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Mild and efficient enzymatic oximolysis by supported *Pseudomonas cepacia* lipases

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Abstract

Pseudonomas cepacia lipase supported on ceramic particles (lipase PS-C) and diatomite (lipase PS-D) catalyzed the acylation of aldoximes and ketoximes. Aliphatic oximes reacted faster than aromatic oximes and polar solvents enhanced the reaction rate. For lipase PS-C, THF was a superior solvent, while for lipase PS-D 1,4-dioxane was the ideal solvent. The amount of enzyme required to catalyze this reaction was optimized. It was found that for lipase PS-C or lipase PS-D, 50 mg was the optimum amount to catalyze 1.0 mmol of substrate.

Keywords: Lipase PS-C; Lipase PS-D; Oxime esters; Solvent effect; Recyclability

1. Introduction

Lipases are used for kinetic resolutions [1–5] as well as to successfully catalyze hydrazide [6] and oxime formation [7,8]. They are also used to perform enantioselective hydrolytic reactions and form ester and amide bonds [9–11]. Lipase PS-C has been successfully employed for synthesis of achiral half esters [12] and for resolution of (±)-2,3-epoxypropyl esters [13]. Oxime esters are versatile intermediates in chemoselective and regioselective acylation of bifunctional compounds such as amino alcohols [14], carbohydrates [15–17] and nucleosides [18,19]. Such nucleoside derivatives are of high significance in some areas of medicinal chemistry [20], showing antineoplastic and antiviral activity [21–23]. Conventional method includes refluxing the ketoximes with acetic anhydride to yield *O*-acetyl ketoximes, whereas, aldoximes cannot be converted by this method as they form the corresponding cyanides [24]. Another method involves treating oximes with acetyl chloride [25] but being a corrosive, flammable liquid with a pungent odor, it is always of current interest to find simple alternative procedure for the preparation of these compounds. Lipases PS-C and PS-D catalyzed the reaction of oximes (aldoximes and ketoximes) with vinyl acetate to yield oxime esters as the sole product (Reaction 1). Oximes did not react with vinyl acetate in the absence of the enzyme.

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2. Experimental

2.1. Reagents and chemicals

THF and 1,4-dioxane were distilled over calcium hydride in order to avoid moisture. Oximes were synthesized using standard procedures. *Pseudomonas cepacia* lipase supported on ceramic particles (lipase PS-C) and diatomite (lipase PS-D) were obtained as gift samples from Amano Pharmaceutical, Japan. Precoated TLC alumina sheets silica gel G F_{254} from Merck were used. Physical constants were taken in open capillaries and are uncorrected. IR spectra were recorded on a Perkin-Elmer 170-X Infrared Fourier Transform Spectrophotometer. ^{1}H NMR was measured in $CDCl_3$/TMS, at 300 MHz on a Bruker AC-300 spectrometer.

2.2. General procedure for the enzymatic oximolysis

In a round bottom flask, oxime (1.0 mmol) was added to a mixture of 10 ml of solvent (*n*-hexane, isopropyl ether, THF, 1,4-dioxane) and vinyl acetate (3.0 mmol). After about 0.5 h, lipase PS-C or PS-D (50.0 mg) was added and stirring was continued for 10–11 h at room temperature. The reaction progress was monitored by TLC. The reaction was quenched by filtering the enzyme through a celite pad, and solvent was evaporated in vacuum. The residue obtained was stirred with a 20-ml, 1:1 mixture of $CHCl_3$:H_2O. The organic phase was separated, washed several times with H_2O, dried over $MgSO_4$ and evaporated in vacuum to yield pure oxime ester.

2.3. Recyclability of the enzyme

Unlike pure enzymes, immobilized enzymes can be easily recovered from the reaction mixture and can be recycled. Further, they may have enhanced stability, activity and selectivity. In this endeavour, we studied the recyclability of these enzymes by choosing benzophenone oxime as the substrate for five cycles. In a round bottom flask, 2.0 mmol of benzophenone oxime was added to a mixture of THF (10 ml) and vinyl acetate (6.0 mmol) and lipase PS-C (100.0 mg) was added and the mixture was stirred at room temperature for 10 h. The reaction was quenched by filtering the enzyme through a celite pad and solvent was evaporated in vacuum. The residue obtained was stirred with a 20-ml, 1:1 mixture of $CHCl_3$:H_2O. The organic phase was separated and washed several times with H_2O, dried over $MgSO_4$ and evaporated in vacuum to yield pure *O*-acetyl benzophenone oxime. The filtered enzyme was washed with (3×10 ml) of THF, to remove remaining reactant and product, dried thoroughly by air blowing and recycled. The same experiment was performed using lipase PS-D. The results are summarized in Table 2.

2.4. Spectral data for compounds **2(a–h)**

For **2a**, **2c** and **2d**, see Ref. [8].

O-Acetyl butanone oxime (**2b**): oil, IR (neat, cm^{-1}) 1479, 1762. ^{1}H NMR ($CDCl_3$) δ (ppm) 2.05 (t, 3H); 2.12 (s, 3H); 2.21 (s, 3H); 3.17 (q, 2H).

O-Acetyl acetophenone oxime (**2e**): oil, IR (neat, cm^{-1}) 1480, 1771. ^{1}H NMR ($CDCl_3$) δ (ppm) 2.06 (s, 3H); 2.14 (s, 3H); 7.30–7.70 (m, 5H).

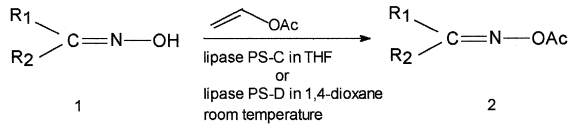
O-Acetyl-*p*-methylacetophenone oxime (**2f**): oil, IR (neat, cm^{-1}) 1479, 1768. ^{1}H NMR ($CDCl_3$) δ (ppm) 2.07 (s, 3H); 2.13 (s, 3H); 2.22 (s, 3H); 7.17 (d, 2H); 7.77 (d, 2H).

O-Acetyl-*p*-nitroacetophenone oxime (**2g**): oil, IR (neat, cm^{-1}) 1481, 1774. ^{1}H NMR ($CDCl_3$) δ (ppm) 2.12 (s, 3H); 2.22 (s, 3H); 7.34 (d, 2H); 7.81 (d, 2H).

O-Acetyl benzophenone oxime (**2h**): solid, mp 62°C, IR (neat, cm^{-1}) 1475, 1710. ^{1}H NMR ($CDCl_3$) δ (ppm) 2.10 (s, 3H); 7.20–7.70 (m, 10H).

3. Results and discussion

Solvents of varying polarity (*n*-hexane, isopropyl ether, THF, and 1,4-dioxane) for this



1 2

Under reaction -1

Reactant	R_1	R_2
1a	CH_3	CH_3
1b	CH_3	C_2H_5
1c	$(CH_2)_5$	
1d	C_6H_5	H
1e	C_6H_5	CH_3
1f	P-$H_3C.C_6H_4$	CH_3
1g	P-$O_2N.C_6H_4$	CH_3
1h	C_6H_5	C_6H_5

Fig. 1. Lipases PS-C and PS-D catalyzed reaction of oximes.

enzymatic oximolysis were used with benzophenone oxime as the substrate and employing lipases PS-C and PS-D. In both cases, polar solvents enhanced the reaction rates, as summarized in Fig. 1. For lipase PS-C, THF was a superior solvent, while in the case of lipase PS-D, 1,4-dioxane was the ideal solvent. Further, to standardize the amount of enzyme required, 1.0 mmol of benzophenone oxime was

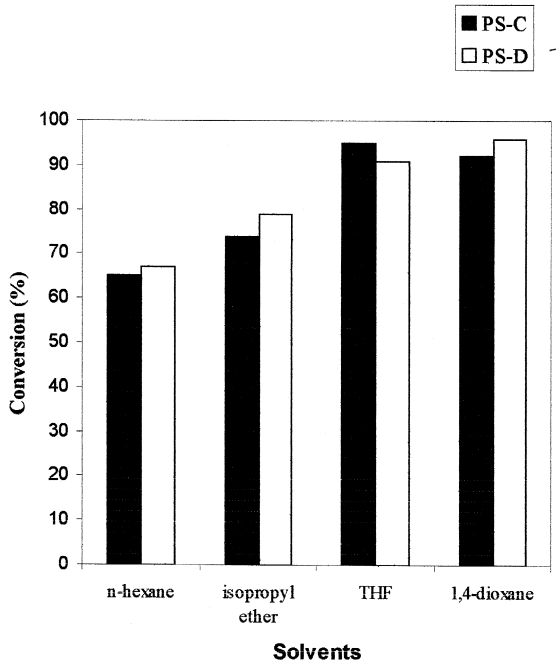


Fig. 2. Solvent effects in lipases PS-C and PS-D using benzophenone oxime as the substrate.

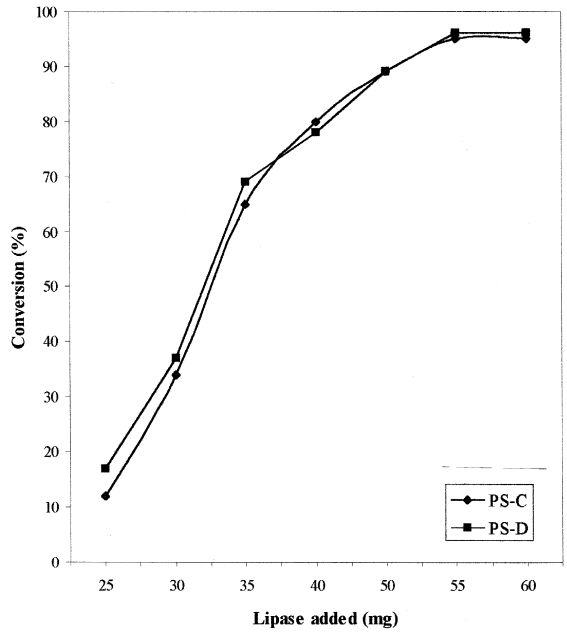


Fig. 3. Standardization of lipases PS-C and PS-D using benzophenone oxime as the substrate.

taken and lipase PS-C or PS-D was added, respectively. The results are summarized in Fig. 2. It can be perceived from Fig. 3 that for lipase PS-C or PS-D, 50.0 mg was the optimum amount to catalyze the conversion of 1.0 mmol of substrate. All other reactions were carried out using these standardized conditions. The results with various substrates employing lipase PS-C in THF and lipase PS-D in 1,4-dioxane are illustrated in Table 1. It can be inferred from Table 1 that aliphatic oximes undergo faster enzymatic oximolysis than aromatic oximes. The stability of the oxime esters was studied by performing

Table 1
Results of enzymatic oximolysis

Product	Lipase PS-C in THF		Lipase PS-D in 1,4-dioxane	
	Time (h)	Yield (%)	Time (h)	Yield (%)
2a	7	93	7	92
2b	7	94	7	91
2c	7	93	7	93
2d	9	92	9	91
2e	9	92	9	93
2f	11	89	11	92
2g	8	94	8	95
2h	9	95	9	96

Table 2
Recyclability of lipases PS-C and PS-D

Cycle no.	Ester yield (%)	
	PS-C	PS-D
1	95	96
2	94	95
3	94	95
4	92	93
5	90	91

control experiments. Oxime esters were stable in anhydrous solvents but in the presence of water, they underwent hydrolysis in 12 h. The recyclability of the enzymes is illustrated in Table 2. From this table, it can be inferred that after the third cycle, the yield decreases marginally.

4. Conclusion

Overall, the results from this study provide a simple alternative to synthesize *O*-acetyl aldoxime and ketoxime using supported lipases with high yields and under mild reaction condition (room temperature).

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