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Relationship between Equilibrium Constant for Synthesis of Acyl Saccharide and Partial Molar Volume of Water Hydrated to Saccharide

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Abstract

The partial molar volume $\bar{V}$ of water hydrated to saccharides was measured through dilatometry. The average $\bar{V}$ value of a saccharide increased with increase in the dynamic hydration number n_{DHN} of the corresponding saccharide. Glucose had the highest average $\bar{V}$ value. Hydration properties of saccharides associated with their conformation greatly influenced $\bar{V}$ values of the hydrated water. Moreover, a high positive correlation between the $\bar{V}$ value and common logarithms of the apparent equilibrium constant K_{C} for enzymatic synthesis of acyl saccharide using an immobilized lipase from *Candida antarctica* in acetonitrile with low water content was observed. We suggest that the evaluation of the $\bar{V}$ value could facilitate the estimation of the K_{C} value.

Keywords: Acyl saccharide, Dilatometer, Dynamic hydration number, Equilibrium constant, Hydrated water, Partial molar volume

1. INTRODUCTION

Acyl saccharide, an ester consisting of saccharides, such as glucose and fructose, and fatty acids, is a surfactant with good emulsifying properties1). Acyl saccharides have been of great interest and have been used in the food, cosmetic, and pharmaceutical industries. Enzymatic synthesis of acyl saccharides using lipase has some key advantages, including moderate reaction conditions, high substrate specificity of the enzyme, and the direct use of unmodified substrates, when compared with conventional chemical synthesis. The equilibrium constant is a critical parameter for predicting the equilibrium yield of the desired product under any conditions. In a previous study2), the apparent equilibrium constants for the enzymatic synthesis of some lauroyl monosaccharides, including glucose, mannose, galactose, and fructose using lipase in acetonitrile with different water contents were estimated (Fig. 1). In addition, it was demonstrated that hydration of monosaccharides influenced the apparent equilibrium constant for the condensation reaction. The volumetric property of a solute is critical in understanding the state of hydrated water because the volume of water hydrated to a solute largely depends on its hydration state. Dilatometry is a precise and relatively simple procedure for measuring the partial molar volume of hydrated water3).

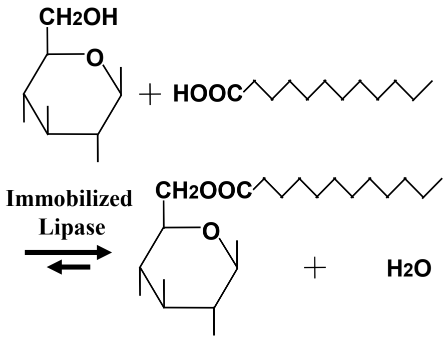

Fig. 1. Scheme of a condensation reaction between D-glucose and lauric acid using an immobilized lipase from *Candida antarctica* in a hydrophilic organic solvent (acetonitrile) with low water content.

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In the present study, we measured partial molar volumes of water hydrated to some saccharides using dilatometry and examined the relationship between the apparent equilibrium constant for enzymatic synthesis of acyl saccharide and partial molar volume.

2. MATERIALS AND METHODS

2.1 Materials

Dodecane, D(+)-glucose, D(+)-mannose, D(+)-galactose, D(+)-fructose, and sucrose were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The chemicals were of analytical grade.

2.2 Measurement of partial molar volume of water hydrated to saccharides by a dilatometer

A schematic diagram of the dilatometer is presented in Fig. 2. The dilatometer consisted of a vertical uniform capillary, an injection port, and a glass bulb. The length of the capillary was *ca*. 0.2 m and the inner diameter ranged from 1.874 to 1.881 mm on calibration with mercury. Each saccharide was dried *in vacuo* at 60°C for 1 day, following which 300 mg of saccharide was put in the bulb. Dodecane was added till it reached approximately the neck of the bulb. After deaeration using a vacuum pump, the capillary was fixed to the bulb. Distilled water (10–40 mg) was injected into the portions through the aperture using a microsyringe. The added weight of distilled water was measured using a semimicrobalance. After each addition of distilled water, the dilatometer was immersed in a water bath whose temperature was controlled at 25 ± 0.01°C. The mixture in the bulb was stirred using a magnetic rotor. The height of the meniscus in the capillary was periodically read to an accuracy of 0.01 mm using a casetometer, until minimal changes in volume were detected.

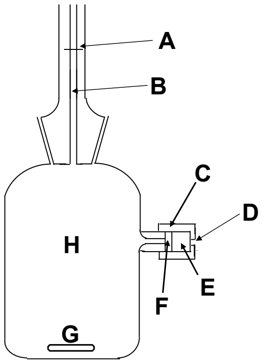

Fig. 2. Diagram of a dilatometer; A: Datum line, B: Uniform capillary, C: Metal fitting, D: Injection aperture, E: Silicone rubber, F: Thin fluorinated rubber, G: Magnetic rotor, H: Glass bulb. The volume of the cell is 15–45 cm^3.

2.3 Statistical analysis

The relationship between average partial molar volume of water hydrated to saccharides and dynamic hydration number of saccharides or average apparent equilibrium constants for lauroyl monosaccharide synthesis was determined by ANOVA. Significant differences were determined by *t*-tests at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Partial molar volume of water hydrated to saccharide

The partial molar volume of water $\bar{V}$ was calculated as follows:

$$\bar{V} = \Delta V/\Delta n \qquad \text{Eq. 1}$$

where ΔV is the change in volume and Δn is the molar number of water added at each instance. The solubility of water in dodecane must be very low, considering that its solubility in pentane and heptane at 25°C are 0.012 g/100 g and 0.015 g/100 g, respectively$^{4)}$. Therefore, the volumetric contribution of monomerically dissolved water to $\bar{V}$ is negligible and can be ignored. Figure 3 illustrates

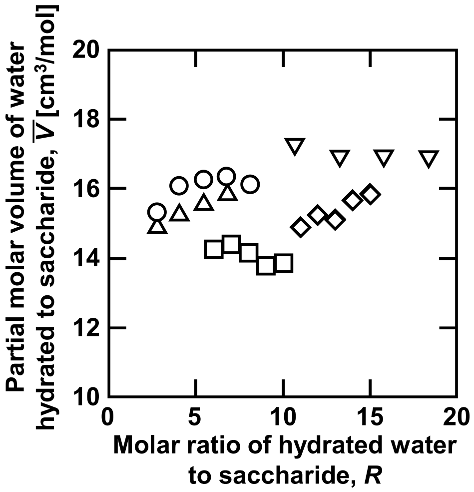

Fig. 3. Influence of the molar ratio *R* on partial molar volume $\bar{V}$ of water hydrated to (○) glucose, (□) mannose, (◇) galactose, (△) fructose, and (▽) sucrose at 25°C.

the influence of molar ratio R of water hydrated to each saccharide on the partial molar volume. Saccharide type influenced partial molar volume of the water hydrated to the saccharide. The R value slightly influenced $\bar{V}$ values of glucose, galactose, and fructose, whereas it did not influence $\bar{V}$ values of mannose and sucrose. A similar trend in mannose and sucrose may also be observed in a lower range of R values compared to the tested values. All $\bar{V}$ values of the saccharides were lower than 18.02 cm^3/mol, which is the partial molar volume of pure water. This indicates that the partial molar volume of water reduces following hydration. However, the reason for such a relationship remains unclear. A slight change in temperature could affect the partial molar volume of water. Consequently, mean of $\bar{V}$ values obtained were used in subsequent investigations in the present study.

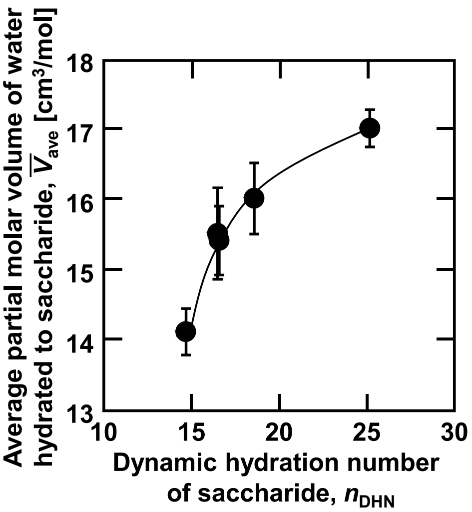

Fig. 4. Relationship between the average partial molar volume $\bar{V}$ of water hydrated to saccharides and dynamic hydration number n_{DHN} of saccharide at 25°C. Statistical significance was determined at $p < 0.05$. The solid curves were empirically drawn.

Figure 4 illustrates the relationship between average partial molar volumes of water hydrated to saccharides and dynamic hydration number n_{DHN} of saccharides at 25°C. The dynamic hydration number is an index for the dynamic state of water molecules hydrated to a solute and is proportional to the entropy of a hydration solute. In addition, the dynamic hydration number of a monosaccharide is known to be proportional to the number of the equatorial hydroxyl group e-OH in a solute molecule$^{5)}$ i.e., higher number of e-OHs in a saccharide molecule corresponds to greater stabilizing effect on water structure. n_{DHN} values in previous reports$^{5,6)}$ were used in Fig. 4. Average $\bar{V}$ values in saccharides increased with increase in n_{DHN} values and that of glucose was the highest. The conformation of a saccharide determines its hydration properties. For example, all hydroxyl groups in β-D-glucose molecule are the equatorial type. Therefore, a model where the β-D-glucose molecule could penetrate the tridymite structure of water has been proposed (Fig. 5)$^{7)}$. These results suggest that the hydration property of saccharides associated with their conformation greatly influences the partial molar volume of the hydrated water.

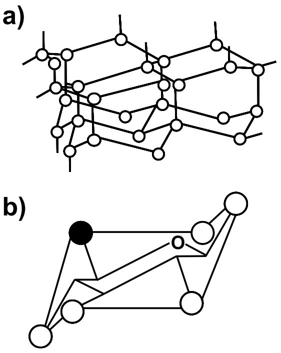

Fig. 5. (a) Tridymite structure of water and (b) β-D-glucose conformation. The open and closed circles represent oxygen atoms and carbon atoms on position C6, respectively.

3.2 Relationship between apparent equilibrium constant for enzymatic synthesis of lauroyl monosaccharide and partial molar volume of water hydrated to saccharide

The apparent equilibrium constant K_C for enzymatic synthesis of lauroyl monosaccharide in acetonitrile by an immobilized lipase from *Candida antarctica* (Fig. 1) is defined by Eq. 2, based on the concentrations of the substrates and products$^{2)}$:

$$K_C = C_{Pe}C_{We}/C_{Se}C_{Fe} \qquad \text{Eq. 2}$$

where C is the concentration in mol/L, and the subscripts S, F, P, W represent the monosaccharide, fatty acid (lauric acid), product (lauroyl monosaccharide), and water, respectively. The subscript e indicates equilibrium. There was a positive correlation between the lnK_C and n_{DHN}, indicating that water plays a key role in the condensation of the microaqueous organic solvent. Monosaccharides having stronger bonds with water molecules would yield larger K_C values, although activities in other components would also be affected by the presence of the monosaccharide. Consequently, it suggests that there is a correlation between the K_C value and the partial molar volume of water hydrated to a monosaccharide.

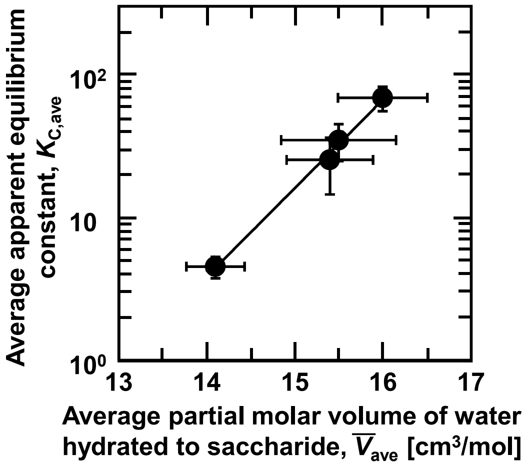

Fig. 6. Relationship between average apparent equilibrium constant K_C for lauroyl monosaccharide synthesis catalyzed using the lipase in acetonitrile, and average partial molar volume of water hydrated to saccharide. Statistical significance was determined at $p < 0.05$. The solid curve represents the correlation between the two values ($R^2 = 0.996$).

Figure 6 illustrates the relationship between the average apparent K_C for the synthesis of lauroyl monosaccharide and average partial molar volumes of water hydrated to saccharides. K_C values averaged from all C_{We} values for each of the products are plotted in the figure, because the K_C value depended on the C_{We}. As expected, a high positive correlation between $K_{C,ave}$ and the average partial molar volume of hydrated water was observed. This suggests that evaluation of the average partial molar volume of water hydrated to saccharide facilitates the estimation of the apparent equilibrium constant for enzymatic synthesis of acyl monosaccharides.

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