Oxidation Kinetics of Linoleic Acid in the Presence of Saturated Acyl L-Ascorbate

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ABSTRACT: The oxidation processes of linoleic acid (LA) in the presence of L-ascorbic acid or saturated acyl L-ascorbate additives were measured at various temperatures and molar ratios of the additive to LA. Higher oxidative stability of LA was observed at higher additive levels for all additives. The addition of the ascorbates lengthened the induction period for the oxidation of LA. An autocatalytic kinetic rate equation was used to model the oxidation processes of LA mixed with the ascorbates, and the dependence of the rate constant, *k*, on acyl-chain carbon number was determined. At any temperature, the use of ascorbate additives decreased the *k* value for LA, and there was a slight tendency for *k* values to decrease with increasing acyl-chain length. The apparent activation energy, E_a, and the frequency factor, k_0, for the rate constant were determined from Arrhenius plots. The calculated E_a and k_0 values also decreased with increasing ascorbate acyl-chain length.

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KEY WORDS: Apparent activation energy, autocatalytic-type kinetics, frequency factor, rate constant, saturated acyl L-ascorbate.

Lipid oxidation in foods causes rancidity and nutritional deterioration. Oxidation is a complicated process including the steps of initiation, propagation, and termination. The kinetics of the oxidation in a bulk lipid have been extensively studied (1–3), and the entire lipid oxidation process has been described by a kinetic expression of the autocatalytic type in terms of the fraction of unoxidized substrate (2). In previous studies, we also applied the expression to the entire oxidation process of n-6 FFA, their esters, and acylglycerols, and we evaluated their kinetic parameters (4–6).

L-Ascorbic acid is a hydrophilic vitamin with high reducing activity due to its enediol-lactone resonant structure; it is widely used as an additive in foods and cosmetics. Its lipophilic derivatives with long acyl chains such as 6-*O*-palmitoyl and 6-*O*-stearoyl L-ascorbates are also used as an additive in foods rich in lipids. Recently, some studies have appeared on the lipase-catalyzed synthesis of acyl ascorbates in an organic solvent (7,8). We also synthesized various 6-*O*-acyl ascorbates by using an immobilized lipase and reported their antioxidative abilities (9–12). Although there have been some studies of the antioxidative ability of palmitoyl ascorbate for the oxidation of oils (13–17), the application of the acyl ascorbates with medium chain lengths has not been reported.

In this study, we analyzed the oxidation processes of linoleic acid (LA) in the presence of L-ascorbic acid or saturated acyl L-ascorbate by applying a kinetic equation of the autocatalytic type to the processes. The dependence of the rate constant on the acyl-chain carbon number of the ascorbates at various temperatures was investigated. Furthermore, the apparent activation energy, E_a, and the frequency factor, k_0, for the rate constant were determined, and the effects of the carbon number on them were demonstrated.

EXPERIMENTAL PROCEDURES

Materials. L(+)-Ascorbic acid and palmitoyl L-ascorbate were obtained from Nacalai Tesque (Kyoto, Japan). Acetone, caprylic acid, and methyl myristate were purchased from Wako Pure Chemical Industries (Osaka, Japan). Lauric acid and trimethylsilyl diazomethane solution were purchased from Sigma Chemical (St. Louis, MO). Immobilized lipase, Chirazyme® L-2 C2 from *Candida antarctica*, was purchased from Roche Molecular Biochemicals (Mannheim, Germany). Linoleic acid (purity >90%) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). All the other chemicals of analytical grade were obtained from either Wako Pure Chemical Industries or Nacalai Tesque.

Enzymatic synthesis and purification of saturated acyl L-ascorbate. L-Ascorbic acid (0.04 mol) and caprylic or lauric acid (0.2 mol) were added to a screw-capped amber glass bottle, and 20 g of Chirazyme® L-2 C2 and 400 mL of acetone were further added to the bottle. The headspace of the bottle was filled with N_2, and the bottle was tightly sealed. The bottle was then immersed in a water bath at 55°C with vigorous shaking. After *ca.* 24 h, each 6-*O*-acyl L-ascorbate was isolated from the reaction mixture according to the reported procedures (8), with a slight modification. The immobilized lipase was removed by filtration from the reaction mixture, and the solvent was removed by rotary evaporation. The concentrate was washed three times with 50 mL of *n*-hexane to remove the unreacted FA. The mixture was dissolved in 150 mL of ethylmethylketone, and water (3 × 50 mL) was added to remove the unreacted ascorbic acid. The organic phase was separated, and excess solvent was removed by rotary evaporation. The purified acyl ascorbate was dried in a desiccator with a petri dish containing phosphorus pentoxide.

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Oxidation of linoleic acid. The autoxidation processes of LA mixed with L-ascorbic acid or saturated acyl L-ascorbate at various molar ratios were measured as follows: First, LA and L-ascorbic acid or capryloyl, lauroyl, or palmitoyl L-ascorbate were dissolved in methanol at concentrations of 0.120 and 0.012 mol/L, respectively. The LA solution (200 μL each, which corresponded to 0.024 mmol of LA) was placed in amber glass vials. Then, 50, 100, 150, 200, or 400 μL of L-ascorbic acid or acyl ascorbate solution was added to the vials to give the molar ratio of 0.025, 0.05, 0.075, 0.1, or 0.2 (ascorbate/LA), respectively. Then 150, 100, and 50 μL of methanol was added to the samples in the molar ratios of 0.025, 0.05, and 0.075, respectively. For the oxidation process of LA without any additive, 200 μL of methanol only was added to the LA solution. The mixture (25 μL each) was placed in flat-bottomed glass cups (1.5 cm i.d. and 3.0 cm height), and the methanol was then evaporated under reduced pressure. The cups were placed in a plastic container in which a petri dish filled with a saturated lithium chloride solution was placed to maintain the relative humidity (RH) at 12%. The container was stored in the dark at a given temperature (37, 50, 65, or 80°C). Samples were periodically taken, and 300 μL of methyl myristate solution [methanol/benzene/methyl myristate = 20:80:0.05 (by vol)] was added. Methyl myristate was used as the internal standard for GC analysis of unoxidized LA. Thirty microliters of a 2.0 mol/L trimethylsilyl diazomethane solution (hexane as a solvent) was then poured into the cup to convert unoxidized LA to methyl linoleate (18). The mixture was left for 30 min at room temperature. After evaporation of methanol, benzene, and hexane under reduced pressure, the remainder was then dissolved with 500 μL of hexane, which was used for the GC analysis with an FID (19).

RESULTS AND DISCUSSION

Oxidation process of linoleic acid mixed with L-ascorbic acid or capryloyl L-ascorbate. Figure 1 shows the oxidative stabilities at 37°C and 12% RH of LA mixed with (a) L-ascorbic acid and (b) capryloyl ascorbate at various molar ratios of the ascorbates to LA. The higher the molar ratio was, the more strongly the oxidation of LA was suppressed in both cases. The induction period for the oxidation of LA was lengthened by the addition of ascorbates. The oxidation processes of LA mixed with lauroyl and palmitoyl ascorbates were similar (data not shown). Capryloyl ascorbate had a greater antioxidative ability than ascorbic acid. The oxidation of LA when mixed with capryloyl ascorbate at the molar ratio of 0.1 was suppressed for at least 130 h. Frankel *et al*. (15) reported that hydrophilic ascorbic acid was more efficient as an antioxidant than lipophilic palmitoyl ascorbate for the oxidation of oil in a bulk system. Our results are contradictory to theirs. A possible reason for the inconsistency is a difference in the concentration of the ascorbate. The concentrations of the ascorbates in our experiments were higher by two to three orders of magnitude than those in the experiments by Frankel *et al.* (15). Another possible reason is a difference in the substrate. We used LA, whereas they used soybean oil.

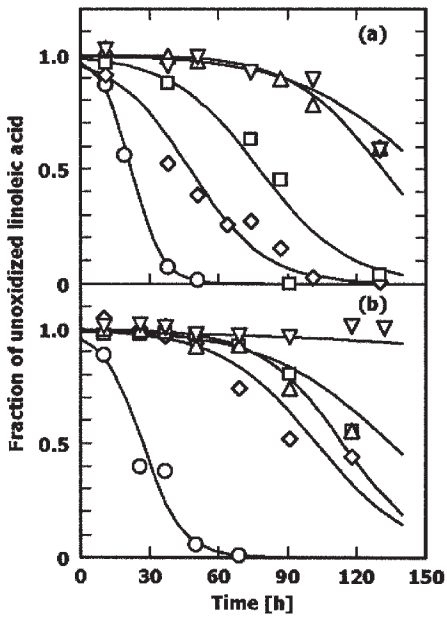

FIG. 1. Oxidation processes of linoleic acid mixed with (a) L-ascorbic acid or (b) capryloyl L-ascorbate at various molar ratios and at 37°C and 12% RH. The ratios of the additives to linoleic acid were (○) 0, (◇) 0.025, (□) 0.05, (△) 0.075, and (▽) 0.1. The solid curves were obtained from the estimated values of k and Y_0 for the oxidation processes.

Applicability of the kinetic equation of the autocatalytic type to the oxidation process of linoleic acid mixed with a saturated acyl L-ascorbate. Figure 2 shows the oxidation processes at 80°C and 12% RH of LA with no additive and of LA mixed with L-ascorbic acid or capryloyl, lauroyl, or palmitoyl ascorbate at the molar ratio (additive/LA) of 0.05. LA with no additive was quickly oxidized. When ascorbic acid was added to LA, the oxidative stability of LA was slightly improved. Capryloyl, lauroyl, and palmitoyl ascorbates retarded the oxidation of LA more than ascorbic acid. There seemed to be little difference in the antioxidative ability among the three acyl ascorbates.

We showed previously (4,6) that the entire oxidation process of LA could be expressed by the following kinetic equation of the autocatalytic type:

$$\frac{dY}{dt} = -kY(1-Y) \qquad [1]$$

where Y is the fraction of the unoxidized substrate, t is the time, and k is the rate constant. Under the condition of $Y = Y_0$ at $t = 0$, the integration of Equation 1 gives

$$\ln\frac{1-Y}{Y} = kt + \ln\frac{1-Y_0}{Y_0} \qquad [2]$$

where Y_0 is the initial fraction of unoxidized substrate and determines the induction period due to the mathematical format of the equation. The applicability of Equation 2 to the oxidation processes of LA mixed with various ascorbates was examined. Linear plots of $\ln[(1 - Y)/Y]$ vs. t for the oxidation process are shown in Figure 3. Based on a linear regression analysis,

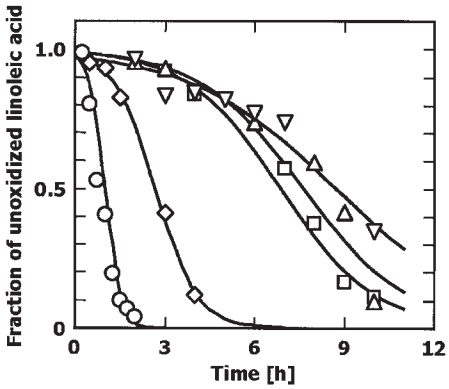

FIG. 2. Oxidation processes of (○) linoleic acid with no additive and that mixed with (◇) L-ascorbic acid, (□) capryloyl L-ascorbate, (△) lauroyl L-ascorbate, or (▽) palmitoyl L-ascorbate at the molar ratio of 0.05 and at 80°C. The solid curves were drawn using the k and Y_0 values estimated in Figure 3.

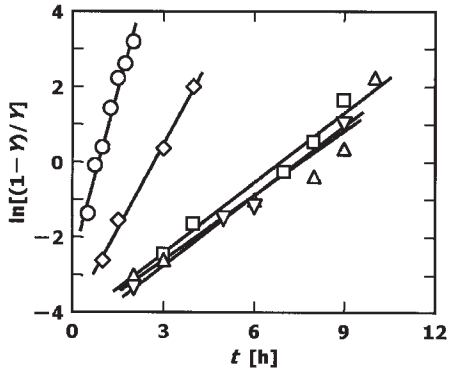

FIG. 3. Determination of the rate constant, k, in the rate expression of the autocatalytic type at 80°C and with the molar ratio of 0.05. The symbols are the same as those in Figure 2. Y denotes the fraction of unoxidized linoleic acid. The solid curves were drawn based on Equation 2.

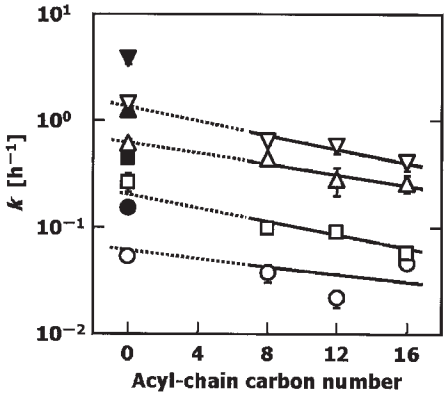

FIG. 4. Relationship between acyl-chain carbon number of ascorbates and the rate constant k at (○) 37, (□) 50, (△) 65, and (▽) 80°C. Open symbols represent the rate constants (mean ± SE) for the oxidation of linoleic acid mixed with various ascorbates, and the closed symbols that for no additive.

the k and Y_0 values were determined from the slope and the intercept, respectively (Table 1). The k and Y_0 values for the oxidation processes of LA at 37, 50, and 65°C were also estimated in the same manner.

Dependence of the rate constants on acyl-chain carbon number of ascorbates. Figure 4 shows the relationship between the acyl-chain carbon number of the ascorbates and the k value at various temperatures. At any temperature, the k value for LA with no additive (closed symbols) was greater than that for LA with ascorbic acid or ascorbate (open symbols). When capryloyl, lauroyl, or palmitoyl ascorbate was added to LA, there was a tendency for the k value in the presence of ascorbates with larger acyl-chain carbon numbers to be slightly smaller. The k value for LA mixed with ascorbic acid (acyl-chain carbon number equal to zero) seemed to be greater than that for LA mixed with the acyl ascorbates at any temperature.

Figure 5 shows the dependence of the acyl-carbon number of the ascorbates on the $\ln[(1 - Y_0)/Y_0]$ value in the rate expression of the autocatalytic type (Eq. 2). As mentioned above, the Y_0 value reflects the initial state of the substrate, which affects the induction period. The large Y_0 value indicates the elongation of the induction period. The Y_0 values for the additives were greater than that for LA with no additive although there were some exceptions. This indicated that the addition of an additive delayed the induction period in the oxidation process of LA.

The temperature dependence of the rate constant k was analyzed based on the Arrhenius equation:

$$k = k_0 \exp(-E_a / RT) \quad [3]$$

where k_0 is the frequency factor, E_a is the apparent activation energy, R is the gas constant, and T is the absolute temperature. The Arrhenius plots using the results shown in Figure 4, in each case, produced a straight line for use in evaluating E_a and k_0 from the slope and the intercept of the line, respectively. Figure 6 shows the relationship between the acyl-chain carbon number and the

TABLE 1
The Rate Constant k and the Parameter Y_0 for the Oxidation of Linoleic Acid at 80°C[a]

Additive	None	Ascorbic acid	Capryloyl ascorbate	Lauroyl ascorbate	Palmitoyl ascorbate
k [h^{-1}]	3.81 ± 0.49	1.44 ± 0.065	0.634 ± 0.043	0.571 ± 0.081	0.405 ± 0.071
$\ln[(1 - Y_0)/Y_0]$	−3.78 ± 0.62	−3.83 ± 0.16	−4.36 ± 0.31	−4.36 ± 0.56	−3.53 ± 0.43
Y_0(mean)	0.978	0.979	0.987	0.987	0.972

[a]The values are mean ± SE expected for the Y_0 value.

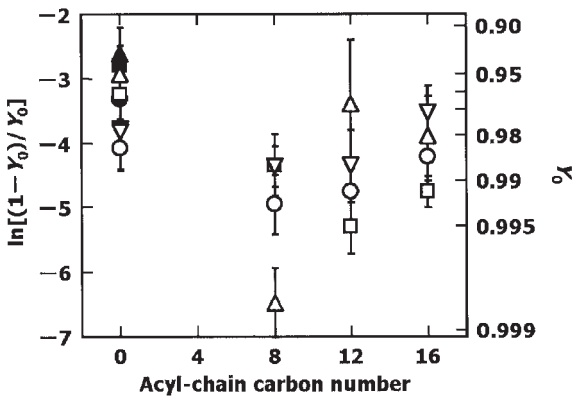

FIG. 5. Dependence of the $\ln[(1 - Y_0)/Y_0]$ value on acyl-chain carbon number of ascorbates at various temperatures. The symbols are the same as those in Figure 4. Open symbols represent the $\ln[(1 - Y_0)/Y_0]$ values (mean ± SE) in the autocatalytic-type rate expression for the oxidation of linoleic acid mixed with various ascorbates, and the closed symbols that for no additive.

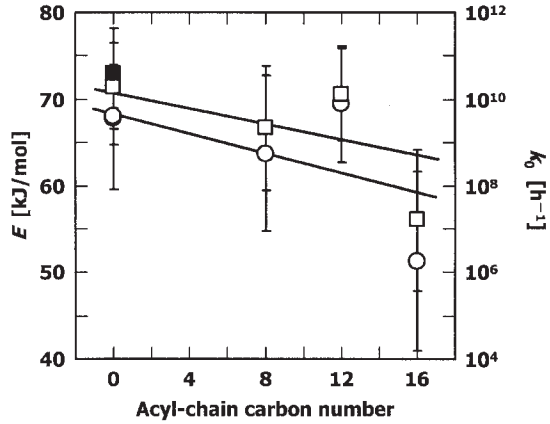

FIG. 6. Effect of acyl-chain carbon number of ascorbates on (○) the apparent activation energy, E_a, or (□) the frequency factor, k_0, for the rate constant (mean ± SE). Open symbols represent the oxidation processes of linoleic acid mixed with various ascorbates, and the closed symbols that for no additive.

E_a or k_0 value for the rate constant. The E_a values for LA mixed with every ascorbate were 50 to 70 kJ/mol, and there was a tendency for E_a value to decrease with increasing acyl-chain carbon number. The k_0 values for LA mixed with ascorbates were smaller than that for LA with no additive or mixed with ascorbic acid. The k_0 value also decreased with increasing acyl-chain length. This would indicate that the presence of acyl ascorbate lowers both the height of the energy barrier for the oxidation of LA and the probability of the reaction resulting in the oxidation of LA.

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REFERENCES

1. Cosgrove, J.P., D.F. Church, and W.A. Pryor, The Kinetics of the Autoxidation of Polyunsaturated Fatty Acids, *Lipids 22*:299–304 (1987).
2. Özilgen, S., and M. Özilgen, Kinetic Model of Lipid Oxidation in Foods, *J. Food Sci. 55*:498–501, 536 (1990).
3. Brimberg, U.I., On the Kinetics of the Autoxidation of Fats, *J. Am. Oil Chem. Soc. 70*:249–254 (1993).
4. Adachi, S., T. Ishiguro, and R. Matsuno, Autoxidation Kinetics for Fatty Acids and Their Esters, *Ibid. 72*:547–551 (1995).
5. Minemoto, Y., E. Ishido, S. Adachi, and R. Matsuno, Autoxidation Kinetics for Polyunsaturated Acylglycerols, *Food Sci. Technol. Res. 5*:104–107 (1999).
6. Ishido, E., Y. Minemoto, S. Adachi, and R. Matsuno, Autoxidation Process of Linoleic Acid and Methyl Linoleate Mixed with Saturated Fatty Acid or Its Methyl Ester, *Lebensm. Wissen. Technol. 34*:234–238 (2001).
7. Tang, L., H. Zhang, M.M. Shehate, and Y. Sun, A Kinetic Study of the Synthesis of Ascorbate Fatty Acid Esters Catalyzed by Immobilized Lipase in Organic Media, *Biotechnol. Appl. Biochem. 32*:35–39 (2000).
8. Yan, Y., U.T. Bornscheuer, and R.D. Schmid, Lipase-Catalyzed Synthesis of Vitamin C Fatty Acid Esters, *Biotechnol. Lett. 21*:1051–1054 (1999).
9. Watanabe, Y., S. Adachi, K. Nakanishi, and R. Matsuno, Condensation of L-Ascorbic Acid and Medium-Chain Fatty Acids by Immobilized Lipase in Acetonitrile with Low Water Content, *Food Sci. Technol. Res. 5*:188–192 (1999).
10. Watanabe, Y., Y. Minemoto, S. Adachi, K. Nakanishi, Y. Shimada, and R. Matsuno, Lipase-Catalyzed Synthesis of 6-*O*-Eicosapentaenoyl L-Ascorbate in Acetone and Its Autoxidation, *Biotechnol. Lett. 22*:637–640 (2000).
11. Watanabe, Y., S. Adachi, K. Nakanishi, and R. Matsuno, Lipase-Catalyzed Synthesis of Unsaturated Acyl L-Ascorbates and Their Ability to Suppress the Autoxidation of Polyunsaturated Fatty Acids, *J. Am. Oil Chem. Soc. 78*:823–826 (2001).
12. Watanabe, Y., X. Fang, Y. Minemoto, S. Adachi, and R. Matsuno, Suppressive Effect of Saturated Acyl L-Ascorbate on the Oxidation of Linoleic Acid Encapsulated with Maltodextrin or Gum Arabic by Spray-Drying, *J. Agric. Food Chem. 50*:3984–3987 (2002).
13. Cort, W.M., Antioxidant Activity of Tocopherols, Ascorbyl Palmitate, and Ascorbic Acid and Their Mode of Action, *J. Am. Oil Chem. Soc. 51*:321–325 (1974).
14. Gwo, Y.Y., G.J. Flick, Jr., and H.P. Dupuy, Effect of Ascorbyl Palmitate on the Quality of Frying Fats for Deep Frying Operations, *Ibid. 62*:1666–1671 (1985).
15. Frankel, E.N., S.W. Huang, J. Kanner, and J.B. German, Interfacial Phenomena in the Evaluation of Antioxidants: Bulk Oils vs. Emulsions, *J. Agric. Food Chem. 42*:1054–1059 (1994).
16. Hamilton, R.J., C. Kalu, G.P. McNeill, F.B. Padley, and J.H. Pierce, Effects of Tocopherols, Ascorbyl Palmitate, and Lecithin on Autoxidation of Fish Oil, *J. Am. Oil Chem. Soc. 75*:813–822 (1998).
17. Lee, K.H., H.S. Yook, J.W. Lee, W.J. Park, K.S. Kim, and M.W. Byun, Quenching Mechanism and Kinetics of Ascorbyl Palmitate for the Reduction of the Gamma Irradiation-Induced Oxidation of Oils, *Ibid. 76*:921–925 (1999).
18. Hashimoto, N., T. Aoyama, and T. Shioiri, A Simple Efficient Preparation of Methyl Esters with Trimethylsilyldiazomethane ($TMSCHN_2$) and Its Application to Gas Chromatographic Analysis of Fatty Acids, *Chem. Pharm. Bull. 29*:1475–1478 (1981).
19. Minemoto, Y., K. Hakamata, S. Adachi, and R. Matsuno, Oxidation of Linoleic Acid Encapsulated with Gum Arabic or Maltodextrin by Spray-Drying, *J. Microencapsul. 19*:181–189 (2002).

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