluorescence emission spectra of catechin has also shown us that oxidation of catechin depends on the pH values of the aqueous basic solution. When the deaerated basic catechin solutions were left in the dark at room temperature for a long time, their fluorescence quantum yields were found to be nearly zero. This result allow us to predict that oxidation of catechin is carried out a sequence of multi-step reactions in the deaerated basic buffer solution.

Acknowledgments. This research was support by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0023938).

References

1. Caturla, N.; Vera-Samper, E.; Villalain, J.; Mateo, C. R.; Micol, V. *Free radical Biol. and Med.* **2003**, *34*(6), 648.
2. Yang, C. S.; Chung, J. Y.; Yang, G.; Chhabra, S. K.; Lee, M. J. *J. Nutr.* **2000**, *130*, 472.
3. Yang, C. S.; Landau, J. M.; Huang, M. T.; Newmark, H. L. *Annu. Rev. Nutr.* **2001**, *21*, 381.
4. McKay, D. L.; Blumberg, J. B. *J. Am. Coll. Nutr.* **2002**, *21*, 1.
5. Wang, H.; Provan, G. J.; Helliwell, K. *Trends food Sci. Tech.* **2000**, *11*, 152.
6. Rice-Evans, C. *Biochem. Soc. Symp.* **1995**, *61*, 103.
7. Gradisar, H.; Pristovsek, P.; Plaper, A.; Jerala, R. *J. Med. Chem.* **2007**, *50*, 264.
8. Katiyar, S.; Elmets, C. A.; Katiyar, S. K. *J. Nutr. Biochem.* **2007**, *18*, 287.
9. Frankel, E. N. *J. Agric. Food Chem.* **1995**, *43*, 890.
10. Torreggiani, A.; Jurasekova, Z.; Sanchez-Cortes, S.; Tamba, M. *Journal of Raman Spectroscopy* **2008**, *39*, 265.

11. Park, H. R.; Liu, H. B.; Shin, S. C.; Park, J. K.; Bark, K. M. *Bull. Korean. Chem. Soc.* **2011** *32*, 981.
12. Jovanovic, S. V.; Hara, Y.; Steenken, S.; Simic, M. G. *J. Am. Chem. Soc.* **1995**, *117*, 9881.
13. Jovanovic, S. V.; Steenken, S.; Simic, M. G.; Hara, Y. *Flavonoids in Health and Disease*; Rice-Evans, C. A., Packer, L., Eds.; Marcel Dekker, Inc.: New York, U.S.A., 1998; p 137.
14. Bors, W.; Heller, W.; Michel, C.; Saran, M. *Methods. Enzymol.* **1990**, *186*, 343.
15. Sies, H. *Eur. J. Biochem.* **1993**, *215*, 213.
16. Komatsu, Y.; Suematsu, S.; Hisanobu, Y.; Saigo, H.; Matsuda, R.; Hara, K. *Bioscience Biotechnology & Biochemistry* **1993**, *57*, 907.
17. Jovanovic, S. V.; Simic, M. G.; Steenken, S.; Hara, Y. *J. Chem. Soc. Perkin Trans* **1998**, *2*, 2365.
18. Eaton, D. F. *Reference Compounds for Fluorescence Measurement*; IUPAC Organic Chem. Division: Wilmington, U.S.A., 1987; p 1.
19. Bark, K. M.; Force, R. K. *Spectrochim. Acta* **1993**, *49*(A), 1605.
20. Demas, J. N.; Grosby, G. A. *J. Phys. Chem*. **1971**, *75*, 2463.
21. Morrison, R. T.; Boyd, R. N. *Organic Chemistry, 3th ed.*; Allyn and Bacon, Inc.: Boston, USA, 1973; p 798.
22. Gichinga, M. G.; Striegler, S. *J. Amer. Chem. Soc.* **2008**, *130*, 5150.
23. Koval, I. A.; Gamez, P.; Belle, C.; Selmeczi, K.; Reedijk, J. *J. Chem. Soc. Rev.* **2006**, *35*, 814.
24. Belle, C.; Selmeczi, K.; Torelli, S.; Pierre, J.-L. *C. R. Chim.* **2007**, *10*, 271.
25. Kim, E.; Chufan, E. E.; Kamaraj, K.; Karlin, K. D. *Chem. Rev.* **2004**, *104*, 1077.
26. Lewis, E. A.; Tolman, W. B. *Chem. Rev.* **2004**, *104*, 1047.
27. Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Plenum Press: New York, USA, 1983; p 257.