Decomposition Kinetics of 6-O-Monoacyl Ascorbate in Air

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6-O-Monoacyl ascorbates were synthesized by the condensation of L-ascorbic acid with caprylic, capric, lauric, myristic or palmitic acid using immobilized lipase in acetone. The decomposition processes of the acyl ascorbates in air were measured, and the decomposition kinetics were analyzed based on the Weibull equation. The rate constant, k, increased with increasing relative humidity and temperature for all acyl ascorbates; k also depended on acyl chain length, as the decomposition rates of ascorbates increased as acyl chains decreased. The enthalpy-entropy compensation held during the decomposition, suggesting that the decomposition of all acyl ascorbates proceeded by essentially the same mechanism. Furthermore, it was suggested that the oxidative degradation of the ascorbyl moiety of acyl ascorbate primarily proceeded during the decomposition, and was followed by the hydrolysis of the ester bond between the acyl and ascorbyl moieties.

Keywords: Monoacyl ascorbate, decomposition kinetics, acid, immobilized lipase, apparent activation energy

Introduction

L-Ascorbic acid is a water-soluble vitamin known as vitamin C. It is widely used as an additive in foods and cosmetics because of its strong reducing ability. The lipase-catalyzed synthesis of 6-O-monoacyl ascorbate through the condensation of ascorbic acid and fatty acid in an organic solvent has been reported (Humeau et al., 1995; Yan et al., 1999). The enzymatic synthesis of the acyl ascorbates is considered more advantageous than a chemical method because of the simplicity of its reaction process and its high regioselectivity.

Acyl ascorbate, which consists of ascorbic acid and a fatty acid as the hydrophilic antioxidant and lipophilic group, respectively, is an amphiphilic antioxidant. It has been reported that 6-O-palmitoyl ascorbate has an antidepressant activity and metastasis-inhibitory effects (Miwa and Yamazaki, 1986; Nagao et al., 1996). Thus, acyl ascorbate would be expected to be a useful food additive. We have also synthesized acyl ascorbates using an immobilized lipase in a batch or continuous reactor (Watanabe et al., 1999; 2003), estimated their surfactant property (Watanabe et al., 2001), demonstrated their antioxidative ability against unsaturated fatty acid (Watanabe et al., 2005), and used them for the microencapsulation of lipid (Watanabe et al., 2002). However, there have been no reports on the decomposition of acyl ascorbates in air, although knowledge of their stability during storage is required for their effective use.

In this study, the decomposition processes in air of monoacyl ascorbates, the acyl chain lengths of which were from 8 to 16, were measured. The process was expressed by the probabilistic Weibull equation and the kinetic parameters were evaluated for the decomposition of the acyl ascorbates at various relative humidities and temperatures.

Materials and Methods

Materials Immobilized lipase from Candida antarctica, Chirazyme® L-2 c.f. C2, was obtained from Roche Molecular Biochemicals, Mannheim, Germany. L(+)-Ascorbic acid (purity >99.5%) was purchased from Nacalai Tesque, Kyoto, Japan. Caprylic, capric, lauric, myristic and palmitic acids and all other chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan) and Yoneyama Yakuhin Kogyo (Osaka, Japan).

Preparation and purification of 6-O-monoacyl ascorbates L-Ascorbic acid (15 mmol) and caprylic, capric, lauric, my-
ristic or palmitic acid (45 mmol) were weighed in an glass bottle equipped with a screw cap, and 1 g of Chirazyme® L-2 C2 and 300 mL of acetone were added to the bottle. The headspace of the bottle was filled with nitrogen gas, then tightly sealed. The bottle was then immersed in a water-bath at 50°C with vigorous shaking to commence the condensation reaction. After ca. 24 h, each 6-O-saturated acyl L-ascorbate was isolated from the reaction mixture according to the reported methods (Yan et al., 1999) with a slight modification.

**Decomposition of 6-O-acyl ascorbate**  First, synthesized capryloyl, caproyl, myristoyl and palmitoyl ascorbates were placed in a desiccator containing a Petri dish with phosphorus pentoxide in the dark for 1 day. The dried acyl ascorbate (30 mmol) was weighed in flat-bottomed glass cups (1.5 cm i.d. and 3.0 cm height). The cups were placed in a plastic container with a Petri dish containing a saturated aqueous solution of lithium chloride, potassium carbonate and sodium chloride to regulate the relative humidity at 12, 44 and 75%, respectively. The container was stored in the dark at 37, 50, 65 and 80°C. At appropriate intervals, a cup was removed, and 1 mL of methanol was added to the cup to fully dissolve the acyl ascorbate. The concentration of the acyl ascorbate in the solution was measured using an HPLC (LC-10AT, Shimadzu, Kyoto, Japan) with an ODS column (4.6 mmφ x 150 mm, Cosmosil 5C18-AR, Nacalai Tesque) and a UV detector (245 nm, SPD-10A, Shimadzu) after appropriate dilution. The solution (20 μL) was applied to the column and eluted with an eluent, methanol/water/phosphoric acid mixture (90:10:0.1 by vol) at 1.0 mL/min.

**Analysis of fatty acid-formed liberated acyl ascorbate**  Lauroyl ascorbate was stored at 65°C and 75% relative humidity by the previously mentioned procedures. At appropriate intervals, a cup was removed from the container, and 300 μL of a mixture of methanol, benzene and methyl myristate (20:80:0.05 by vol.) was added to the cup. The lauric acid liberated from the lauroyl ascorbate during the storage was converted to its methyl ester by adding 20 μL of 2 mol/L trimethylsilyldiazomethane in hexane and allowing it to stand at room temperature for 30 min (Hashimoto et al., 1981). After evaporation of the solvent under reduced pressure, the remainder was dissolved in 500 μL of hexane and used for the GC analysis. Methyl myristate was used as the internal standard for the GC analysis. The amount of lauric acid was analyzed by a gas chromatograph (G-3500, Hitachi, Tokyo, Japan) equipped with a hydrogen ionization detector. Two microliters of the hexane solution was injected into a capillary column 0.25 mm in diameter and 30 m in length, along with polyethylene glycol (ZB-WAX, Shimadzu). The injection and detection temperatures were 200°C, and the column temperature was 180°C.

**Results and Discussion**  
**Effect of relative humidity on the decomposition**  Figure 1 shows the transient changes in the fractions of the remaining capryloyl, caproyl, lauroyl, myristoyl and palmitoyl ascorbates at 80°C and at 12, 44 and 75% relative humidity. The relative humidity significantly affected the decomposition of the acyl ascorbates. The decomposition rate of all ascorbates increased with increasing relative humidity. At 12 and 44% relative humidity the rate of ascorbates increased as acyl chains decreased.

The decomposition kinetics of the acyl ascorbates were empirically expressed by the Weibull equation, which is flex-
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Decomposition rate constants. The decomposition rates at 80˚C and at (□) 12%, (○) 44% and (△) 75% relative humidity.

\[ Y = \exp[-(kt)^n] \]  

(1)

where \( Y \) is the fraction of the remaining acyl ascorbate at time \( t \), \( k \) is the rate constant, the reverse of which is called the scale parameter, and \( n \) is the shape constant. The kinetic parameters, \( k \) and \( n \), were evaluated by fitting the experimental results by nonlinear regression using Excel. The curves in Fig. 1 were drawn based on the equation, using estimated parameters.

Figure 2 shows the acyl-chain length dependencies of the \( k \) or \( n \) values at different relative humidities for the decomposition of acyl ascorbate at 80˚C. The \( k \) values increased with increasing relative humidity for all acyl ascorbates. The acyl chain length affected the \( k \) value at 44% relative humidity, and the \( k \) value increased as acyl chain length decreased. The \( n \) value for the decomposition of any acyl ascorbate at 75% relative humidity was nearly equal to or slightly less than unity, while the \( n \) values at 12% and 44% relative humidities were higher than unity. In particular, the \( n \) value at 44% relative humidity strongly depended on the acyl chain length of the ascorbate, and the \( n \) value for the decomposition of an ascorbate having a long acyl chain was high. This indicates that the decomposition of the ascorbate with a longer acyl chain progressed via a longer induction period. It is presumed that sorption of water onto the ascorbate with a longer acyl chain was weak at 44% relative humidity due to its high hydrophobicity, and that the induction period was consequently prolonged.

Decomposition at different temperatures. Figure 3 shows the decomposition processes of the acyl ascorbates at 37, 50 and 65˚C and at a 75% relative humidity. The relative humidity was, for convenience, selected to obtain the results within a short time because the decomposition proceeded fastest at that humidity. All acyl ascorbates decomposed fast as temperature increased. The acyl-chain length also affected the decomposition of the ascorbates, and ascorbates with shorter acyl chains decomposed faster. This tendency was significant at 65°C. The decomposition of all acyl ascorbates at all temperatures was analyzed based on the Weibull equation in

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order to estimate the kinetic parameters. The curves in the figure were drawn using the estimated $k$ and $n$ values.

Figure 4 shows the temperature dependencies of the $k$ and $n$ values for the decomposition of the acyl ascorbates at 75% relative humidity. Although the $k$ value increased with increasing temperature, there was no strong temperature dependency for the $n$ value and the values were almost 1.0 ± 0.4. The $k$ values for the decomposition of ascorbates increased as acyl chains decreased.

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

$$ k = k_0 \exp(-E / RT) $$

where $k_0$ is the frequency factor, $E$ is the activation energy, $R$ is the gas constant, and $T$ is the absolute temperature. The Arrhenius plots in Fig. 4, in each case, produced a straight line to evaluate the activation energy $E$ and the frequency factor $k_0$ from the slope and the intercept of the line, respectively. Figure 5 shows the relationships between the acyl chain length and the $E$ or $k_0$ value for the rate constant. Both the $E$ and $k_0$ values for the ascorbate increased as acyl chain decreased.

The $E$ values are plotted versus the natural logarithms of the $k_0$ values in the inset of Fig. 5. The plots lie on a straight line ($R^2 = 0.997$). Equation 3 is one of the expressions describing the enthalpy-entropy compensation (Leffer, 1955; Exner, 1964; Liu and Guo, 2001).

$$ E = RT\beta \ln k_0 + \gamma $$

where $T\beta$ is an isokinetic temperature and $\gamma$ is a constant. The linear relationship indicated that the compensation held during the decomposition and that the decomposition of every acyl ascorbate essentially proceeded by the same mechanism. The $T\beta$ value was estimated to be 101°C from the slope. At this temperature, the rate constant $k$ for the decomposition of all acyl ascorbates would be the same.

**Liberation of fatty acid during the decomposition** The decomposition of an acyl ascorbate would consist of the oxidative degradation of the ascorbyl moiety and the hydrolysis of the ester bond between the acyl and ascorbyl moieties.

Figure 6 shows the transient changes in the fractions of the remaining lauroyl ascorbate and lauric acid liberated by the hydrolysis of lauroyl ascorbate at 65°C and 75% relative humidity. During the early stage during the storage, the decomposition of lauroyl ascorbate rapidly proceeded, but free lauric acid was scarcely formed. Lauric acid gradually formed when the fraction of the remaining lauroyl ascorbate became less than 0.5. The relationship between the fractions of the consumed lauroyl ascorbate and liberated lauric acid is shown in the inset of Fig. 6. The amount of consumed lauroyl ascorbate was calculated by subtracting the fraction of the remaining lauroyl ascorbate, which was estimated using the parameters, $k$ and $n$, by the Weibull equation, from unity. The solid line in the inset of Fig. 6 was drawn assuming that the disappearance of the lauroyl ascorbate was ascribed to its hydrolysis. All the plots were under the broken line; thus,
no lauric acid was liberated during the early stage of the decomposition of lauroyl ascorbate. This indicated that the oxidative degradation of the ascorbyl moiety occurred first, and then the hydrolysis of the ester bond followed to liberate the lauric acid; that is, the decomposition of acyl ascorbate appears to be a consecutive process.

References


