# Copper(II)-Catalyzed Oxidation of *d*-α-Tocopherol by Oxygen in Aqueous Solution

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**α-Tocopherol (α-Toc) was solubilized in aqueous solutions using 13 solubilizing agents and the products of oxidation by oxygen in the presence and the absence of Cu(II) were analyzed by HPLC. In the presence of Cu(II), the oxidation was accelerated and 5-formyl-7,8-dimethyltocol and α-tocoquinone were the major oxidation products. Their yields greatly increased in the presence of Cu(II). The yields and the rates of formation of the products were dependent on the properties of solubilizing agents and other conditions as well as the presence of Cu(II) or other metal ions. It is suggested that slight changes in the structure of the solubilizing agents affect the course of the reaction.**

**Key words** *d*-α-tocopherol; oxidation; Cu(II) catalysis; 5-formyl-7,8-dimethyltocol; α-tocoquinone; sodium deoxycholate

*d*-α-Tocopherol (α-Toc) is a lipophilic antioxidant which is distributed in the biomembrane and protects against lipid peroxidation. Its antioxidant activity has attracted much attention and there are many studies concerning its reactivity in organic solvents.[1] On the other hand, few reports have appeared on the reactions and their products of α-Toc in aqueous solution because it is insoluble in water.[2]

In the previous paper,[3] we reported that α-Toc was oxidized by oxygen in aqueous solutions in the presence of solubilizing agents to form 5-formyl-7,8-dimethyltocol (5-FDT),[4] 7-formyl-5,8-dimethyltocol (7-FDT) and α-tocoquinone (α-TQ). 5-FDT and 7-FDT were concluded to be responsible for the coloration of the aqueous solutions. However, the yields of the three oxidation products were too low to clarify the whole oxidation paths of α-Toc by oxygen in aqueous solutions.

In the course of further study on oxidation of α-Toc by oxygen, we found that the yields of 5-FDT and α-TQ greatly increased in the presence of Cu(II). The yields and rates of formation of the products were dependent on the properties of the solubilizing agents and other conditions as well as the presence of metal ions. The present paper describes these results in detail.

## Experimental

**Material and methods** α-Toc was purchased from Sigma Chemical Co. (MO, U.S.A.) and purified by silica gel column chromatography. α-TQ was obtained from Nutritional Biochemical Co. (Cleveland OH, U.S.A.). 5-FDT was prepared by oxidation of α-Toc with oxygen in aqueous deoxycholate solution and purified by preparative TLC and HPLC. Biochemical grade of sodium deoxycholate (DOC), sodium cholate (CO), acetonitrile for HPLC, and diethyl ether were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Sodium dodecylsulfate (SDS), dodecyltrimethylammonium bromide (C12-TBr), tetradecyltrimethylammonium bromide (C14-TBr), hexadecyltrimethylammonium bromide (C16-TBr), sodium chenodeoxycholate (ChenoDOC), sodium ursodeoxycholate (UDOC), sodium taurodeoxycholate (TDOC), sodium taurochenodeoxycholate (TchenoDCO), sodium taurocholate (TCO), sodium tauroursodeoxycholate (TUDOC) were obtained from Sigma Chemical Co. Stearyltrimethylammonium bromide (C18-TBr) was obtained from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan). The other chemicals were of reagent grade and obtained commercially. Water was used after distillation and the ion exchange treatment with Milli-Q SP Reagent Water System.

The inductively coupled plasma mass spectra was recorded on an HP4500 mass spectrometer at Yokogawa Analytical Systems Inc.

**Procedure** α-Toc (0.1 mM) was dissolved in 25 mM aqueous solutions of solubilizing agents. The solution was filtered with a membrane filter and the filtrate was kept in the dark in a bath thermostated at 50—85 °C. The reaction was initiated by introduction of gaseous oxygen and the solution was kept under oxygen atmosphere with stirring during the reaction. In the reactions in the presence of metal ions, an aqueous solution (1—5 μM) of the salts of polyvalent metals was added immediately before the introduction of oxygen. The metal salts used in the study were $CuSO_4(NH_4)_2SO_4$, $Cu(ClO_4)_2$, $Fe(ClO_4)_3$, $Ni(ClO_4)_2$, $Co(ClO_4)_2$, and $Mn(ClO_4)_2$. Samples were withdrawn at intervals and a 50-μl volume was injected into the HPLC. α-Toc and the products were determined as described previously.[3]

**HPLC conditions** Column, SHISEIDO CAPCELL PAK C18 UG120 S-5 (4.6ID×250 mm); eluent, linear gradient from $CH_3CN : H_2O$ (95 : 5) to $CH_3CN : (C_2H_5)_2O$ (2 : 3); flow rate, 1.0 ml/min; detector, two Hitachi L-4000 UV detectors (268 and 290 nm) connected in series.

## Results

**Effects of Metal Ion on Oxidation of α-Toc by Oxygen** The catalytic effects of Cu(II) ion on oxidation of α-Toc by gaseous oxygen in aqueous DOC at 80 °C were studied and the results are included in Fig. 1. The oxidation of α-Toc was accelerated by the addition of 0.01 eq of $Cu^{2+}$ ion, and the yield of 5-FDT was increased to 42% after 3 h, while only 0.6% of 5-FDT was formed in the absence of $Cu^{2+}$ ion. Thus, the catalytic activity was examined for the other biologically relevant transition metals such as Mn(II), Fe(III), Co(II), and Ni(II). Figure 1 shows the consumption of α-Toc and the formation of 5-FDT and α-TQ when 0.01 eq of each metal ion was added at 80 °C. Only a trace amount of 7-FDT was formed under these experimental conditions. The results show that $Cu^{2+}$ ion is the most effective catalyst for the formation of 5-FDT. In addition, it was found that all metal ions used above more or less accelerated the formation of 5- FDT, whereas the yields of α-TQ remained low.

**Effects of Contaminated Metal Ions on the Reactions** The above results on the catalytic effect of Cu(II) and the other metal ions gave rise to a question whether the reactions without added metal ions described in the previous[3] and present reports proceeded by a trace amount of metal ion contaminated in the reaction mixture. To examine the possible participation of metal contaminants in the reaction solvent, the following measurements were carried out.

Metal ions which might be contained in water used in the

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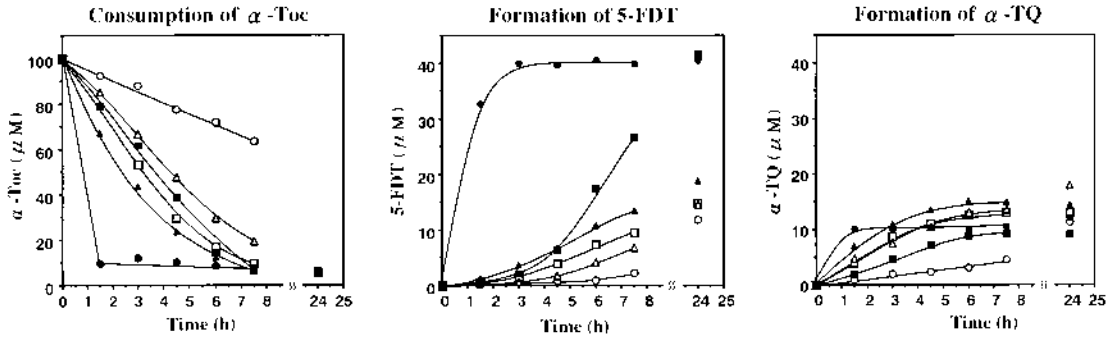



Fig. 1. Catalytic Effects of Metal Ions on Oxidation by Oxygen of $\alpha$-Toc in Aqueous DOC at 80 °C

Concentration of metal ions was 1.0 $\mu$M; concentration of DOC, 25 mM; initial concentration of $\alpha$-Toc, 100 $\mu$M. ○, metal ion free; □, Co(II); ●, Cu(II); ■, Mn(II); △, Fe(III); ▲, Ni(II).

Table 1. Oxidation by Oxygen of $\alpha$-Toc

Residual Amount of $\alpha$-Toc ($\mu$M)

| Solubilizing agent<br>Time (h) | None<br>1 $\mu$M $Cu^{2+}$ | 25 mM DOC<br>1 $\mu$M $Cu^{2+}$ | 25 mM CO<br>1 $\mu$M $Cu^{2+}$ | 25 mM SDS<br>1 $\mu$M $Cu^{2+}$ | 25 mM $C_{16}$-TBr<br>1 $\mu$M $Cu^{2+}$ |
|---|---|---|---|---|---|
| 0.0 | 100.0 (100.0) | 100.0 (100.0) | 100.0 (100.0) | 100.0 (100.0) | 100.0 (100.0) |
| 1.5 | 25.7 (—) | 21.2 ( 92.4) | 0.0 ( 31.0) | 6.4 ( 74.7) | 79.6 (100.0) |
| 3.0 | — | 5.3 ( 87.7) | 0.0 ( 10.3) | 0.0 ( 58.9) | 63.9 ( 97.9) |
| 6.0 | 9.4 ( 69.1) | 3.7 ( 71.8) | 0.0 ( 0.0) | — ( 34.6) | 32.9 ( 97.2) |
| 24.0 | 0.0 ( 27.0) | 0.0 ( 5.7) | 0.0 ( 0.0) | — ( 2.9) | 0.0 ( 82.9) |

Formed Amount of 5-FDT ($\mu$M)

| Solubilizing agent<br>Time (h) | None<br>1 $\mu$M $Cu^{2+}$ | 25 mM DOC<br>1 $\mu$M $Cu^{2+}$ | 25 mM CO<br>1 $\mu$M $Cu^{2+}$ | 25 mM SDS<br>1 $\mu$M $Cu^{2+}$ | 25 mM $C_{16}$-TBr<br>1 $\mu$M $Cu^{2+}$ |
|---|---|---|---|---|---|
| 0.0 | 0.0 (0.0) | 0.0 ( 0.0) | 0.0 (0.0) | 0.0 (0.0) | 0.0 (0.0) |
| 1.5 | 0.8 (—) | 11.3 ( 0.8) | 6.4 (1.5) | 1.3 (0.5) | 1.7 (0.4) |
| 3.0 | — | 30.5 ( 0.7) | 6.1 (2.9) | 1.3 (0.6) | 4.4 (0.5) |
| 6.0 | 1.8 (0.6) | 50.5 ( 1.0) | — | — (1.3) | 11.3 (0.8) |
| 24.0 | 2.0 (2.8) | 57.4 (11.8) | 3.2 (6.1) | — (2.9) | 35.8 (1.6) |

Formed Amount of $\alpha$-TQ ($\mu$M)

| Solubilizing agent<br>Time (h) | None<br>1 $\mu$M $Cu^{2+}$ | 25 mM DOC<br>1 $\mu$M $Cu^{2+}$ | 25 mM CO<br>1 $\mu$M $Cu^{2+}$ | 25 mM SDS<br>1 $\mu$M $Cu^{2+}$ | 25 mM $C1_6$-TBr<br>1 $\mu$M $Cu^{2+}$ |
|---|---|---|---|---|---|
| 0.0 | 0.0 (0.0) | 0.0 ( 0.0) | 0.0 (0.0) | 0.0 ( 0.0) | 0.0 (0.0) |
| 1.5 | 1.9 (—) | 9.0 ( 1.0) | 15.5 (0.0) | 39.1 ( 3.6) | 2.2 (—) |
| 3.0 | — | 10.0 ( 1.9) | 15.3 (0.0) | 40.3 ( 7.2) | 4.8 (0.0) |
| 6.0 | 3.7 (0.1) | 8.5 ( 3.1) | — (0.3) | — (11.3) | 9.3 (0.0) |
| 24.0 | 4.4 (1.7) | 9.7 (11.2) | 13.9 (0.4) | — (21.9) | 18.0 (0.2) |

Reaction temperature, 80 °C. The numbers in parentheses show the yields in the absence of $Cu^{2+}$ ion.

study were analyzed by means of the inductively coupled plasma mass spectrometry. The results showed that the only metal ion present at greater than 1 $\mu$g/l was sodium ion (2.3 $\mu$g/l), and that all the other metal ions present were less than 1 $\mu$g/l. Solubilizing agents used for the reactions can be assumed as the other contamination source. The fact that the reaction rates are retarded under high concentration of the solubilizing agents (data not shown) is one reason to exclude this assumption.

The reactions were followed in the presence of disodium ethylenediamine tetraacetate (EDTA). The residual amount of $\alpha$-Toc was slightly smaller and the amounts of 5-FDT and $\alpha$-TQ practically unchanged 6 h after in aqueous DOC in the presence of 0.1—10.0 $\mu$M EDTA. From these results, the reactions without added polyvalent metal ions are believed to proceed without catalysis of metal ions.

**Effects of Solubilizing Agents on the Oxidation of $\alpha$-Toc** In our previous study,[3] we found that the oxidation of $\alpha$-Toc by oxygen was greatly influenced by the structure of the solubilizing agents used for the reaction medium. The results prompted us to investigate the effect of the solubilizing agents on the reaction using $Cu^{2+}$ ion. The reaction was monitored by both the consumption of $\alpha$-Toc and the formation of 5-FDT and $\alpha$-TQ, the results of which are shown in Table 1. In this experiment, four solubilizing agents were selected from those shown in Chart 1, that is, DOC, CO, SDS, and C16-TBr. The results in the absence of Cu(II), which are listed in parentheses in Table 1, showed that the consumption

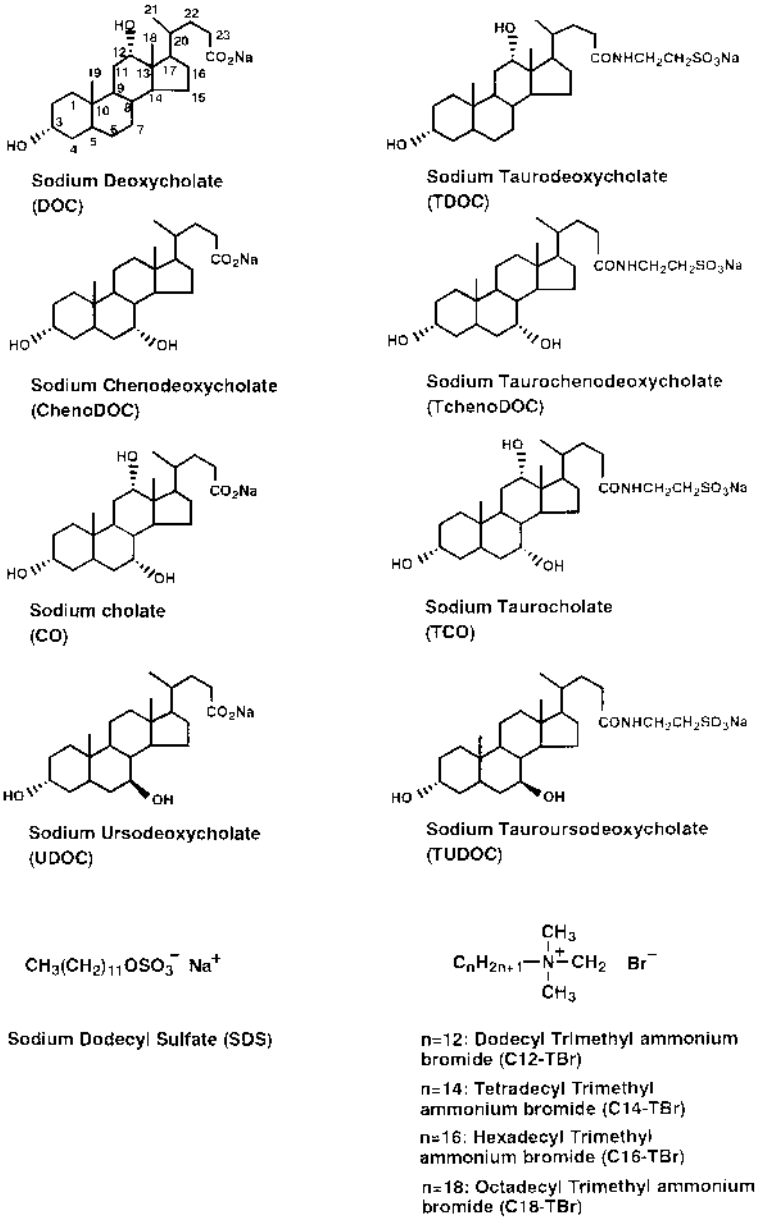



Chart 1. Sodium Cholate Derivatives, Alkyltrimethylammonium Bromide and Sodium Dodecyl Sulfate Used as Solubilizing Agents

of $\alpha$-Toc was faster in the order of CO>SDS>none≧DOC>C16-TBr. By the addition of Cu(II) ion, the reaction was accelerated in every case, and the yield of 5-FDT was increased to 42% in DOC (3 h), and 36% in C16-TBr (24 h). The yield of $\alpha$-TQ was highest in the solution of SDS (40%, 3 h).

**Effect of Sodium Cholate Derivatives on the Oxidation of $\alpha$-Toc** The previous[3] and the present studies (Table 1) showed that the products and the rates of the reactions of $\alpha$-Toc in aqueous solutions with oxygen were greatly dependent on solubilizing agents. Thus, reactions using various sodium cholate derivatives shown in Chart 1 were studied. The consumption of $\alpha$-Toc and the formation of 5-FDT and $\alpha$-TQ with time using 1 $\mu$M Cu(II) at 80 °C are shown in Fig. 2. The upper and lower figures show the results by solutions of non-conjugated cholates and of taurine-conjugated cholates, respectively. The consumption of $\alpha$-Toc was fast in solutions of non-conjugated cholates, and the yields of $\alpha$-TQ were lower than 10%. The yields of 5-FDT were, however, as high as 51% in DOC, and 33% in ChenoDOC after 6 h. Since the lipophilicity of non-conjugated cholate was reported to be in the order of DOC>ChenoDOC>CO>UDOC,[5] the yields of 5-FDT seem to increase with the lipophilicity of the cholates. Because of the low solubility of $\alpha$-Toc in the aqueous solution of UDOC, the initial $\alpha$-Toc concentration in the solution was 24 mM and the yield of 5-FDT was estimated as 37.5% (9 mM/24 mM). In the cases of taurine-conjugated cholates, the consumption of $\alpha$-Toc became slower in the order of TDOC>TchenoDOC>TCO, which is also the order of the lipophilicity of the agents. The yield of $\alpha$-TQ increased considerably in the solution of TDOC and the results are of interest with compared to those of DOC, since the yield of 5-FDT was highest in DOC. In other words, taurine conjugation alters the main reaction product from 5-FDT to $\alpha$-TQ.

**Effect of the Reaction Temperature on the Oxidation of**

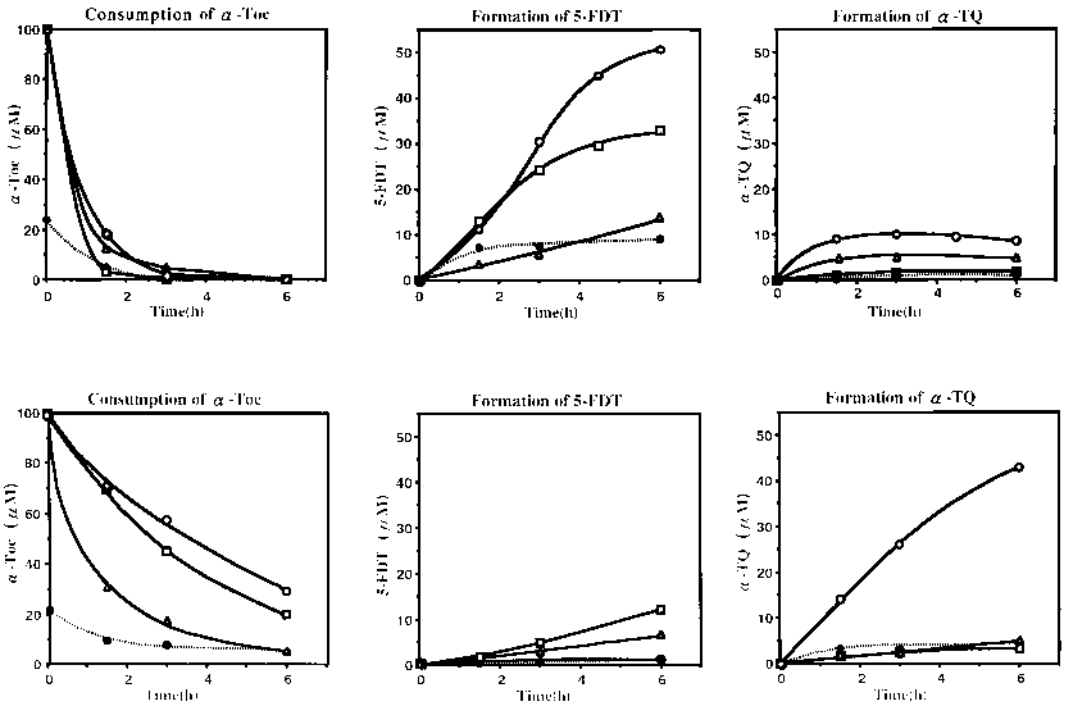



Fig. 2. Effects of Sodium Cholates on Oxidation by Oxygen of $\alpha$-Toc in the Presence of 1 $\mu$M Cu(II) at 80 °C

Concentration of cholate ions was 25 mM. Upper figures: ○, DOC; □, ChenoDOC; △, CO; ●, UDOC. Lower figures: ○, TDOC; □, TchenoDOC; △, TCO; ●, TUDOC.

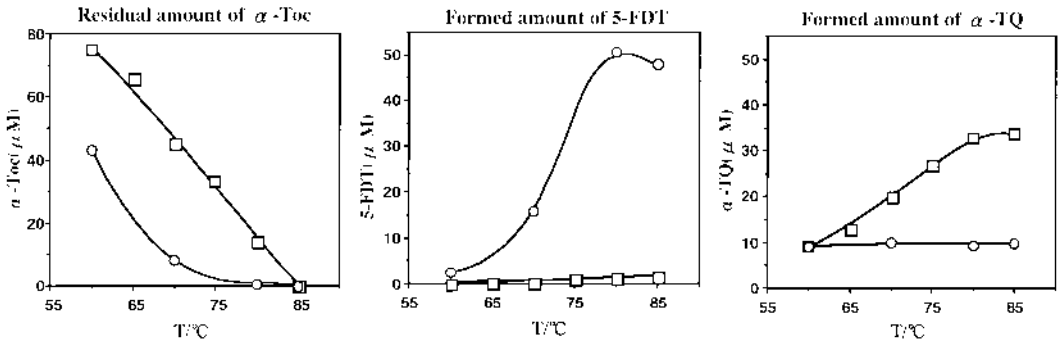



Fig. 3. Effect of Temperature on Oxidation by Oxygen of $\alpha$-Toc in Aqeous DOC and TDOC

Reaction time, 6 h; initial concentration of $\alpha$-Toc, 100 $\mu$M; concentration of DOC and TDOC, 25 mM; concentration of Cu(II), 1.0 $\mu$M. ○, DOC; □, TDOC.

**$\alpha$-Toc** The effects of temperature on the reaction in DOC and TDOC solubilized $\alpha$-Toc were studied and the results are shown in Fig. 3. In the case of DOC, the consumption of $\alpha$-Toc and the formation of 5-FDT became faster as the reaction temperature was raised, but the formation of $\alpha$-TQ was less than 10% and only slightly affected by the temperature. On the other hand, $\alpha$-TQ was the major product in TDOC, although the temperature dependency was less than that of 5-FDT in DOC.

**Effects of Alkyltrimethylammonium Bromides** The oxidation by oxygen was studied on $\alpha$-Toc in aqueous solutions solubilized by alkyltrimethylammonium bromides with alkyl groups of C12, C14, C16 and C18. Table 2 summarizes the results, which show that the shorter the alkyl group in the alkyltrimethylammonium bromides, the greater the consumption of $\alpha$-Toc in 24 h reaction. The yield of 5-FDT was highest when tetradecyltrimethylammonium bromide was used at 80 °C in the presence of 1 $\mu$M Cu(II). $\alpha$-TQ was formed much more than 5-FDT in the presence of 5 $\mu$M Cu(II) at 50 °C.

The effect of the reaction temperature was further investigated using three solubilizing agents (C12, C14, C16) at 60—85 °C and the results are summarized in Fig. 4. The yield of 5-FDT increased with the increase of temperature in solutions of the tetradecyl and hexadecyl derivatives and the yield raised up to 55% in solutionts of the tetradecyl derivative at 80 °C. The yield, however, decreased with the increase of temperature in solution of dodecyltrimethylammounium bromide. The formation of $\alpha$-TQ gradually decreased with temperature in solutions of the three alkyltrimethylammonium bromides.

## Discussion

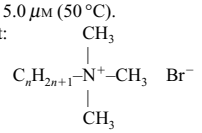
$\alpha$-Toc was solubilized in aqueous solutions using various solubilizing agents and the products of oxidation of $\alpha$-Toc by oxygen were analyzed in the presence and the absence of $Cu^{2+}$ ion. With the solubilizing agents used in the present study, 5-FDT and $\alpha$-TQ were the major oxidation products.

Table 2. Effect of the Chain Length of Alkyltrimethylammonium Bromide on Cu(II)-Catalyzed Oxidation by Oxygen of $\alpha$-Toc

| Solubilizing agent | $\alpha$-Toc unreacted ($\mu$M) | | | | 5-FDT($\mu$M) | | | | $\alpha$-TQ ($\mu$M) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $C_{12}$ | $C_{14}$ | $C_{16}$ | $C_{18}$ | $C_{12}$ | $C_{14}$ | $C_{16}$ | $C_{18}$ | $C_{12}$ | $C_{14}$ | $C_{16}$ | $C_{18}$ |
| 80 °C +1 $\mu$M $Cu^{2+}$ | 0 | 0 | 5.09 | 16.61 | 5.34 | 54.54 | 41.06 | 25.53 | 0.43 | 19.83 | 17.29 | 27.38 |
| 50 °C +5 $\mu$M $Cu^{2+}$ | 4.92 | 36.91 | 27.02 | 51.14 | 10.40 | 2.48 | 4.34 | 2.34 | 42.25 | 45.33 | 38.11 | 22.50 |

Reaction time, 24 h; reaction temperature, 80 °C, 50 °C; initial concentration of $\alpha$-Toc, 100 $\mu$M; concentration of alkyltrimethylammonium bromide, 25 mM; concentration of Cu(II), 1.0 $\mu$M (80 °C), 5.0 $\mu$M (50 °C).

Solubilizing agent:



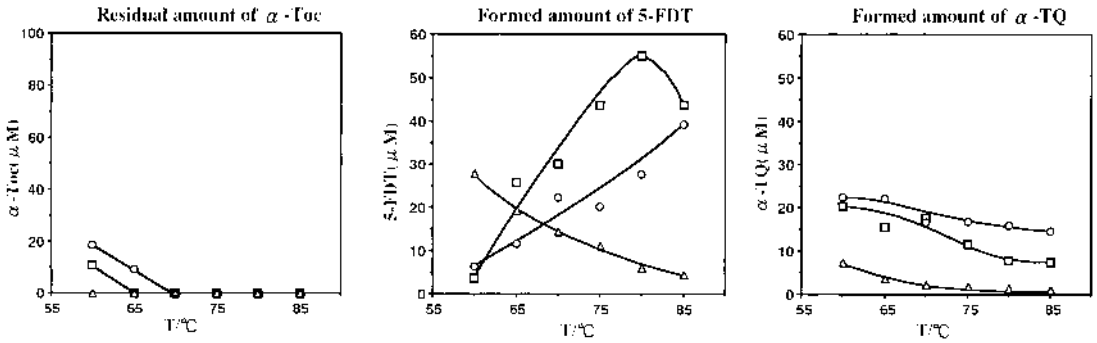



Fig. 4. Effect of Chain Length of Alkyltrimethylammonium Bromide and Temperature on the Reaction

Reaction time, 24 h; initial concentration of $\alpha$-Toc, 100 $\mu$M; concentration of alkyltrimethylammonium bromide, 25 mM; concentration of Cu(II), 2.0 $\mu$M. ○, C16; □, C14; △, C12.

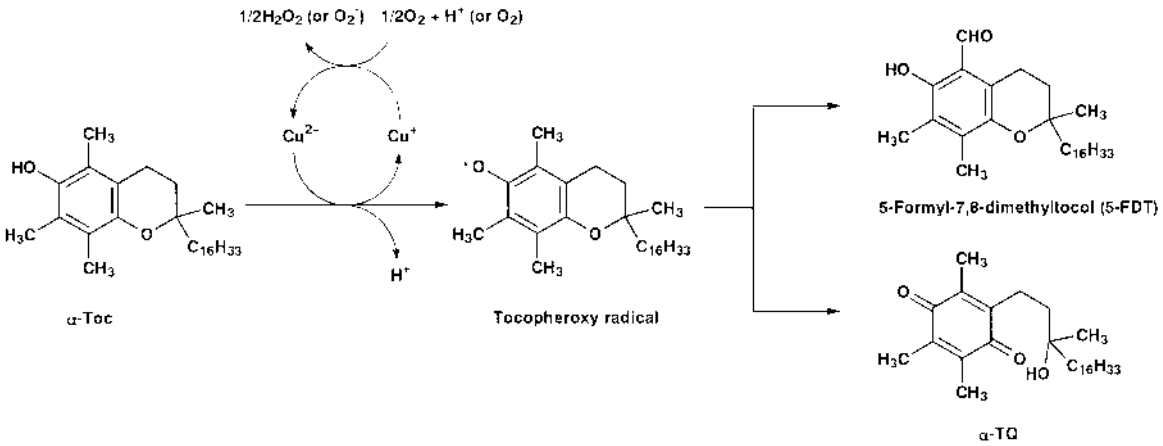



Chart 2. Oxidation by Oxygen of $\alpha$-Toc and Cu(II) Catalysis

Since the formation of 5-FDT was a slower process than the consumption of $\alpha$-Toc, there must be undetected intermediate(s) in the reaction. In DOC solution the formation of 5-FDT increased and the reaction was accelerated by the addition of a catalytic amount of Cu(II) ion. The consumption of $\alpha$-Toc was fastest in CO solution among the solubilizing agents studied, though the yield of 5-FDT was lower than in DOC solution. These facts suggest that a slight change of the structure of the solubilizing agents affects the course of the reaction.

In SDS solution, the major product was $\alpha$-TQ. Though SDS is an anionic detergent as well as DOC and CO, the main course of the reaction was different. It was reported that SDS forms a spherical micelle and the aromatic moiety of $\alpha$-Toc is included in the paricede layer, and the isoprenoid chain is in the inner area of the micelle by hydrophobic interaction.[6] This inclusion process is quite different from that of DOC and CO, which forms a pleats-sheet structure and molecules are included between them. C16-TBr is a cationic detergent. The slow reaction in C16-TBr solution might be caused by the cationic surface of the micelle structure which can present a repulsive interaction toward $Cu^{2+}$ ion.[7,8]

Yoshida *et al.*[10] and Maiorino *et al.*[9] reported that $\alpha$-Toc reduced Cu(II) to Cu(I) and was transformed into $\alpha$-tocopheroxy radical in the course of lipid peroxidation in liposome or micelle. Thus, the proposed reaction mechanism is

as shown in Chart 2.

In the presence of Cu(II), it should react faster than oxygen with $\alpha$-Toc to form Cu(I) and tocopheroxy radical. The Cu(I) ion thus formed would be oxidized by oxygen[11] to regenerate Cu(II), which acts as a catalyst for further oxidation. Tocopheroxy radical formed by the above reaction was transformed into 5-FDT or $\alpha$-TQ depending upon the mode of the inclusion by the solubilizing agent. The conversion to $\alpha$-TQ was thought to occur *via* the addition of an oxygen molecule to the carbon at the 8a position, thus the inclusion of this site with solubilizing agent would alter the reaction pathway.

Study on the reaction in solutions of alkyltrimethylammonium bromides showed that 5-FDT was formed in good yields in the cases of C14–C16 alkyltrimethyl ammonium bromides. $\alpha$-Toc has a long alkyl group, whose straight-chain length is C13 with three methyl groups attached at the 4′-, 8′-, and -12′ positions. Therefore, the length of C14–C16 would include properly the alkyl group and extend to the chroman moiety. Thus the access of an oxygen molecule to C-8a would be hindered and the formation of $\alpha$-TQ might be prevented and that of 5-FDT was favored instead.

In the cases of sodium cholate derivatives, DOC and TDOC were found to afford the major products more than other cholate derivatives, although the main products were different from each other (Figs. 2, 3). The results indicate that $\alpha$-Toc is included more effectively in DOC derivatives than other cholate derivatives, and that the reactive chroman moiety is located near the carboxylate group of DOC to change the reaction path under the influence of the structure of this group. The temperature dependency (Fig. 3) and detailed mechanistic study remain to be clarified.

In this paper, we described that $\alpha$-Toc was oxidized in air in the presence of $Cu^{2+}$ ion in aqueous solubilizing agent to give 5-FDT and $\alpha$-TQ in various ratios depending upon the structure of the solubilizing agents. This is the first finding that the oxidative process of $\alpha$-Toc in water is governed by the properties of the molecular environments rather than oxidants.

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11) In the reaction of Cu(I) and oxygen, it was reported that the reaction pathway was changed under the influences of surroundings such as solvent and ligands. From the stoichiometrical viewpoint, there are two types of the reactions; i) one consists of two Cu(I) ion and one oxygen to give two Cu(II) and hydrogen peroxide[a,b]; ii) the other consists of one Cu(I) and oxygen to give Cu(II) and superoxide.[c,d] See, *a*) ZuberbŸhler A. D., *Helv. Chim. Acta*, **59**, 1448 (1976): *b*) Gorbunova N. V., Purmal A. P., Skurlatov Y. I., Travin S. O., *Zh. Phys. Khim.*, **51**, 1984 (1976); *c*) Graham D. R., Marshall L. E., Reich K. A., Sigman D. S., *J. Am. Chem. Soc.*, **102**, 5419 (1980); *d*) Goldstein S., Czapski G., *ibid.*, **105**, 7276 (1983).