Surface Activity of 6-O-Hexanoyl, Octanoyl, Decanoyl and Dodecanoyl Ascorbates

Yoshiyuki WATANABE1, Shuji ADACHI1,†, Takao FUJI1, Kazuhiro NAKANISHI2, and Ryuichi MATSUNO1

1Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan and 2Department of Bioscience and Biotechnology, Faculty of Engineering, Okayama University, Tsushima-naka, Okayama 700-8530, Japan

The surface tensions of 6-O-hexanoyl, octanoyl, decanoyl and dodecanoyl L-ascorbates, which were synthesized via the immobilized-lipase-catalyzed condensation of ascorbic acid and the corresponding fatty acids in acetonitrile, were measured at 25°C by the Wilhelmy method at their various concentrations, and the critical micelle concentration, CMC, and the residual area per molecule, a, were evaluated for each acyl ascorbate. The CMC was lower for the ascorbate with a longer acyl chain, while the a values were ca. 0.30 nm² for all the acyl ascorbates.

Key words: Acyl ascorbate, surfactant, surface tension

1. Introduction

6-O-Acyl ascorbates are surface-active. Tukamoto et al.[1] reported the surface activities of the dodecanoyl, tetradecanoyl and hexadecanoyl ascorbates, which were chemically synthesized and then dissolved in a 0.4 mol/L phosphate buffer, pH 7.0, at 20°C or 50°C.

In this study, the ascorbates with the shorter acyl chains were enzymatically synthesized according to our previous study [2], and their surface activities were measured at 25°C by the Wilhelmy method using distilled water as the solvent. The acyl ascorbates synthesized and tested were the hexanoyl, octanoyl, decanoyl and dodecanoyl ascorbates. The surfactant properties of the acyl ascorbates were then compared with those of the 6-O-acyl mannosides [3] and 1-alkyl β-D-glucosides [4], the acyl or alkyl chain lengths of which were 8 to 14, respectively.

2. Materials and Methods

2.1 Materials

Immobilized lipase from Candida antarctica, Chirazyme® L-2, was purchased from Roche Molecular Biochemicals, Mannheim, Germany. The L (+) -ascorbic, hexanoic, octanoic, decanoic and dodecanoic acids, acetonitrile, molecular sieves 4A, and silica gel (Wakogel® C-200) were purchased from Wako Pure Chemical Industries, Osaka, Japan. All the other chemicals were obtained from either Wako Pure Chemical Industries or Nacalai Tesque, Kyoto.

2.2 Preparation and purification of 6-O-acyl ascorbates

The acyl ascorbates were synthesized through the immobilized-lipase-catalyzed condensation of ascorbic acid and a fatty acid in acetonitrile according to our previous procedures [2]. Ascorbic acid (15 mmol) and a fatty acid (75 mmol) were dispersed or dissolved in 300 mL of acetonitrile dehydrated over molecular sieves 4A. Six grams of Chirazyme® L-2 was then added to commence the condensation at 50°C with shaking under a nitrogen atmosphere. After a 3-day reaction, the reaction mixture was filtered over a sintered glass. The filtrate was evaporated to remove acetonitrile. The residue was dissolved with a small amount of ethyl acetate, and 40 g of silica gel was then added to adsorb the product and the fatty acid. The gel was packed into a cylindrical glass column, and about 300 mL of ethyl acetate was fed to elute out the fatty acid. The product adsorbed on the gel was eluted out with ca. 300 mL of methanol, and the effluent was then rotary-evaporated. The concentrated effluent (500μL) was applied to an YMC-pack ODS-AQ column (20 mmφ × 250 mm, Kyoto) and eluted with a mixture of methanol and water (65:35, 70:30, 75:25 and 85:15 by vol.) for hexanoyl, octanoyl, decanoyl and dodecanoyl ascorbates, respectively, at a flow rate of 8 mL/min. The effluent at the peak corresponding to the desired product was collected, and the
product was recovered by evaporation. This purification was repeated until a sufficient amount of the product was obtained for the surface-tension measurement.

The condensation product between the ascorbic and dodecanoic acids had been analyzed by $^1$H NMR and identified to be 6-<i>O</i>-dodecanoyl ascorbate [2]. Although the products of the ascorbic acid and the other fatty acids were not analyzed, the lipase would catalyze the acylation of the primary hydroxyl group of ascorbic acid to produce the 6-<i>O</i>-acyl ascorbates.

2.3 Measurement of surface tension by the Wilhelmy method

The products were dissolved with water at various concentrations and their surface tensions were measured at 25°C by the Wilhelmy method using a CBVP-A3 surface tensiometer (Kyowa Kaimenkagaku, Tokyo).

2.4 Determination of the molar volume of ascorbic acid

The molar volume of ascorbic acid, $v$, was determined at 25°C from the relationship between the water and ascorbic acid concentrations, $C_w$ and $C$, given by Eq. (1) under the assumptions that the volumes of water and ascorbic acid are independent and that additivity holds [5]:

$$C_w = \frac{1}{v_w} - \frac{v}{v_w} C \quad (1)$$

where $v_w$ is the molar volume of water ($1.807 \times 10^{-2}$ L/mol).

3. Results and Discussion

3.1 Surface activities

Figure 1 shows the surface tensions of the aqueous solutions of the produced acyl ascorbates observed at the various concentrations and at 25°C by the Wilhelmy method. The critical micelle concentration, CMC, was estimated from the intersection of the two lines for each acyl ascorbate. The surface excess, $\Gamma$, was evaluated from the slope of the line drawn at the low concentrations according to the following equation:

$$-\frac{d\gamma}{d\log C} = 2.30 \frac{RT}{\Gamma} \quad (2)$$

where $\gamma$ is the surface tension, $C$ is the concentration of the acyl ascorbate, $R$ is the gas constant, and $T$ is the absolute temperature. The residual area per molecule, $a$, was calculated from the $\Gamma$ value by:

$$a = \frac{1}{(\Gamma N_A)} \quad (3)$$

where $N_A$ is Avogadro’s number.

The CMCs of the ascorbates with longer acyl chains were lower, while the $\Gamma$ values scarcely depended on the acyl chain length. The CMC of the dodecanoyl ascorbate obtained in this study was $1.32 \times 10^{-4}$ mol/L, while the value of the ascorbate reported by Tukamoto et al. [1] was ca. $1.4 \times 10^{-3}$ mol/L at 20°C. One of the possible reasons for this difference would be the effect of the ionic strength of the solution on the CMC. We measured the surface tensions in distilled water, while they did them in 0.4 mol/L phosphate buffer. The type of coexisting ion and its concentration affect the CMC even for non-ionic surfactants [1,6].

3.2 Comparison of surfactant properties of 6-<i>O</i>-acyl ascorbates with those of acyl or alkyl hexoses

The CMC and $a$ values of the acyl ascorbates are plotted versus the carbon number of the acyl chains in Fig. 2, together with the CMCs at 25°C for 6-<i>O</i>-acyl mannoses [3] and 1-alkyl D-glucosides [4]. The change in the CMC as a function of the chain length $n$ is expressed by [4]

$$\log CMC = -\frac{w}{kT} n + b \quad (4)$$

where $w$ is the cohesive energy change per methylene group passing from the bulk of the solution to the micelle, $k$ is Boltzmann’s constant, and $b$ is a constant. The $w$ values were estimated to be $1.3 \times 10^{-21}$, $2.0 \times 10^{-21}$ and $2.2 \times 10^{-21}$ J for the acyl ascorbates, acyl mannoses and alkyl glucosides, respectively. The $w$ value for acyl ascorbates was much smaller than those for the acyl mannoses and alkyl glucosides.

The $a$ values of ca. 0.40 nm$^2$ were practically the same for the acyl mannoses and alkyl glucosides, while the $a$ values of the acyl ascorbates were ca. 0.30 nm$^2$, which was much smaller than those of the acyl mannoses and alkyl glucosides. Since acyl or alkyl derivatives would be orient-
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The molar volume of ascorbic acid was estimated to be 0.106 L/mol (Fig. 3), from which the cross-sectional area was calculated to be 0.38 nm² under the assumption that the molecule was a sphere. The a value of 0.30 nm² was much smaller than the cross-sectional area. Ascorbic acid has a γ-lactone ring, which is smaller than the pyranose one of mannose or glucose. The γ-lactone ring might act as the hydrophilic moiety of the acyl ascorbate.

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References