A Kinetic Model for Co-oxidation of β-Carotene with Oleic Acid

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ABSTRACT: Oxidation experiments with β-carotene in a lipid solution were conducted under various conditions of temperature, oxygen composition, and lipid content. The experimental results were compared with those using *n*-decane reported previously. Under all conditions, the oxidation rate in oleic acid was faster than that in *n*-decane. A novel kinetic model for the co-oxidation of carotene with a lipid was proposed based on the reaction mechanism, which consisted of the oxidation of carotene, the oxidation of oleic acid, and the cross-reaction of carotene with oleic acid. The model quantitatively described the oxidation behavior of carotene over a wide range of temperatures, oxygen compositions, and lipid contents.

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KEY WORDS: β-Carotene, co-oxidation, cross-reaction, kinetic model, oleic acid.

β-Carotene (β-Car) is an active oxygen quencher and an antioxidant (1,2). However, β-Car is very susceptible to oxidation in air and loses its biological activities. In order to utilize β-Car in a food system, prevention of oxidation during processing and long-term storage is very important. Understanding the oxidation kinetics of β-Car can be a useful tool for predicting oxidation behavior and for providing effective operating conditions. In our previous study (3), a novel kinetic model for the oxidation of β-Car in *n*-decane was proposed. This model was based on an autocatalytic free-radical chain reaction mechanism that included the reactions concerned with the hydroperoxide or the β-Car radical. The model described well the oxidation behavior under various conditions of temperature and oxygen composition.

β-Car is commonly dissolved in a lipid when used as a food additive. The lipid is also susceptible to oxidation; hence, the oxidation of β-Car in such a system proceeds *via* a complicated co-oxidation mechanism accompanying the oxidation of the lipid. Several researchers have investigated the oxidation of β-Car in solution containing lipids (4–10). Most of them have discussed the antioxidative effect of β-Car on the oxidation of the lipid (4–8). However, Budowski and Bondi (9) and Ramakrishnan and Francis (10) have investigated the oxidation of β-Car in an organic solution containing a lipid such as cottonseed oil or methyl linoleate. In their studies, the oxidation rate of β-Car increased with the lipid content in solution. They suggested that the product generated by the oxidation of the lipid affected the oxidation rate
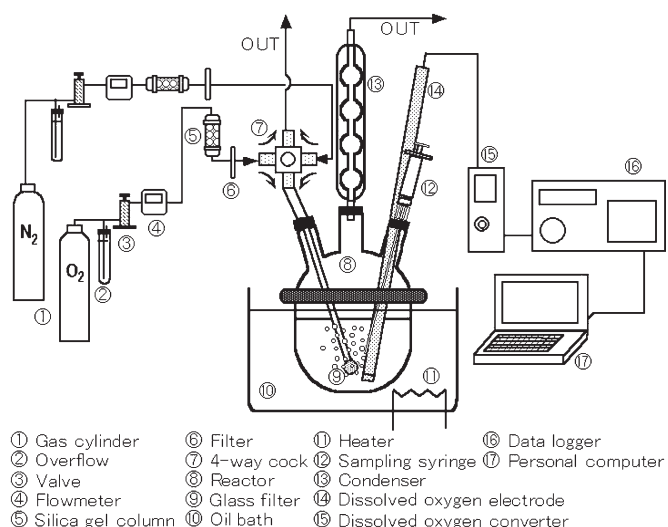
of β-Car. However, the co-oxidation mechanism of β-Car with a lipid is still unclear, and a kinetic model based on the reaction mechanism has never been constructed.

In this study, the oxidation experiments of β-Car were conducted in an organic solution containing the lipid oleic acid. A novel kinetic model was proposed that could describe quantitatively the kinetics of the co-oxidation of β-Car with a lipid. The kinetic and equilibrium constants in the model were estimated by fitting the model with the experimental results obtained under various conditions of temperature and oxygen composition.

EXPERIMENTAL PROCEDURES

Oleic acid was chosen as the model lipid solvent because it has a simple structure and its residue is the oxidized part of triolein, which is a main component of edible oils. Oleic acid was kindly provided by the NOF Co., Tokyo, Japan, and its purity was 92.3%. Other chemicals were of reagent grade and were used without purification.

A diagram of the experimental apparatus (3) is shown in Scheme 1. The reaction vessel was made of stainless steel, and its volume was 5.0×10^{-4} m^3. The working volume was 3.0×10^{-4} m^3 and the initial β-Car concentration was 0.75 mol · m^{-3}. Aeration gas, N$_2$ or a mixture of O$_2$ and N$_2$, was supplied by bubbling through a porous filter at the rate of 1.7×10^{-6} m^3 · s^{-1} at atmospheric pressure. A condenser was placed at the gas outlet to reduce the loss of solvent due to evaporation. The reaction solution in the vessel was suffi-

① Gas cylinder ⑥ Filter ⑪ Heater ⑯ Data logger
② Overflow ⑦ 4-way cock ⑫ Sampling syringe ⑰ Personal computer
③ Valve ⑧ Reactor ⑬ Condenser
④ Flowmeter ⑨ Glass filter ⑭ Dissolved oxygen electrode
⑤ Silica gel column ⑩ Oil bath ⑮ Dissolved oxygen converter

SCHEME 1

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ciently aerated by inert N_2 gas to purge the dissolved oxygen, and then the vessel was immersed in an oil bath. After the solution temperature reached a given constant value, the reaction was started by switching from N_2 gas to the reaction gas mixture. An initiator was not used in this study. The reaction was stopped when β-Car conversion reached 90%. The temperature and oxygen composition in the supplied gas were regulated from 323 to 343 K and from 20 to 100 mol%, respectively, in the oxidation experiments using pure oleic acid as a solvent. On the other hand, the temperature and oxygen composition were fixed at 333 K and 40 mol%, respectively, in the experiments using a mixed solvent of 10 or 50 mol% oleic acid (the residual component was *n*-decane).

The dissolved oxygen (DO) concentration was measured *in situ* using a monitoring system with a DO electrode (OET-8350; TOA Electronics, Ltd., Tokyo, Japan). Sample solutions were withdrawn at specific time intervals. The β-Car concentration in the sample was measured spectroscopically at 450 nm. Oleic acid concentration was measured with a high-performance liquid chromatography system (L-7100; Hitachi, Tokyo, Japan) equipped with an Inertsil octadecylsilane column (particle size 5×10^{-6} m, i.d. 4.6×10^{-3} m, length 0.25 m; GL Science Inc., Tokyo, Japan) and an ultraviolet detector (L-7400, Hitachi) at 210 nm. The mobile phase was acetonitrile/water (90:10, vol/vol), and the flow rate was 0.5×10^{-8} m$^3 \cdot$ s^{-1}.

RESULTS AND DISCUSSION

The results of the oxidation experiments using pure oleic acid as a solvent are shown in Figures 1 and 2. The experiments were repeated under conditions in which the temperature and the oxygen composition were 333 K and 40 mol%, respectively. Relative errors from the replicated experiments were less than 9%, and the reproducibility of the data was confirmed to be good. For comparison, the results of the oxidation experiments using *n*-decane as the solvent reported previously (3) are also shown in these figures. β-Car oxidized more rapidly in oleic acid than in *n*-decane (Fig. 1). The DO concentrations were similar in both solvents (Fig. 2). The oleic acid concentration decreased slightly throughout the experiment, as indicated by an oxidative conversion of less than 1% (data not shown). The same tendencies were observed under other conditions of temperature and oxygen composition.

In our previous study (11), oleic acid was found to be oxidized by an autocatalytic free-radical chain reaction mechanism similar to β-Car. The peroxyl radical, having a high reactivity, is considered to react preferentially with the β-Car rather than the oleic acid owing to the polyene structure of β-Car. This reaction may contribute to an increase in the oxidation rate of β-Car in the presence of oleic acid. On the basis of this concept, a kinetic model was constructed for the co-oxidation of β-Car with a lipid.

Construction of the kinetic model. The co-oxidation of β-Car with a lipid is considered to consist of the oxidation of β-Car itself, the oxidation of oleic acid itself, and the cross-reaction of β-Car with the oleic acid. As previously reported

(3), the oxidation of β-Car proceeds by a series of mechanisms in which a secondary initiation, a reverse reaction of propagation, and a termination concerned with the C-centered radical were involved as
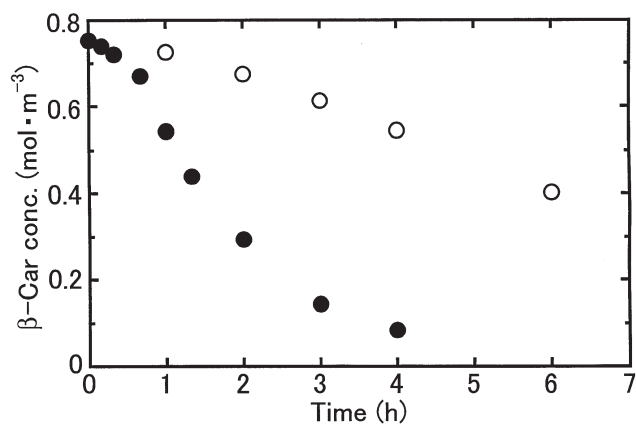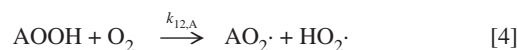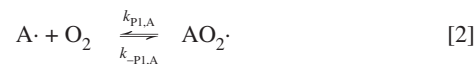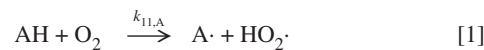
$$AH + O_2 \xrightarrow{k_{11,A}} A\cdot + HO_2\cdot \qquad [1]$$

$$A\cdot + O_2 \underset{k_{-P1,A}}{\overset{k_{P1,A}}{\rightleftharpoons}} AO_2\cdot \qquad [2]$$

$$AH + AO_2\cdot \xrightarrow{k_{P2,A}} AOOH + A\cdot \qquad [3]$$

$$AOOH + O_2 \xrightarrow{k_{12,A}} AO_2\cdot + HO_2\cdot \qquad [4]$$

$$AO_2\cdot + AO_2\cdot \xrightarrow{k_{T1,A}} \text{nonradical stable product} \quad [5]$$

$$A\cdot + AO_2\cdot \xrightarrow{k_{T2,A}} \text{nonradical stable product} \quad [6]$$

FIG. 1. Experimental data for β-carotene concentration (β-Car) obtained at an oxygen composition of 40 mol% and a temperature of 333 K: (●), in oleic acid; (○), in *n*-decane.
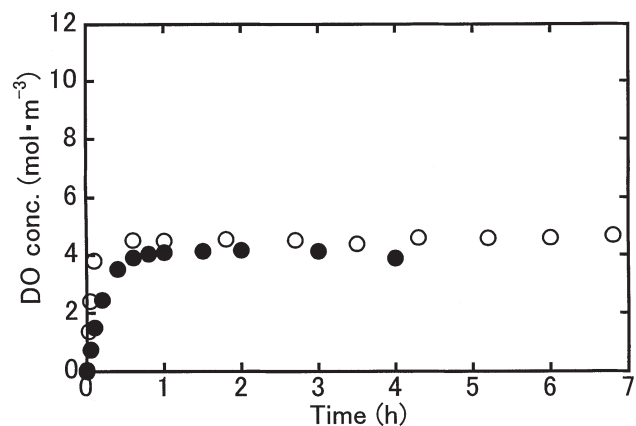

FIG. 2. Experimental data for dissolved oxygen (DO) concentration obtained at an oxygen composition of 40 mol% and a temperature of 333 K: (●), in oleic acid; (○), in *n*-decane.

Here, Equation 1 is the chain initiation (I1) process, Equations 2 and 3 are the propagation (P1,P2) processes, Equation 4 is the secondary initiation (I2) process, and Equations 5 and 6 are the termination (T1,T2) processes. AH is β-Car, A· and AO_2· are the C-centered and peroxyl radicals derived from β-Car, respectively, and AOOH is hydroperoxide. It has been reported that decomposition products such as β-ionone are generated by the β-Car oxidation (12). However, only small amounts of decomposition products are present when β-Car remains in the system (13). In this study, about 10% of the β-Car remained at the end of the experiment; hence, the reactions concerned with the decomposition products were not taken into account.

As described in the Results and Discussion section, oleic acid is also oxidized by a mechanism similar to β-Car, in which the chain initiation is given as

$$LH + O_2 \xrightarrow{k_{I1,L}} L· + HO_2· \qquad [7]$$

L· generated by the above reaction reacts rapidly with oxygen to form the peroxyl radical LO_2·, as

$$L· + O_2 \xrightarrow{k_{P1,L}} LO_2· \qquad [8]$$
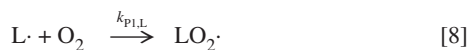
Unlike the oxidation of β-Car, L· is unstable; thus, the reverse reaction of Equation 8 does not need to be included in the model.

In this study, the oleic acid concentration decreased slightly throughout the experiment, suggesting that the oxidation of oleic acid remained in the initial stage of the chain reaction. Therefore, it is sufficient to take into account only Equations 7 and 8.

LO_2· generated by Equation 8 preferentially reacts with the β-Car rather than with the oleic acid, as described in the Results and Discussion section. Therefore, the cross-reaction of β-Car with oleic acid is taken into account as

$$AH + LO_2· \xrightarrow{k_{cross}} LOOH + A· \qquad [9]$$

Here, LOOH is hydroperoxide. The co-oxidation of β-Car with oleic acid proceeds by a series of mechanisms, as shown in Equations 1–9.

Assuming the pseudo steady-state for concentrations of the respective radicals in the system, the rates of change in the concentrations of the β-Car and hydroperoxide are

$$\frac{dC_{AH}}{dt} = -k_{I1,A}C_{AH}C_{O_2} - k_{PT}\left(1 + \frac{k_R}{C_{O_2}}\right)^{-1/2} C_{AH}(r_I + r_{cross})^{1/2} - r_{cross} \quad [10]$$

$$\frac{dC_{AOOH}}{dt} = -k_{I2,A}C_{AOOH}C_{O_2} + k_{PT}\left(1 + \frac{k_R}{C_{O_2}}\right)^{-1/2} C_{AH}(r_I + r_{cross})^{1/2} \quad [11]$$

The initial conditions are

$$t = 0; \ C_{AH} = C_{AH}(0), \ C_{AOOH} = 0 \qquad [12]$$

The initiation rate, r_I, the kinetic constants, k_R and k_{PT}, and the cross-reaction rate, r_{cross}, in Equations 10 and 11 are given, respectively, as

$$r_I = K_{I1,A}C_{AH}C_{O_2} + k_{I2,A}C_{AOOH}C_{O_2} \qquad [13]$$

$$k_R = \frac{k_{T2,A} \cdot k_{-P1,A}}{k_{T1,A} \cdot k_{P1,A}} \qquad [14]$$

$$k_{PT} = k_{P2,A}/(2k_{T1,A})^{-1/2} \qquad [15]$$

$$r_{cross} = k_{I1,L}C_{LH}C_{O_2} \qquad [16]$$

Because the oleic acid concentration hardly changed throughout the experiment, C_{LH} in r_{cross} is assumed to be constant at the initial concentration, $C_{LH}(0)$. Thus, Equation 16 is substituted by the following equation:

$$r_{cross} = k_{I1,L}C_{LH}(0)C_{O_2} \qquad [17]$$

In this study, the period in which the DO concentration increased was about 1 h and was not negligibly short compared to the total reaction time of 4–6 h, as shown in Figures 1 and 2. Therefore, the variation in the DO concentration at the beginning of the reaction should be included in this model. The mass balance equation for oxygen in the solution is expressed as

$$\frac{dC_{O_2}}{dt} = k_L a\left(C_{O_2}^* - C_{O_2}\right) - \left(r_I + r_{cross}\right) \qquad [18]$$

Here, $k_L a$ is the volumetric mass transfer coefficient and $C_{O_2}^*$ is the saturated DO concentration. The second term on the right side of Equation 18 is the rate of oxygen consumption by the reaction. Thus, in this model, there are seven unknown constants including the kinetic constants for the oxidation of β-Car itself, $k_{I1,A}$, $k_{I2,A}$, k_{PT}, and k_R, the kinetic constant for the cross-reaction, $k_{I1,L}$, and the constants in the mass balance equation for oxygen, $k_L a$ and $C_{O_2}^*$.

Application of the kinetic model. (i) Estimation of constants in the mass balance equation for oxygen. Generally, $k_L a$ is hardly affected by the temperature or oxygen composition and hence is assumed to be constant under all conditions. $C_{O_2}^*$ is assumed to be proportional only to the mole fraction of oxygen. The rate of oxygen consumption by the reaction is considered to be negligible compared to that of the gas–liquid mass transfer during the period in which the DO concentration increases at the beginning of the reaction. Therefore, the mass balance equation for oxygen in the solution, Equation 18, is simplified to

$$\frac{dC_{O_2}}{dt} = k_L a\left(y_{O_2}C_{pureO_2}^* - C_{O_2}\right) \qquad [19]$$

Here, y_{O_2} is the mole fraction of oxygen in the supplied gas and $C^*_{pureO_2}$ is the saturated DO concentration when 100 mol% O_2 gas is supplied to the solution. The constants, $k_L a$ and $C^*_{pureO_2}$, were estimated by fitting Equation 19 with the experimental results for the DO concentration under all conditions. In the calculation, the experimental results for reaction times up to 2 h were used. The fitting procedure was similar to that reported previously (11).

The fitted and experimental results are shown in Figure 3. The symbols represent the experimental results, and the solid lines represent the results fitted by the model. Under all conditions, the calculated lines were in agreement with the experimental results and described the drastic increase at the beginning of the reaction. The estimated values of $k_L a$ and $C^*_{pureO_2}$ are 1.31×10^{-3} s^{-1} and 1.02×10^1 mol · m^{-3}, respectively.

(ii) Estimation of other kinetic constants. In free-radical chain reactions such as in the oxidation of organic compounds, it has been reported that differences in the physical properties of solvents have little effect on the reaction rates (14). Therefore, the kinetic constants for oxidation of β-Car itself in oleic acid, $k_{I1,A}$, $k_{I2,A}$, k_{PT}, and k_R, were assumed to be the same values as those for *n*-decane reported in a previous study (3). These values are listed in Table 1.

To estimate the kinetic constant for the cross-reaction, $k_{I1,L}$, a temperature dependence of the Arrhenius type was introduced. The constant, $k_{I1,L}$, is expressed using the pre-exponential factor, B, and the activation energy, E_a as

$$k_{I1,L} = B \exp(-E_a/RT) \quad [20]$$

These constants, B and E_a, were estimated by the model equations, Equations 10 and 11, with six sets of experimental results for the β-Car concentrations.

Figures 4 and 5 show the effects of temperature and oxygen composition on the oxidation of β-Car in oleic acid.
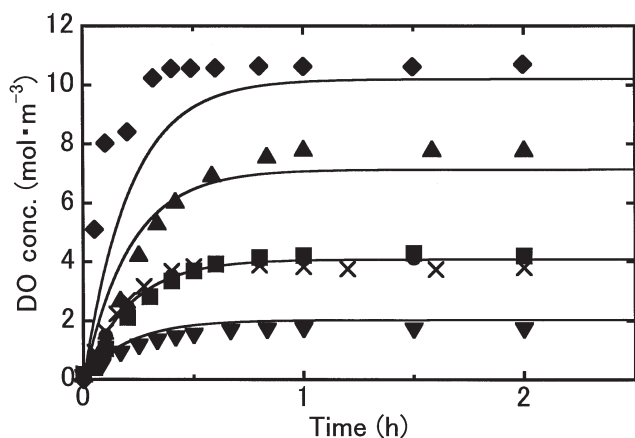
Under all conditions, the calculated lines were in good agreement with the experimental results. The model described quantitatively the oxidation behavior of β-Car in oleic acid over a wide range of temperatures and oxygen compositions. The estimated value of $k_{I1,L}$ is given as

$$k_{I1,L}(\text{mol}^{-1} \cdot \text{m}^3 \cdot \text{s}^{-1}) = 2.03 \times 10^{-7}\exp(-3.16 \times 10^4/RT) \quad [21]$$

To elucidate the sensitivity of the novel constant, $k_{I1,L}$, estimated in this study, the sum of the squares of the relative error, S, was calculated by numerical simulation. The value of $k_{I1,L}$ was changed in the range of the estimated value ±20% at a temperature of 333 K.

The simulated results are listed in Table 2. The values of S obtained by changing the constant were larger than the minimum values, S_{min}, by at least 27.7%. Therefore, the reliability of the novel constant, $k_{I1,L}$, obtained in this study is considered to be high. The activation energy of the kinetic constant concerned with oxidation is generally known to be in the range of 20 to 150 kJ · mol^{-1} (15). Therefore, the estimated value, 31.6 kJ · mol^{-1}, is considered to be reasonable.

(iii) Simulation of oxidation behavior for various oleic acid contents. As described in the section above, the rate of the cross-reaction of β-Car with oleic acid, r_{cross}, is expressed by the following equation:

$$r_{cross} = k_{I1,L}C_{LH}(0)C_{O_2} \quad [17]$$

TABLE 1
Estimated Values of Constants for β-Carotene Oxidation in *n*-Decane (3)

| Constants	Pre-exponential factor B	Activation energy E_a (J · mol^{-1})
$k_{I1,A}$	5.82×10^{10} (mol^{-1} · m^3 · s^{-1})	1.38×10^5
k_{PT}	2.30×10^4 (mol$^{1/2}$ · m$^{3/2}$ · s$^{1/2}$)	2.78×10^4
$k_{I2,A}$	5.30×10 (mol^{-1} · m^3 · s^{-1})	6.60×10^4
k_R	2.02×10^3 (mol · m^{-3})	1.87×10^4

FIG. 3. Experimental data and fitted results by model for DO concentration: (▼), 20; (●), 40; (▲), 70; (♦), 100 mol% of oxygen compositions at 333 K: (■), 323; (×), 343 K at 40 mol% of oxygen composition; solid lines, fitted results by model. For abbreviation see Figure 2.
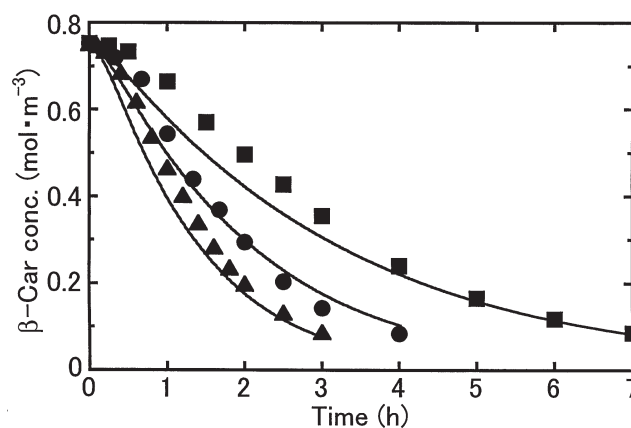

FIG. 4. Experimental data for β-Car concentration obtained at an oxygen composition of 40 mol% and fitted results by the model: (■), 323; (●), 333; (▲), 343 K temperatures; solid lines, fitted results by model. For abbreviation see Figure 1.
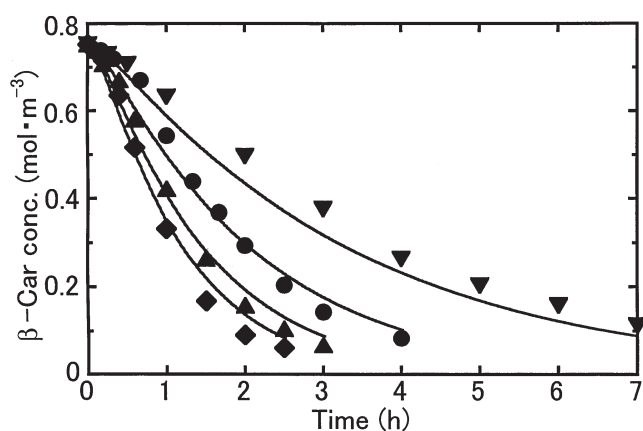
FIG. 5. Experimental data for β-Car concentration obtained at a temperature of 333 K and fitted results by model: (▼), 20; (●), 40; (▲), 70; (◆) 100 mol% of oxygen compositions; solid lines, fitted results by model. For abbreviation see Figure 1.

TABLE 2
Sensitivity of Constants in the Model

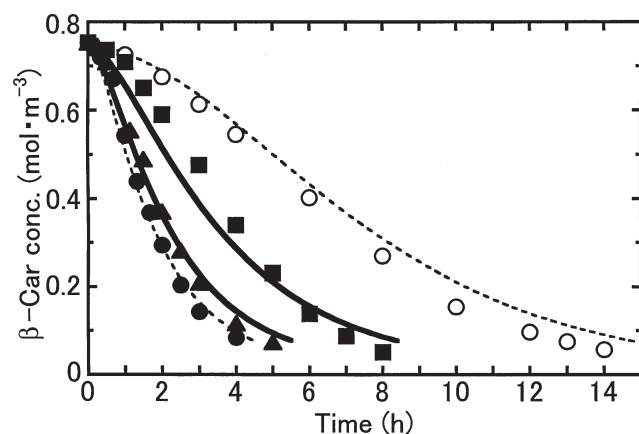| Constant	Value	S	Deviation (%) $(S - S_{min})/S_{min}$
$k_{l1,L}$	5.54×10^{-9}	0.353	+184.5
	7.21×10^{-9}	0.124	—
	8.65×10^{-9}	0.158	+27.7

FIG. 6. Time course of β-Car concentration obtained at an oxygen composition of 40 mol% and a temperature of 333 K : (○), 0 (in n-decane); (■), 10; (▲), 50; (●), 100 mol% of oleic acid contents; solid lines, simulated results; broken lines, fitted results.

The cross-reaction rate increases with the initial oleic acid concentration, $C_{LH}(0)$; hence, the oxidation rate of β-Car is considered to increase with $C_{LH}(0)$. The oxidation rates were estimated by the model using various oleic acid contents.

The results are shown with the experimental data in Figure 6. For reference, the experimental and fitted results for

the oleic acid contents of 0 and 100 mol% are also shown in this figure. The results for 0 mol% correspond to those of n-decane reported previously (3). The oxidation rate of β-Car was found to increase with the oleic acid content, and the model simulated the experimental results well.

ACKNOWLEDGMENTS

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