Oxidation Kinetics for *cis*-9,*trans*-11 and *trans*-10,*cis*-12 Isomers of CLA

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ABSTRACT: The autoxidation processes of the *cis*-9,*trans*-11 (*c*9,*t*11) and *trans*-10,*cis*-12 (*t*10,*c*12) isomers of CLA were separately observed at *ca.* 0% RH and different temperatures. The *t*10,*c*12 CLA oxidized faster than the *c*9,*t*11 isomer at all tested temperatures. The first half of the oxidation process of *t*10,*c*12 CLA obeyed an autocatalytic-type rate expression, but the latter half followed first-order kinetics. On the other hand, the entire oxidation process of *c*9,*t*11 CLA could be expressed by the autocatalytic-type rate expression. The apparent activation energies and frequency factors for the autoxidation of the isomers were estimated from the rate constants obtained at various temperatures based on the Arrhenius equation. The apparent activation energies for the CLA isomers were greater than those for the nonconjugated n-6 and n-3 PUFA or their esters. However, the enthalpy-entropy compensation held during the autoxidation of both the CLA and PUFA. This suggested that the autoxidation mechanisms for the CLA and PUFA were essentially the same.

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KEY WORDS: CLA, enthalpy-entropy compensation, kinetics, oxidation.

The term CLA is used to describe a group of positional and geometric isomers of linoleic acid (LA). The autoxidation, at 37°C, of CLA proceeds at a rate similar to that of nonconjugated LA in the free acid form (1). However, the extent of oxidation, as assessed by the PV, indicated that CLA oxidized more slowly than LA (2). The latter result could be explained by the fact that a conjugated diene is prone to form a cyclic endoperoxide and dioxoetane rather than hydroperoxides (3). CLA is much more susceptible to oxidation in air than LA (4,5) and, similar to DHA, CLA is extremely unstable in both the FFA and TAG forms (5). The foregoing investigations were performed using a mixture of CLA isomers, and the susceptibility of each isomer to autoxidation has not been elucidated. We have recently developed an effective method for fractionating CLA isomers through selective esterification using *Candida rugosa* lipase to produce *cis*-9,*trans*-11 (*c*9,*t*11) and *trans*-10,*cis*-12 (*t*10,*c*12) isomers in purities of about 95% based on the total contents of the two isomers (6).

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In this context, we examined the autoxidation processes in air of the *c*9,*t*11 and *t*10,*c*12 isomers in single-component systems at various temperatures to compare their susceptibility to autoxidation. The rate expressions for describing their autoxidation processes are described, and the rate constants in the expressions were evaluated at various temperatures for both isomers to estimate the apparent activation energies and frequency factors.

EXPERIMENTAL PROCEDURES

Materials. The *t*10,*c*12 and *c*9,*t*11 isomers of CLA were prepared according to our previous method comprising selective esterification with *C. rugosa* lipase, molecular distillation, and urea adduct fractionation (6), except that ethanol was used as a solvent for urea adduct fractionation. The FA compositions of the *t*10,*c*12 preparation were 94.7% (w/w) *t*10,*c*12 CLA, 3.2% *c*9,*t*11 CLA, 1.3% other CLA, and 0.8% oleic acid, and those of the *c*9,*t*11 preparation were 82.4% *c*9,*t*11 CLA, 4.0% *t*10,*c*12 CLA, 13.6% oleic acid, and <0.1% other CLA. Palmitic acid (purity: >95%) used as the internal standard in the GC analysis was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). All the FA were stored at −30°C until used. The other chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan) and were of analytical grade.

Autoxidation process. Either the *t*10,*c*12 or *c*9,*t*12 isomer of CLA (about 30 mg) was dissolved in 1 mL of hexane. Five microliters of that solution was placed in a flat-bottomed cup (15 mm i.d. and 40 mm height) using a microsyringe, and the hexane was removed under a gentle N_2 stream. About 20 cups were prepared for the oxidation of each isomer at a given temperature. The cups were placed in a desiccator, the RH in which was regulated at *ca.* 0% with phosphorus (V) oxide. The desiccator was stored in the dark in a temperature-controlled chamber (40 to 80°C). Periodically, a cup was removed to analyze the amount of unoxidized CLA.

Five microliters of the palmitic acid dissolved in hexane at a concentration of 30 mg/mL was added to the cup as the internal standard for the GC analysis. Unoxidized CLA and palmitic acid were methylesterified according to the method of Murao *et al.* (7). DMSO (1 mL) and a mixture (1 mL) of sulfuric acid and methanol (1:115 by vol) were added to the cup. After being

vigorously mixed, the mixture was allowed to stand at 80°C for 30 min. The mixture was then cooled by immersing the cup in tap water. A saturated sodium chloride solution (1 mL) and hexane (1 mL) were added to the mixture to extract the lipophilic substances into the hexane phase.

The hexane phase was analyzed using a Shimadzu GC-8A gas chromatograph with an FID (Kyoto, Japan) and a column (3.2 mm i.d., 3.0 m length) packed with Thermon-3000 on Shincarbon A (Shinwa Kako, Kyoto, Japan). The analytical conditions were as follows: the volume of sample injected was 5 μL, the temperatures of the injector and detector were both 230°C, and the flow rate of N_2 as the carrier gas was 50 mL/min. The amounts of unoxidized CLA and methyl palmitate, which was derived from palmitic acid through methyl esterification, were determined from their areas under the peaks. The fraction of the unoxidized CLA was calculated from the ratio of these areas.

RESULTS AND DISCUSSION

The autoxidation processes of $t10,c12$ and $c9,t11$ CLA in a single component system were observed at various temperatures. Figures 1A and 1B show the autoxidation processes at 50–80°C for $t10,c12$ and $c9,t11$ CLA, respectively. $t10,c12$ CLA oxidized faster than $c9,t11$ CLA regardless of temperature. The entire autoxidation process of LA (8) and its acylglycerols (9) can be expressed by the following autocatalytic-type equation:

$$\frac{dY}{dt} = -k_1 Y(1-Y) \qquad [1]$$

where Y is the fraction of unoxidized FA, t is the time, and k_1 is the rate constant. The applicability of Equation 1 to the autoxidation processes of $t10,c12$ and $c9,t11$ CLA was examined using Equation 2, which can be derived by integrating Equation 1 under the condition of $Y = Y_0$ at $t = 0$.

$$\ln \frac{1-Y}{Y} = k_1 t + \ln \frac{1-Y_0}{Y_0} \qquad [2]$$

Y_0 is a parameter that was introduced to solve Equation 1 and reflects the initial state of a substrate used in the autoxidation experiment (8). Figures 2A and 2B show the plots of $\ln[(1 - Y)/Y]$ vs. t for $t10,c12$ and $c9,t11$ CLA, respectively. The plots for the first half of the autoxidation process of $t10,c12$ CLA, at a given temperature, lie on a straight line that allows us to evaluate the rate constant k_1 and the parameter Y_0. However, the plots for the latter half of the process deviated from the line as shown by the broken line. A similar phenomenon was observed in the latter half of the oxidation process of n-3 PUFA, and simple first-order kinetics was applicable to describe the process (8):

$$\frac{dY}{dt} = -k_2 Y \ (Y < 0.5) \qquad [3]$$

where k_2 is the rate constant. The integration of Equation 3 under the condition of $Y = 0.5$ at $t = t_{0.5}$ gives
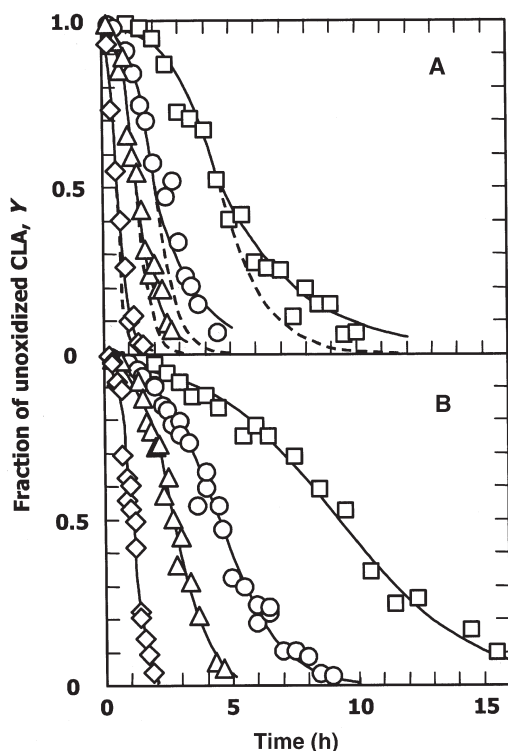

FIG. 1. Autoxidation processes of (A) *trans*-10,*cis*-12 CLA and (B) *cis*-9, *trans*-11 CLA at (□) 50, (○) 60, (△) 70, and (◇) 80°C and *ca.* 0% RH.
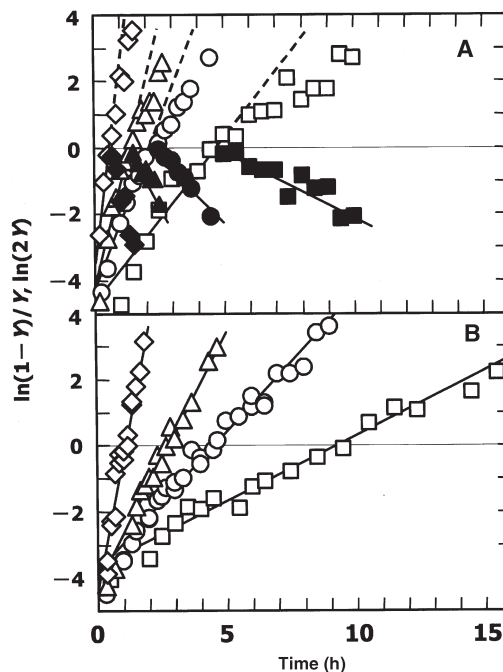

FIG. 2. The applicability of Equations 2 and 4 to changes in the fraction of unoxidized CLA. The conditions and keys are the same as those in the legend for Figure 1. The closed symbols represent $\ln(2Y)$.

$$\ln(2Y) = -k_2 (t - t_{0.5}) \qquad [4]$$

where $t_{0.5}$ is the time when Y reaches 0.5 and can be obtained from Equation 2 as follows:

$$t_{0.5} = -\frac{1}{k_1} \ln \frac{1 - Y_0}{Y_0} \qquad [5]$$

The solid symbols in Figure 2A indicate the plots of $\ln(2Y)$ vs. t for the latter half of the autoxidation processes of $t10,c12$ CLA. The plots could be connected by a line that passed through the point $Y = 0.5$ at $t_{0.5}$, estimated from Equation 5. The value of the rate constant k_2 at each temperature was estimated from the slope of the line. The solid curves in Figure 1A were drawn using the evaluated k_1, Y_0, and k_2 values. The broken curves represent Equation 1 for $Y < 0.5$.

Figure 2B shows that the entire autoxidation process of $c9,t11$ CLA could be expressed by Equation 2 at any temperature, similar to the oxidation process of LA (8). The values of k_1 and Y_0 were evaluated from the slope and the intercept of each line. The solid curves in Figure 1B were calculated using these values.

As shown above, the entire autoxidation process of $c9,t11$ CLA and the first half of the autoxidation of $t10,c12$ CLA could be expressed by the rate expression of the autocatalytic type. However, the latter half of the oxidation process of $t10,c12$ CLA obeyed first-order kinetics. These results indicate the possibility that the oxidation routes of $t10,c12$ and $c9,t11$ CLA are different, although we did not identify the oxidation products. Leung and Liu (10) reported that $t10,c12$ CLA exhibited stronger radical scavenging capacity than $c9,t11$ CLA. Yurawecz *et al.* (11) determined various furan FA as oxidation products of CLA. However, the oxidation products of each isomer of CLA have not been discussed.

The temperature dependencies of k_1 and k_2 for $t10,c12$ CLA and of k_1 for $c9,t11$ CLA were analyzed according to the Arrhenius equation:

$$k_i = k_{i0} \exp(-E_i / RT) \quad (i = 1 \text{ and } 2) \qquad [6]$$

where k_{i0} is the frequency factor, E_i is the apparent activation energy, R is the gas constant, and T is the absolute temperature. Figure 3 shows the Arrhenius plots of k_1 and k_2 for $t10,c12$ CLA and k_1 for $c9,t11$ CLA. The contents of $t10,c12$ and $c9,t11$ CLA were 94.7 and 82.4%, respectively. We reported that a saturated FA or its ester acted as a diluent in a mixed system of unsaturated and saturated FA or their esters and that the rate constant for the unsaturated substrate to be oxidized was proportional to its fraction in the mixture (12). Therefore, the rate constants obtained from Figure 2 were divided by the contents to estimate the intrinsic constants of the pure isomers under the assumption that other components in the samples acted as the diluent similar to saturated FA. The plots for each rate constant could be connected by a line, and the k_{i0} and E_i values were obtained from this line (Table 1). The broken line in Figure 3 represents the Arrhenius plot of k_1 for LA, which is cited from our
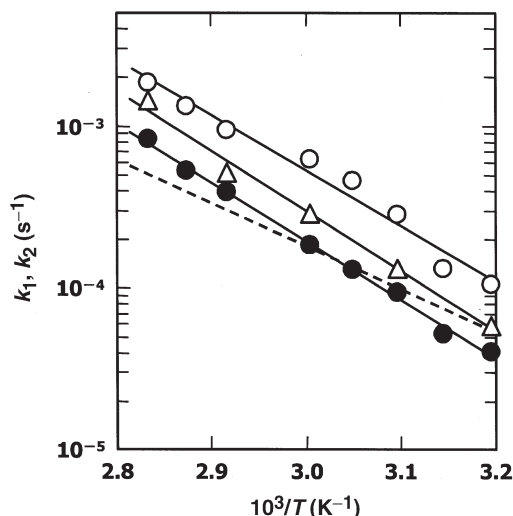

FIG. 3. Arrhenius plots for (○) k_1 and (●) k_2 of *trans*-10,*cis*-12 CLA and for (△) k_1 of *cis*-9,*trans*-11 CLA. The broken line represents the relationship for k_1 of the nonconjugated linoleic acid (8).

previous study (8). The results in the literature (8) were obtained for a mixture of LA and methyl palmitate at a weight ratio of 1:1. Therefore, the rate constants for LA in a single component system should, based on the aforementioned reason, be approximately double the constants in the mixed system. The broken line was drawn using this approximation. The E_i values for $t10,c12$ and $c9,t11$ CLA were greater than those for the nonconjugated n-6 and n-3 PUFA, which were in the range of 50 to 60 kJ/mol (8). This indicates that the autoxidation of CLA at a high temperature is more significantly accelerated than that of the nonconjugated FA. Zhang and Chen (5) reported that CLA oxidized more rapidly than LA at 90°C, while Hofman and Elmer (1) found similar oxidation rates for CLA and LA at 37°C. The inconsistency in susceptibility to oxidation of CLA and LA could be explained by the difference in the apparent activation energy between CLA and LA, although the oxidation of CLA was assessed using a mixture of CLA isomers in the previous studies.

We reported that the enthalpy-entropy compensation held during the autoxidation of the PUFA and their acylglycerols (9). Equation 7 is one of the expressions describing this compensation (13):

$$E_i = RT_\beta \ln k_{i0} + \gamma \qquad [7]$$

TABLE 1
Frequency Factors (k_{10} and k_{20}) and Apparent Activation Energies (E_1 and E_2) of the Rate Constants k_1 and k_2 for the Oxidation in Air of *trans*-10,*cis*-12 and *cis*-9,*trans*-11 CLA at 0% RH

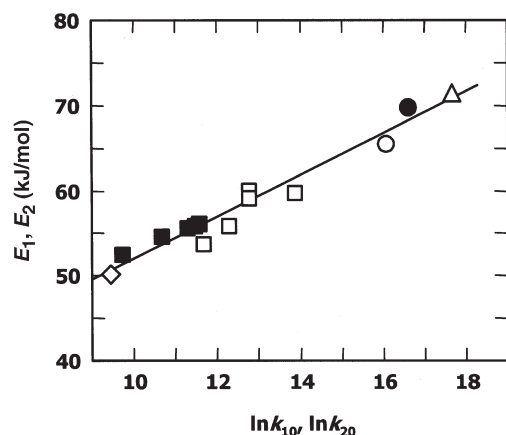| Isomer of CLA	k_{10} (s^{-1})	E_1 (kJ/mol)	k_{20} (s^{-1})	E_2 (kJ/mol)
trans-10,*cis*-12	9.64×10^6	65.4	4.75×10^7	71.5
cis-9,*trans*-11	6.09×10^7	72.1	—	—

FIG. 4. The relationship between the apparent activation energies E_1 and E_2 and the natural logarithms of the frequency factors k_{10} and k_{20} for the rate constants k_1 and k_2. The keys for the symbols, $\bigcirc$, $\bullet$, and $\triangle$, are the same as those in the legend for Figure 3. The symbol $\diamond$ represents the rate constant k_1 for the nonconjugated linoleic acid. Symbols $\square$ and $\blacksquare$ indicate the rate constants k_1 and k_2, respectively, of the other nonconjugated PUFA or their esters. The values represented by the symbols $\diamond$, $\square$, and $\blacksquare$ are cited from our previous study (8).

where T_β is a parameter called the isokinetic temperature and γ is a constant. The E_i and natural logarithm of k_{i0} ($\ln k_{i0}$) for CLA are plotted in Figure 4 together with the E_i and $\ln k_{i0}$ for the nonconjugated PUFA, which are cited from our previous study (8). Because the k_{i0} values in the previous study were obtained for the substrate mixed with an equal weight of methyl palmitate, they were doubled and plotted. All the plots lie on a straight line, indicating that the compensation holds during the autoxidation of both CLA and PUFA. This suggests a common mechanism for the oxidation of CLA and nonconjugated PUFA.

ACKNOWLEDGMENT

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