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Equilibrium constant for the lipase-catalyzed synthesis of fatty acid butyl ester in various organic solvents

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Abstract

The equilibrium constants for the formation of fatty acid butyl esters by the lipase-catalyzed condensation were estimated in nitriles, tertiary alcohols and their mixtures. The equilibrium constant for the synthesis of butyl decanoate did not depend on the alkyl chain length of the nitriles or alcohols, but significantly depended on the type of solvent polar group. The constant in the mixture of a nitrile and a tertiary alcohol increased as the molar fraction of the nitrile in the mixture increased. The IR spectra of the C=O bond of decanoic acid and butyl decanoate were measured in various solvents. The measurements indicated that the interaction between the decanoic acid and *t*-butyl alcohol was stronger than that between butyl decanoate and the alcohol. The interaction would be a reason for the dependence of the equilibrium constant on the fraction of nitrile in a mixed solvent.

Keywords: Equilibrium constant; Reaction medium; Lipase; Condensation; IR spectrum

1. Introduction

Over the past two decades, much attention has been paid to the enzymatic reactions in organic solvents. The lipase-catalyzed esterification has been widely used for the synthesis of stereoselective compounds, cosmetics, foods and pharmaceuticals [1–3]. The solvents usually used for the esterification are acetonitrile, acetone, tertiary alcohols such as *t*-butyl alcohol and *t*-amyl alcohol, and hydrophobic solvents such as hexane. The esterification in an organic solvent can be shifted toward the product side by decreasing the concentration of water, one of the products, in the reaction mixture. Although the equilibrium constant is an important factor for predicting the equilibrium yield of the desired product, only a few studies have reported the factors affecting the constant for the lipase-catalyzed esterification in organic solvents. Some reports have dealt with the thermodynamics of the ester formation in various reaction media [4–12]. It has been reported that the equilibrium constant based on substrate and product concentrations depends on the type of reaction medium [7–12], although the thermodynamic equilibrium constant should be characteristic of the reaction and does not depend on the type of medium. This phenomenon is widely observed for various sets of reaction media and reactants. The shift in the equilibrium constants is related to the solvent properties such as the partition coefficient to 1-octanol [8–10], the dielectric constant [11], relative permittivity [9,10] and water solubility in organic solvent [12]. However, our knowledge seems

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to be still insufficient to elucidate the reason for the change in the equilibrium constant depending on the nature of the reaction medium. Furthermore, a mixture of two or more solvents is scarcely used for the enzyme-catalyzed synthesis except for the reactions in biphasic systems, although the nature of the reaction medium can be easily changed by mixing solvents.

In this study, the equilibrium constants for the lipase-catalyzed condensation of 1-butanol and fatty acids with acyl chain lengths of 8–14 in some water-miscible solvents were estimated. The solvents used were nitriles, 1,4-dioxane, tertiary alcohols and mixtures of nitriles and tertiary alcohols. The effect of the alkyl chain length of the solvents on the equilibrium constant was also examined. The change in the equilibrium constant was discussed based on the IR spectra of a fatty acid and an ester measured in various solvents.

2. Materials and methods

2.1. Materials

Butyl octanoate, butyl decanoate, octanoic, decanoic, lauric and myristic acids, and 1-butanol were purchased from Wako Pure Chemical Industries (Osaka, Japan). Butyl laurate and butyl myristate were purchased from Tokyo Chemical Industries (Tokyo, Japan). Acetonitrile, propionitrile, butyronitrile, 1,4-dioxane, *t*-butyl alcohol and *t*-amyl alcohol used as the reaction medium were from Wako. Two kinds of lipases, Chirazyme® L-2 (C2-carrier-fixed) and Chirazyme L-2 (free) from *Candida antarctica*, were obtained from Roche Diagnostics (Manheim, Germany). All other chemicals were from either Wako or Nacalai Tesque (Kyoto, Japan).

2.2. Condensations

One of the solvents or a mixture of two solvents at a specific volumetric ratio was used as a reaction medium for the lipase-catalyzed esterification. A typical esterification was conducted as follows: a specific amount of water was added to the reaction medium to adjust its water concentration in the range of 30–80 mmol/l, which was precisely determined by Karl–Fischer titration. The fatty acid butyl ester, fatty acid and 1-butanol were placed in a 50 ml glass vial, and lipase (250 mg of immobilized lipase or 50 mg of free lipase) and 50 ml of the reaction medium were then added to the vial. The concentrations of both the fatty acid butyl ester and the fatty acid were changed in the range of 20–100 mmol/l, and the concentration of 1-butanol was fixed at 50 mmol/l. The vial was tightly screw-capped and vigorously shaken at 160 rpm at 50 °C. At appropriate intervals, 10 μl of the reaction mixture was sampled and used for the HPLC analysis.

2.3. Estimation of equilibrium constant

The reaction considered here can be described as follows:

$$\mathrm{A} + \mathrm{F} \leftrightarrows \mathrm{E} + \mathrm{W} \tag{1}$$

where A, F, E and W represent the alcohol, fatty acid, ester and water, respectively. The equilibrium constant, K, for the ester formation can be defined by Eq. (2) based on the concentrations of the reactants.

$$K = \frac{C_\mathrm{E} C_\mathrm{W}}{C_\mathrm{A} C_\mathrm{F}} \tag{2}$$

where C_i represents the concentration of the component i (i = E, W, A or F).

The reaction was conducted at five or six different initial concentrations of E, F and W for each reaction medium to determine the equilibrium constant in the medium. After the reaction reached equilibrium, the concentrations of the reactants were determined. The $C_\mathrm{E}C_\mathrm{W}$ values were then plotted versus the $C_\mathrm{A}C_\mathrm{F}$ values. The K value was evaluated from the slope of a regression line connecting the plots and passing through the origin.

2.4. Analysis

The water concentration, C_W, was determined using the MKS-510 Karl–Fischer titrator (Kyoto Electronics Manufacturing, Kyoto). The volume of injected sample was 5 or 10 ml. The concentrations of the fatty acid butyl esters and fatty acids were determined by the HPLC equipped with the Shimadzu LC-10AT pump (Kyoto), SPD-10AV UV-Vis detector (220 nm, Shimadzu) and Cadenza CD-C18 column (3.0 mm × 30 mm, Imtakt, Kyoto). The eluents used were methanol/water mixtures of 85/15, 92/8,

92/8, 95/5 and 65/35, 75/25, 80/20, 90/10 (v/v) for butyl-octanoate, decanoate, laurate and myristate, and octanoic, decanoic, lauric and myristic acids, respectively, at 0.5 ml/min. The concentration of 1-butanol was calculated from the material balance of the reactants.

2.5. IR spectra

The IR spectra of the decanoic acid and butyl decanoate dissolved in the nitriles, tertiary alcohols or the mixtures of acetonitrile and *t*-butyl alcohol at various ratios were measured using the FTIR-8300 spectrometer (Shimadzu) by the ATR method. The concentrations of decanoic acid and butyl decanoate were both fixed at 100 mmol/l.

3. Results and discussion

3.1. Equilibrium constant for the ester formation in various media

Fig. 1 shows the transient changes in the butyl decanoate concentration during its synthesis by the immobilized lipase-catalyzed condensation in acetonitrile at 50 °C. The estrification reached equilibrium within 10 h in all cases when the initial concentrations of butyl decanoate and decanoic acid were varied at the fixed concentrations of 1-butanol and water. Therefore, in this study, the concentrations of the reactants at a reaction time of 24 h were regarded as the equilibrium ones in every case. The equilibrium constant, K, was evaluated by plotting the $C_E C_W$ values versus the $C_A C_F$ values according to Eq. (2) (Fig. 2). A straight line passing through the origin could be obtained in every case, indicating that the equilibrium constant did not depend on the reactant concentrations for the solvents tested.

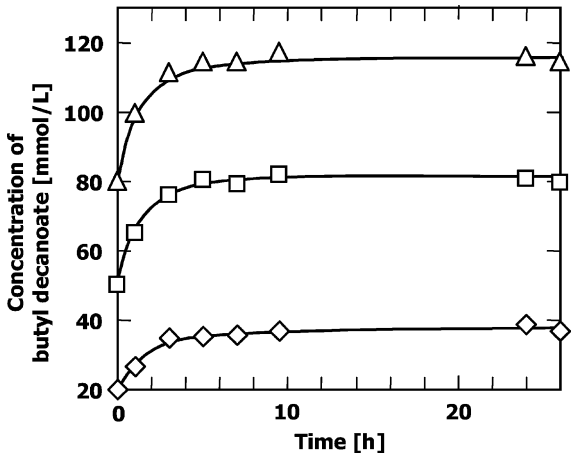

Fig. 1. Transient changes in the concentration of butyl decanoate during the immobilized lipase-catalyzed condensation of 1-butanol with decanoic acid in acetonitrile at 50 °C. The initial concentrations of 1-butanol and water were both 50 mmol/l, and those of butyl decanoate and decanoic acid were both (◇) 20 mmol/l, (□) 50 mmol/l and (△) 80 mmol/l.

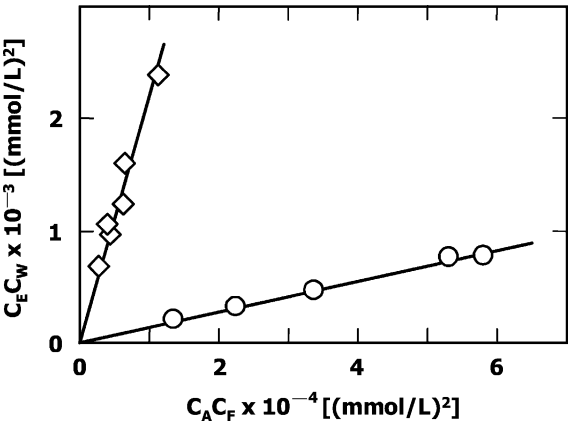

Fig. 2. Estimation of the equilibrium constant for the immobilized lipase-catalyzed synthesis of butyl decanoate in (○) *t*-butyl alcohol or (◇) acetonitrile at 50 °C.

Fig. 3 shows the equilibrium constants for the formation of butyl decanoate in various solvents. The

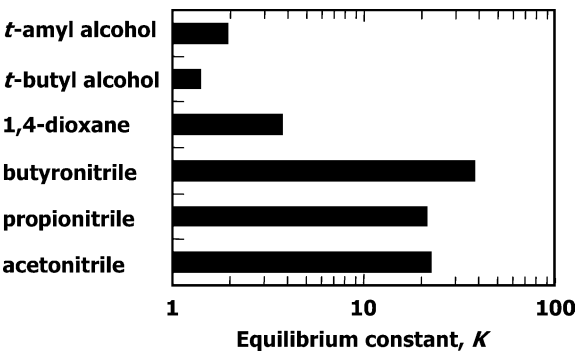

Fig. 3. The equilibrium constants for the immobilized lipase-catalyzed synthesis of butyl decanoate in various reaction media at 50 °C.

equilibrium constants in *t*-amyl alcohol and *t*-butyl alcohol were 1.9 and 1.4, while those in the nitriles were from 21 to 38. The constant in 1,4-dioxane was midway between the values in the nitriles and those in the tertiary alcohols. The values in the nitriles were almost first-order greater than those in tertiary alcohols. The alkyl chain length of the nitriles or tertiary alcohols scarcely affected the constant.

The equilibrium constants were estimated for the condensation carried out using the immobilized lipase. To examine the effect of the enzyme support on the equilibrium constant, a free lipase from the same origin as the immobilized one was employed for the synthesis of the fatty acid esters with different acyl chain lengths in acetonitrile. The equilibrium constants evaluated by using the free lipase were compared with those evaluated by using the immobilized lipase. There was no significant effect of the enzyme support on the equilibrium constant. The effect of the acyl chain length on the equilibrium constant was also insignificant. These results indicated that the property of the reaction medium (solvent) was a major factor affecting the equilibrium constant. Therefore, the immobilized lipase was used in the following experiments.

The equilibrium constant for the synthesis of butyl decanoate was estimated in the mixtures with nitriles and tertiary alcohols at various volumetric ratios. The nitriles used were acetonitrile, propionitrile and butyronitrile, and the alcohols were *t*-butyl alcohol and *t*-amyl alcohol. Fig. 4 shows the relationship between the equilibrium constant and the molar fraction of the nitrile in the mixtures, which was calculated from the volumetric fraction based on the density and molecular mass of the solvents. All the plots could be connected by a curve, and the equilibrium constant increased as the molar fraction of the nitrile in the mixture increased. The alkyl chain length of the nitrile or tertiary alcohol only slightly affected the equilibrium constant. These results would suggest that the equilibrium is controllable to a certain degree using a mixture of a nitrile and a tertiary alcohol as the reaction medium.

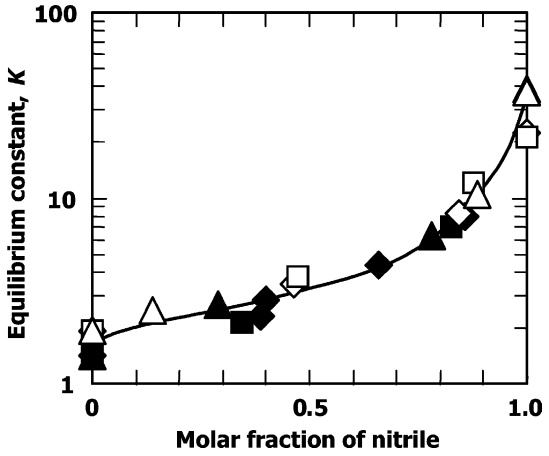

Fig. 4. The equilibrium constants for the formation of butyl decanoate in mixtures of alcohols and nitriles of various molar ratios at 50 °C. The open and closed symbols represent the mixtures of *t*-butyl alcohol and *t*-amyl alcohol, respectively, with (◇, ◆) acetonitrile; (□, ■) propionitrile; and (△, ▲) butyronitrile.

3.2. IR spectrum

The thermodynamically-defined equilibrium constant for a reaction should be intrinsic at specific temperature and pressure. However, the equilibrium constant for the synthesis of butyl decanoate, which was defined by the concentrations of the reactants instead of the activities, largely depended on the type of reaction medium. To elucidate the effect of a reaction medium on the equilibrium constant for the synthesis of butyl decanoate, the IR spectra of decanoic acid and butyl decanoate were measured in various solvents. Although four reactants were included in the present reaction system, the spectra of decanoic acid and butyl decanoate were measured because they have a carbonyl double bond (C=O), which absorbs in the IR range of 1700–1800 cm^{-1}, and the reaction media used in this study do not have a C=O double bond. The spectra of decanoic acid in various solvents or their mixtures are shown in Fig. 5. The wavenumber at the absorption peak of a C=O double bond did not depend on the alkyl chain length of the medium for both the nitriles and tertiary alcohols (Fig. 5(a)), but the peak shifted to a higher wavenumber when the volumetric fraction of acetonitrile in the mixture with *t*-butyl alcohol was higher (Fig. 5(b)). Butyl decanoate showed two peaks in the range of wavelengths when the volumetric fraction of acetonitrile in the mixture was equal to or lower than 0.25 (Fig. 6), and the stronger peak was adopted in this study because it was observed at any fraction. These results indicate that the intermolecular interaction of *t*-butyl alcohol with the reactants is stronger than that of acetonitrile.

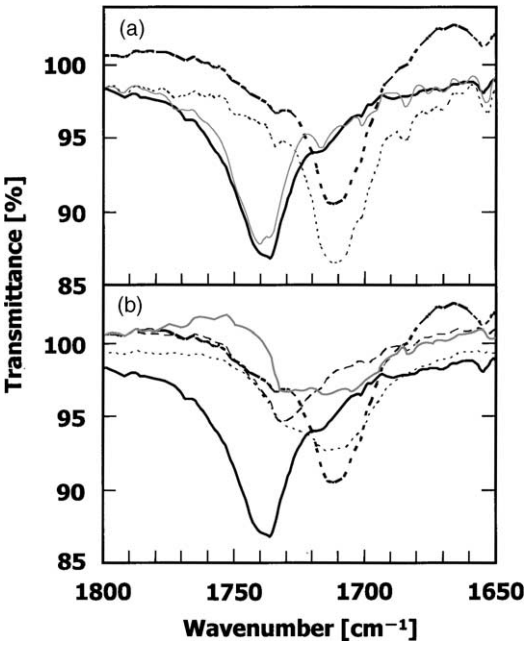

Fig. 5. The IR spectra of decanoic acid in (a) various solvents and (b) mixtures of acetonitrile with *t*-butyl alcohol. The concentration of decanoic acid was 100 mmol/l in all cases. The solvents used in (a) were (▬) acetonitrile; (▪▪▪) propionitrile; (_) *t*-butyl alcohol and (....) *t*-amyl alcohol. The volumetric ratios of acetonitrile to *t*-butyl alcohol in (b) were (▬) 1/0, (– – –) 3/1, (····) 1/1, (- - -) 1/3 and (...) 0/1.

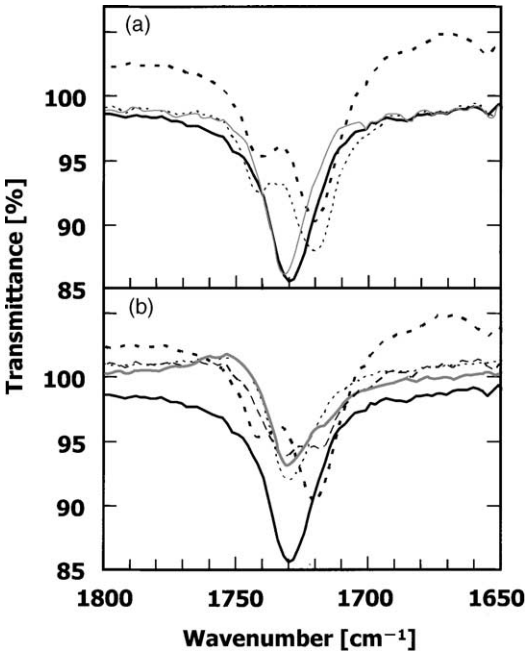

Fig. 6. The IR spectra of butyl decanoate in (a) various solvents and (b) mixtures of acetonitrile with *t*-butyl alcohol. The concentration of butyl decanoate was 100 mmol/l in all cases. The line designations are the same as in Fig. 5.

Fig. 7 shows the relationships between the wavenumber at the absorption peak of a C=O double bond and the molar fraction of acetonitrile in the mixture with *t*-butyl alcohol for decanoic acid and butyl decanoate. The wavenumber for decanoic acid increased with an increase in the fraction of acetonitrile, while the change in wavenumber for butyl decanoate was smaller than that for decanoic acid. These results suggest that the hydroxyl group of decanoic acid interacts with the hydroxyl group of *t*-butyl alcohol via the hydrogen bond, while the hydrogen bonding between butyl decanoate and the hydroxyl group of *t*-butyl alcohol is weak because butyl decanoate does not have a hydroxyl group. Thus, the stronger interaction of decanoic acid with *t*-butyl alcohol than that of butyl decanoate allows the reaction to shift toward the hydrolysis in the mixture with higher fraction of *t*-butyl alcohol.

The equilibrium constant, *K*, estimated in the mixture of acetonitrile and *t*-butyl alcohol at 50 °C could be linearly, on a semi-logarithmic scale, correlated to the wavelength at the peak of the C=O double bond of decanoic acid, as shown in Fig. 8. The correlation would allow us to predict the equilibrium constant for

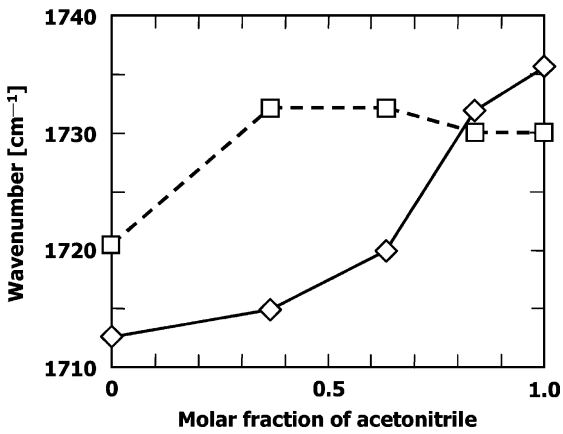

Fig. 7. Relationships between the molar fraction of acetonitrile in its mixture with *t*-butyl alcohol and the wavenumbers where (◇) decanoic acid and (□) butyl decanoate showed a peak for C=O.

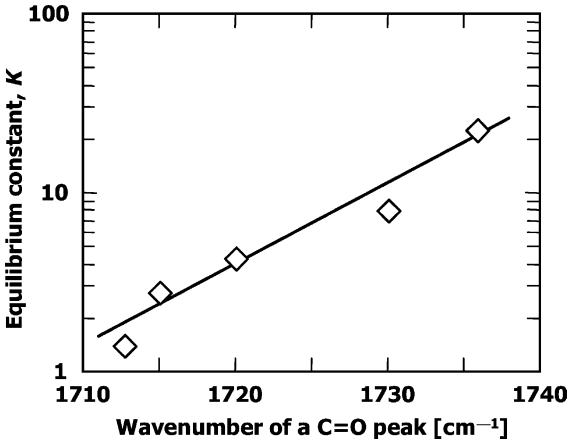

Fig. 8. Relationship between the equilibrium constant, *K*, for the synthesis of butyl decanoate and the wavenumber for the C=O peak of decanoic acid. The *K* value was estimated in the mixture of acetonitrile and *t*-butyl alcohol at 50 °C.

the synthesis of a fatty acid ester in a mixture of a tertiary alcohol and nitrile by measuring the IR spectrum of the C=O double bond of the fatty acid in the mixture. However, this is rather limited because the other reactants, water and 1-butanol, are not taken into account. It is possible that the reactants also more strongly interact with the tertiary alcohol than with the nitrile because they have a hydroxyl group. Further investigations of the interactions of reactants and the reaction medium are required for better understanding the phenomena occurring in the reaction system.

4. Conclusion

The equilibrium constant for the formation of butyl decanoate was subject to change in various media or their mixtures. The alkyl chain length of tertiary alcohols or nitriles used as a reaction medium did not affect the equilibrium constant, but the type of a solvent polar group was a significant factor. The equilibrium constant changed depending on the volumetric fraction of the nitrile in the mixtures with tertiary alcohols, and nitriles are more favorable for ester formation than tertiary alcohols. The equilibrium constant for the synthesis of butyl decanoate was discussed based on the IR spectra of decanoic acid and butyl decanoate in various solvents. The interaction of decanoic acid with *t*-butyl alcohol suggested affecting the equilibrium constant.

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