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Studies on the Interaction between Catechin and Metal Ions

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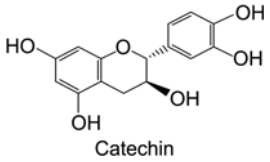
Polyphenolic substances have been widely studied in recent years due to their numerous biological activities such as anticarcinogenic, antiatherosclerotic, antimicrobial, and antioxidant properties.[1-5] Flavanol derivatives are a group of natural substances with a diversity of phenolic structures which can be found in plants, vegetables, fruits and teas. Catechin (5,7,3',4'-tetrahydroxyflavan-3-ol), a major constituent of green tea, is one of the flavanol compounds.[6,7] This substance is well known as an excellent antioxidant agent against lipid peroxidation and its antioxidant capacity has been demonstrated in several *in vitro* and *ex vivo* systems.[2-4]

The particular importance of catechin is their unique ability to repair vitamin E. In addition, these tea phenols are very efficient scavengers of biologically damaging oxy radicals such as the superoxide radical and singlet oxygen.[8,9] The reason for this biological activity is reported as being due to their ability to act as free radical acceptors.[7] Various metal ions such as Cu^{2+}, Zn^{2+}, Co^{2+}, Fe^{2+}, Mg^{2+}, and Ca^{2+} exist in the human body. These divalent metal ions are vital for all type of biological functions such as gene expression, apoptosis, enzyme regulation and neurotransmission.[10,11] These metal ions have acidic properties, and may combine with Lewis-basic compounds to form acid-base complexes.[12,13] Although many studies concerning the biological activity of this catechin have been performed, few papers tried to correlate these activities with molecular interactions between metal ions and catechin molecules.[5] Some papers have reported that metal ions were bound with catechin, leading to metal-catechin complexes.[14-16] On the other hand, a study reported that catechin undergoes an oxidation process.[17] It is therefore interesting to study the interaction of catechin with metal ions.

In this paper, we carried out spectroscopic studies between catechin and metal ions, such as Cu^{2+}, Zn^{2+}, Co^{2+}, and Fe^{3+} in methanol using UV-vis spectrophotometry and fluorescence spectroscopy in an attempt to clarify the interaction between the two type of molecules.

Experimental Section

Catechin (5,7,3',4'-tetrahydroxyflavan-3-ol) and methanol were obtained from the Sigma Chemical Co. (St. Louis, U.S.A.) and were used without further purification.



Catechin

Scheme 1

The chemicals used as metal cations, $ZnCl_2$, $CuCl_2 \cdot 2H_2O$, $CoCl_2 \cdot 6H_2O$, and $FeCl_3 \cdot 6H_2O$ were purchased from Junsei Chemical Co. (Japan, ACS reagent grade) and the other chemicals were of reagent grade and used as received. The deaerated methanolic stock solution of catechin (1 mM) was prepared using air-free methanol which was saturated by bubbling with high purity argon for about 90 min. The sample solution was then prepared by dissolving an aliquot of the stock solution to the air-free methanol. The prepared stock solution was saturated again with argon for a short time before and after using the solution. The metal ions were also prepared by dissolving an aliquot of ionic compounds in deaerated methanol. The UV-vis absorption spectra of the solutions were measured using a UV-vis Spectrophotometer (Uvikon, model 943, Italy). The steady-state fluorescence emission spectra were obtained on a varian Cary Eclipse spectrofluorometer with 5 nm slits at room temperature. The reduction yield of Fe^{+3} to Fe^{+2} ions was measured by a spectrophotometric method.[18] Aliquot (1 mL) of the sample solutions were treated with 0.1% aqueous o-phenanthrolin solution and 1 M sodium acetate dissolved 1 N H_2SO_4 solution as a complexing reagent. The complex formed reveals absorption maximum peak at 512 nm in methanolic solution. This method was not interfered by the presence of Fe^{+3} ion under this experimental condition.

Results and Discussion

100 μM of the prepared deaerated methanolic catechin solution was left in the dark at room temperature for a long time to examine the oxidation of the solution, since the aqueous catechin solution is easily oxidized and turns to a yellowish color.[19] The solution did not display a yellowish color, even though it was left in the dark at room temperature

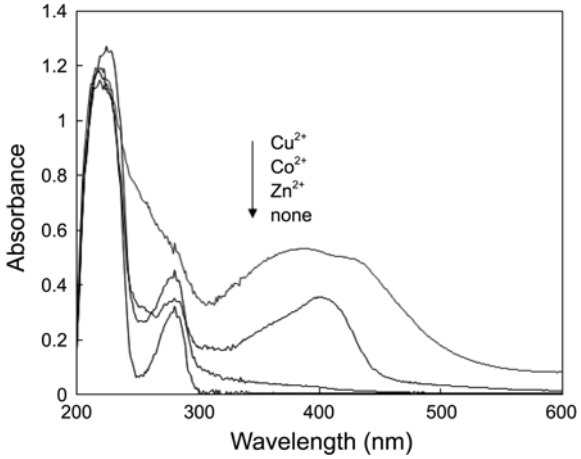


Figure 1. UV-vis absorption spectra of 100 μM catechin with an equivalent concentration of various metal ions in deaerated methanol 48 h after the addition of metal ions to the solution.

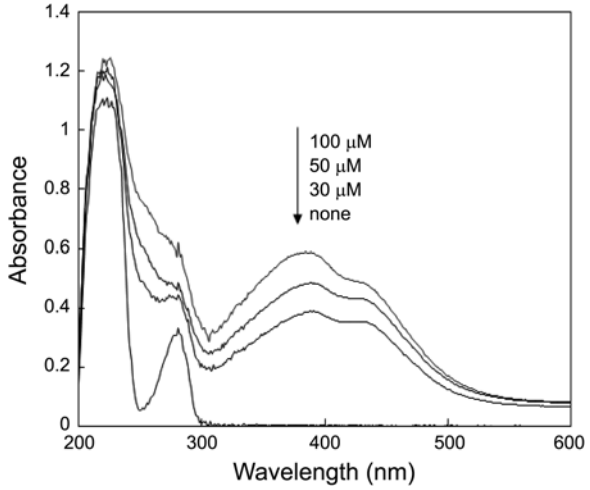


Figure 2. UV-vis absorption spectra of 100 μM catechin with various concentrations of Cu^{2+} ions in deaerated methanol 72 h after the addition of metal ions to the solution.

for two weeks. The UV-vis absorption spectrum of the 100 μM catechin revealed a very sharp and strong absorption maximum peak at 225 nm with a weak maximum peak at 280 nm in methanol. The solution did not absorb wavelengths above 300 nm. The absorption spectrum of 100 μM deaerated methanolic catechin solution did not change, even though the solution was left in the dark at room temperature for 12 days. This means that 100 μM methanolic catechin solution is not oxidized under this experimental condition.

To investigate the interaction between catechin and divalent metal ions in deaerated methanol, we measured the UV-vis absorption spectra of the methanolic catechin reacted with metal ions as a function of reaction time. As shown in Figure 1, a new absorption peak appeared at wavelengths above 300 nm, by time lapse after the addition of metal ions to the solution. This indicates that there are some interactions between catechin and divalent metal ions in methanol. A probable interaction between two substances is the formation of metal complex, since metal ions are Lewis acids and the catechin molecule can serve as a Lewis base. Catechin and metal ions dissolved in methanol reacted with each other and the reaction reached equilibrium. As a result, an isosbestic point on their UV-vis absorption spectra was observed within the variation of the concentration of metal ions. Thus, the measurement was done on the change of the UV-vis absorption spectra of catechin in reaction with divalent metal ions in methanol. The concentration of catechin was held constant while the concentration of divalent metal cations was raised from zero up to 200 μM. Figure 2 shows the absorption spectra of 100 μM catechin with various concentration of Cu^{2+} ions in deaerated methanol. The spectra showed the new absorption maximum peaks at the wavelengths of 395 nm, and 430 nm, which increased at first after adding catechin to the methanol solution. However, we could not find any isosbestic points on the UV-vis absorption spectra in the solution, although the spectra were also monitored as a function of time. Besides, the absorption spectra of the solution changed according to lapse of time

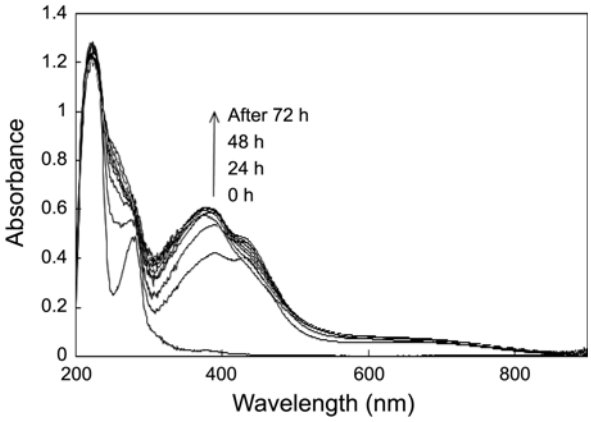


Figure 3. UV-vis absorption spectra of 100 μM catechin with an equivalent concentration of Cu^{2+} ions in deaerated methanol at various times.

after adding Cu^{2+} ion to the deaerated methanolic catechin solution as shown in Figure 3. The new maximum peaks at 395 nm, and 430 nm were increased in the initial state and then the peaks become one as time passed after adding Cu^{2+} ion to the deaerated methanolic catechin solution. These results are quite similar to the case of aqueous catechin solutions. In the previous work, we reported that new peaks appeared and then the peaks became one peak as a result of oxidation of aqueous catechin.[19]

It is also possible that divalent metal ions can participate in the oxidation of catechin. In such case, the divalent metal ions serve as an oxidizing agent. The standard reduction potential for Cu^{2+} ion to Cu is 0.34 eV in acidic solution.[20] Thus, the reduction of Cu^{2+} ion is very favorable. Because of this, the oxidation of catechin took place in deaerated methanol. To ascertain this hypothesis, the oxidation of catechin was examined with ferric ion as an oxidizing reagent, because Fe^{3+} ion is easily reduced to Fe^{2+} ion. The amount of Fe^{2+} ion formed during the reaction of 100 μM catechin with 200 μM of Fe^{3+} ion in deaerated methanol was determined

by a spectrophotometric method. The amount of Fe^{2+} ion formed varied with stored duration of the solution. About 90% of Fe^{3+} ion was converted into Fe^{2+} ion 1 h following addition of 200 μM Fe^{3+} ion into the deaerated methanolic catechin solution. This means that Fe^{3+} ion is reduced to Fe^{2+} ion in deaerated methanolic catechin solution. During the redox reaction, catechin is oxidized in the deaerated methanolic catechin solution. This finding explains the reason why the new absorption peak appeared at a wavelength above 300 nm in the reaction of catechin with metal ions. The formation of ferrous ion is more highly detected in the case of deaerated methanol than in the case of aerated methanol. The reason of this might be due to the competition of Fe^{3+} ion against oxygen in the oxidation of catechin. In contrast to the standard reduction potentials for Cu^{2+} ion and Fe^{3+} ions, those for Zn^{2+} ion and Co^{2+} ion are −0.76 eV and −0.28 eV in acidic solution, respectively.[20] The negative sign for the standard reduction potentials simply means that the substance is not as easily reduced into H^+ ion. However, catechin can be oxidized receiving help from Zn^{2+} ion and Co^{2+} ion, even if the standard reduction potentials for Zn^{2+} ion and Co^{2+} ion have a negative sign. Some papers reported that oxidation of catechin is mainly carried out in the dissociation of catechol moiety in the initial state.[7,19] It might be therefore concluded that the -OH part of the catechol moiety bound to catechin molecule is first dissociated its anion form and then the ionized chemical species are converted into the oxidized form called quinones receiving help from metal ions.

The steady-state fluorescence emission spectra of 100 μM catechin were measured in deaerated methanol. Their emission spectra were obtained in the range of 280 nm to 450 nm with an emission maximum peak at 315 nm in the initial state. As time passed after adding metal ions to the deaerated methanolic catechin solutions, the fluorescence intensity decreased and a new emission maximum peak appeared at 460 nm, as presented in Figure 4. These fluorescence spectral changes depended strongly on the storage time of the solution. This is also quite similar to the case of the aqueous catechin solution. Substances that display significant fluorescence generally possess delocalized electrons that are formally present in conjugated double bonds.[21] Since catechin molecules contain delocalized electrons, the substances emit fluorescence. However, the change of their fluorescence spectra along with lapse of time after preparation of the solution allows us to predict that the oxidation of catechin was carried out by a sequence of multi-step reactions in deaerated methanol solution. Unfortunately, we are not able to further investigate the oxidation reaction. Further studies will be performed in the future to explain the sequence of multi-step reactions.

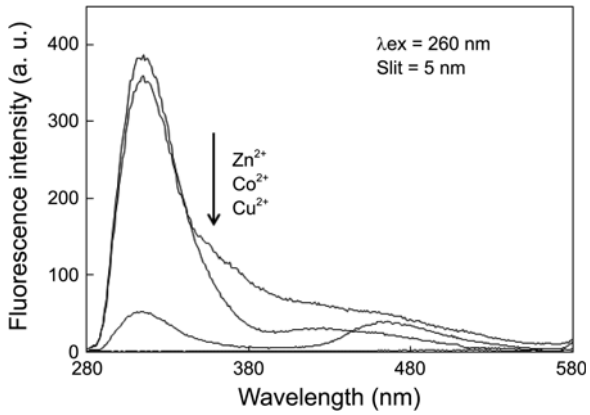


Figure 4. Fluorescence spectra of 100 μM catechin with an equivalent concentration of various metal ions in deaerated methanol 168 h after the addition of metal ions to the solution.

In conclusion, the UV-vis absorption spectra of the deaerated methanolic solution reacted with metal ions such as Cu^{2+}, Zn^{2+}, Co^{2+}, and Fe^{3+} were changed as time passed after adding catechin followed by addition of catechin to methanol. This is strongly dependent not only on the presence of metal ion but on the storage time of the solution. The change has relevance to the oxidation of catechin. Oxidation of catechin is first initiated by the dissociation of -OH part of the catechol moiety in methanol and then the ionized anion forms are converted into their oxidized forms called quinones. The higher the standard reduction potential for metal ion, the faster the oxidation occurs. The steady-state fluorescence emission spectra of catechin changed depending on the storage time of the solution. This finding indicates that oxidation of catechin is undergone by a sequence of multi-step reactions in deaerated methanol solution.

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