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RESEARCH NOTES

Stability of Surfactant-coated *Candida Rugosa* Lipase in Isooctane*

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Abstract The stability of *Candida rugosa* lipase coated with glutamic acid didodecyl ester ribitol amide was investigated taking esterification of lauryl alcohol and lauric acid in isooctane as a model reaction. At 30℃, the half-life of the activity of the coated lipase was ca 10 h, the enzyme activity became less changed after 12 h and the residual activity was 39% of the initial value. The coated lipase obeyed a first-order deactivation model with a deactivation energy of 29.9 J·mol^{-1}.

Keywords activity, *Candida rugosa*, lipase, organic solvent, stability, surfactant

1 INTRODUCTION

Surfactant-coated enzymes have been extensively studied as a promising biocatalyst in organic solvents over the past 10 years or so[1−3]. The advantages of the surfactant-coated enzymes include simple preparation procedure, good solubility in a wide range of organic solvents[4] and less activity loss as compared with other methods such as the enzyme modification by polyethylene glycols[5] and entrapment in reverse micelles[6]. However, most studies in this field concentrated on the activity of the surfactant-coated enzymes[7,8], and less attention is paid to their stability, which is also an important factor affecting their commercial applications.

In this work, the stability of surfactant-coated *Candida* rugosa lipase is investigated taking the esterification of lauric acid and lauryl alcohol in isooctane as a model reaction.

2 MATERIALS AND METHODS

2.1 Materials

Lipase (EC 3.1.1.3) from *Candida rugosa* (Type VII) was purchased from Sigma. The nonionic surfactant, glutamic acid didodecyl ester ribitol amid used for coating the lipase, was synthesized following the method of Goto *et al.*[9]. All other chemicals used were of analytical grade and obtained commercially.

2.2 Preparation of the surfactant-coated lipase

The surfactant-coated lipase was prepared according to the method of Goto *et al.*[4] with a slight modification by dissolving the surfactant in 40℃ ethanol rather than in a buffer solution. Lipase (500 mg) was dissolved in 250 ml 0.05 mol·L^{-1} phosphate buffer of pH 6.8 and stirred for 20 min at 4℃. A solution containing 500 mg surfactant in 10 ml warm ethanol was added dropwise to the enzyme solution in an ultrasonic cleaner. The solution was incubated in a refrigerator for 24 h. The precipitate was collected by centrifugation and dried under vacuum to give the surfactant-coated lipase in white powder with a yield of about 20%. The specific activity of the coated lipase prepared in this way was ca. 150% higher than that prepared following the original method of Goto *et al.*[4].

2.3 Protein assay

The protein content in the surfactant-coated lipase was determined based on the UV absorption of aromatic amino acid residue in proteins at 280 nm following the procedures of Okahata and Ijiro[1].

2.4 Determination of lipase activity and stability

The reaction system consisted of lauric acid (0.12 mol·l^{-1}), lauryl alcohol (0.12 mol·l^{-1}) and lipase (2—4 mg·ml^{-1}) in isooctane. The reaction mixture (20 ml) was incubated at 30℃ for 3 h with continuous stirring. Then 1 ml sample was withdrawn and mixed with 10 ml ethanol/acetone (1:1, v/v) to stop the reaction. The remaining acid was determined by titration with 0.01 mol·mol^{-1} NaOH. Specific activity of the enzyme was expressed as μmol·min^{-1} per milligram protein.

Lipase stability was analyzed by incubating the enzyme in isooctane at constant temperature. The samples were withdrawn at intervals and the residual activities were detected at 30℃. Relative activity (Ra) was defined as the ratio of the residual activity to the initial activity at 30℃.

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3 RESULTS AND DISCUSSION

3.1 Time course of lipase activity at different temperatures

Figure 1 shows that the enzyme deactivated rapidly within the first several hours. The coated lipase deactivated more severely at higher temperatures. At 30℃, the half-life of the coated lipase was ca. 10 h, while it decreased to ca. 1 h when the temperature was raised to 50℃. It should be mentioned that the stability of native *Candida rugosa* lipase is very poor. For example, in AOT/isooctane reverse micelles the half-life of the same lipase at 30℃ was only 1.5 h[6]. Therefore the stability of *Candida rugosa* lipase was obviously improved after being coated with surfactant. It is noted that the enzyme activity became stable after long time of contact with the solvent. The final residual activities at 30℃ and 50℃ were 39% and 3% of the initial activity at 30℃, respectively.

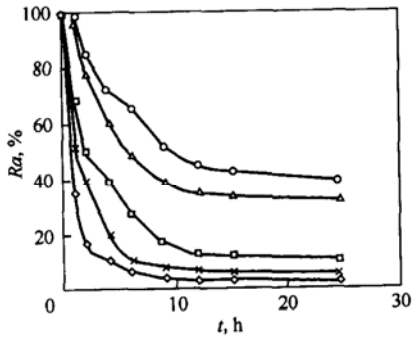

Figure 1 Deactivation curves of the surfactant-coated *Candida rugosa* lipase in isooctane at different temperature

—○— 30℃; —△— 34℃; —□— 38℃; —×— 45℃, —◇— 50℃

3.2 Kinetic model of enzyme deactivation

Assuming that lipase follows the first-order deactivation model[10]

$$\mathrm{E} \xrightarrow{k_d} \mathrm{E_d} \tag{1}$$

Where E and E_d represent active and partially deactivated enzymes, respectively, and k_d is the deactivation rate constant.

The concentrations of active [E] and partially deactivated [E_d] enzymes can be expressed as

$$[\mathrm{E}] = E_0 \exp(-k_d t) \tag{2}$$

$$[\mathrm{E_d}] = E_0[1 - \exp(-k_d t)] \tag{3}$$

Where E_0 represents the initial enzyme concentration and t is time.

Assuming that the active and partially deactivated enzymes are of the same Michaelis constant (K_m), the relative activity (Ra) can be calculated as

$$Ra = \frac{k_{cat}[\mathrm{E}] + \alpha k_{cat}[\mathrm{E_d}]}{k_{cat}E_0} = \alpha + (1-\alpha)\exp(-k_d t) \tag{4}$$

Where k_{cat} is the catalytic activity constant, and α represents the ratio of the specific enzyme activity at the final state (E_d) to that at the initial state (E_0).

For determination of the deactivation rate constant (k_d), Eq. (4) is transformed to the following form

$$\ln(Ra - \alpha) = \ln(1 - \alpha) - k_d t \tag{5}$$

The plots of $\ln(Ra - \alpha)$ against t (Fig. 2) at different temperatures show excellent linear correlations ($r^2 > 0.993$), confirming the assumption of the first-order deactivation model. The values of k_d and α at different temperature are listed in Table 1. It is seen that the value of k_d increases almost linearly with temperature while the value of α sharply decreases. At 30℃, the deactivation constant of the coated lipase is 0.20 h^{-1}, while that of the native lipase in AOT/isooctane reverse micelles is 0.77 h^{-1}[6], indicating that the stability of the coated lipase is 3.8 times higher. This might be ascribed to the more rigid structure of the enzyme-surfactant complex than the lipase entrapped in reverse micelles as well as the less water content in the former case. Klibanov[11] claimed that the increased conformational rigidity of enzyme molecules in organic solvents and the less water environment are favorable to keeping their stability.

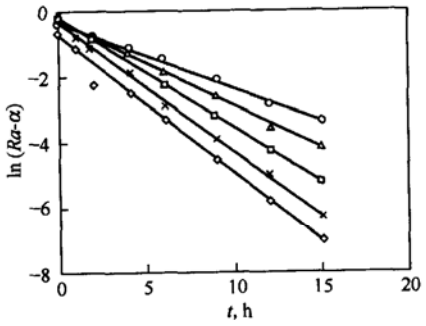

Figure 2 Plot of $\ln(Ra - \alpha)$ against time

—○— 30℃; —△— 34℃; —□— 38℃; —×—45℃; —◇— 50℃

Table 1 Deactivation parameters of the surfactant-coated lipase in isooctane at different temperature

T, ℃	k_d, h^{-1}	α
30	0.20	0.39
34	0.26	0.32
38	0.31	0.11
45	0.40	0.06
50	0.42	0.03

substrates: lauric acid (0.12 mol·L^{-1}) + lauryl alcohol (0.12 mol·L^{-1}); lipase: 2—4 mg·ml^{-1}; pH: 6.8

The effect of temperature on deactivation constant is represented by the Arrhenius equation

$$k_d = A_d \exp(-E_a/RT) \tag{6}$$

where, A_d is the exponential factor and E_a is the activation energy of the deactivation reaction. The plot of $\ln k_d$ against $1/T$ shows a straight line (Fig. 3). From the slope and intercept E_d and A_d are calculated as $29.9\,J\cdot mol^{-1}$ and $3.1 \times 10^4\,h^{-1}$, respectively.

The activation energy of the esterification reaction catalyzed by the coated lipase was estimated to be 60—70 $kJ\cdot mol^{-1}$ according to the temperature-dependence of enzyme activity (data not shown). Therefore the activation energy of the enzyme deactivation reaction was less than 1‰ of that of the enzymatic reaction, indicating that the enzyme deactivation proceeds much easier than the enzymatic reaction. However, for a practical reaction system, the presence of substrates or products may be favorable to the maintenance of enzyme stability[12].

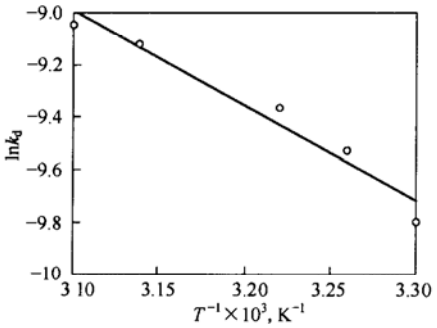

Figure 3 Plot of $\ln k_d$ against $1/T$

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