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Full Length Research Paper

High regioselective acetylation of vitamin A precursors using lipase B from *Candida antarctica* in organic media

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The effect of different reaction parameters was explored on the acylation of primary hydroxyl group of 1,6-diol by lipase B from *Candida antarctica* catalysis in organic solvent. First, the effect of the organic solvents was investigated, and the highest conversion rate was obtained in *n*-hexane. Then, the effect of the acyl donor was studied. Among several reactants, including acetic acid and two different acetates, vinyl acetate gave the best yield. A maximum monoester yield of 98.5% was obtained using vinyl acetate as acyl donor in n-hexane at 50°C. The substrate concentration was 25 mmol/L, while the diol to vinyl acetate molar ratio was 1:3. Substrate concentration had to be limited due to an inhibitory effect on enzyme by the diol that caused a decrease on initial reaction rate. To promote initial reaction rate, excess vinyl acetate was used. Under the optimum conditions, the conversion rate and mono-acylation selectivity were 98.5 and 100%, respectively. The produced monoester was 6.1 mg/ml, and this amount can be further optimized base on the results presented here.

Key word: Acetylation, regioselectivity, immobilized lipase B, biocatalytic processes, vitamin A precursors.

INTRODUCTION

Vitamin A (retinol) is an essential component in animal tissue which plays a number of important roles in the organism, including cell differentiation and growth and involvement in the visual process (Marks, 1985; John and Hähnlein, 1996). In the classical vitamin A synthesis, retinyl acetate (3) is obtained from intermediate diol (1) via partial acetylation (mixture of mono- and diacetylated compounds) and subsequent dehydration/isomerization. Higher yield is obtained in the last step when pure monoester (2) is used (Figure 1).

Biocatalytic processes for the preparation of monoester product would offer numerous advantages as compared to conventional, chemical methods such as increased selectivity and product purity, mild reaction conditions and the omission of toxic catalysts. Since lipases are well known for their capability to hydrolyze and synthesize various esters (Torres et al., 2008), it seemed highly attractive to utilize these biocatalysts for the direct monoacylation of primary hydroxyl group of the diol 1 for vitamin A synthesis.

Different resources of lipases were widely used to catalyze regioselectivity monoacylation reaction between various diol and ester in organic solvent. The mono-acylation of diol using lipases from *Pseudomonas* sp. (Akita et al., 1994), *Mucor miehei* (Berger et al., 1992), *Pergillus niger* (Hsiao et al., 1996), *Aspergillus oryzae* (Molinari et al., 2000), *Rhizomucor miehei* (Hari Krishna et al., 2001) and *Candida antarctica* (Martinelle and Huit, 1995; Romero et al., 2005) were reported. Among them, lipase B from *C. antarctica* has shown to be an excellent catalyst in selective monoacylation of long-chain mass size diol (Torres et al., 2008).

In this study, lipase B from *C. antarctica* was the selected catalyst to acetyl transfer for the monoacylation of primary hydroxyl group of diol 1 by using acetyl donors in organic solvent (Figure 2). The potential of lipase B catalyzed monoacylation of the diol 1 with various acyl donors in different organic solvents was investigated. Once the optimal reaction organic solvent and acyl donor

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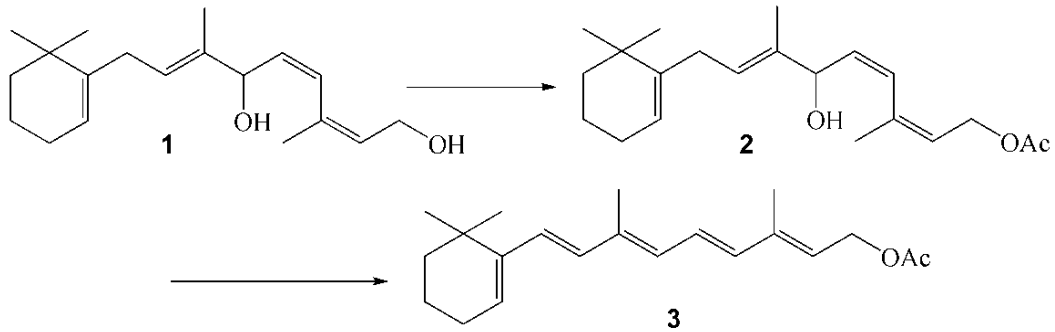

Figure 1. Vitamin A acetate synthesis by acetylating prepared monoester retinol 2.

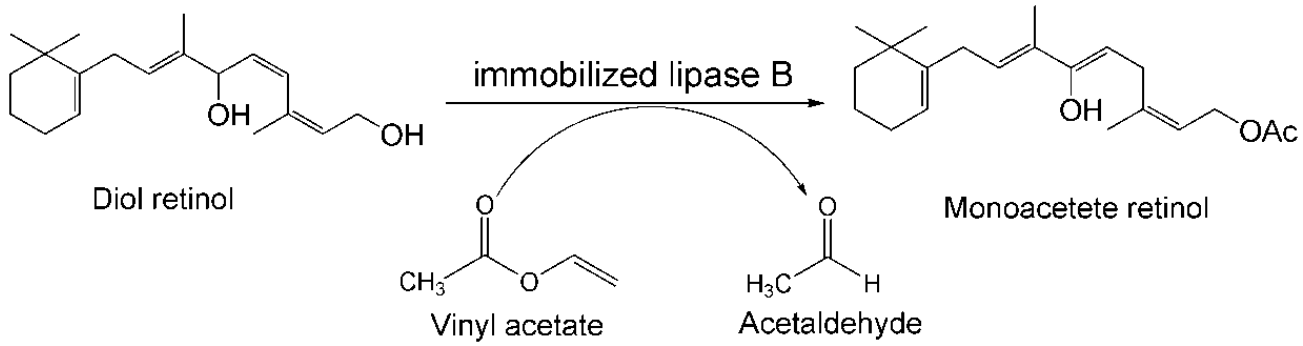

Figure 2. Lipase-catalyzed mono-acylating of primary-secondary diol with vinyl acetate.

were fixed, the effect of several reaction parameters such as temperature, rotation speed, molar ratio of acyl acceptor to donor, substrate concentration and stability of lipase was investigated.

MATERIALS AND METHODS

Novozym 435 (lipase B from *C. antarctica,* immobilized on a macroporous acrylic resin with a water content of 1 to 2% (w/w)) was kindly provided by Novo Nordisk. 7, 10-Dihydro-10-hydroxy-(11-cis, 13-cis)-retinol (diol 1) was prepared and purified as described in the patent (Orsat et al., 2001). All other chemicals were supplied by Sigma Chemical Co. The solvents were all of analytical grade.

Enzymatic reactions

Reactions were conducted in screw-cap vials. The reaction condition was carried at 50°C and 200 rpm by adding a certain amount of diol 1 and acyl donor and 5 mg lipase into 5 ml of solvent for 90 min. The reaction conditions of control test was used as earlier described, expect without adding lipase. The solvent employed had been previously dried using 3 Å molecular sieve (water activity $a_w = 0.04$).

Analytical method

Samples were analyzed by normal phase HPLC (Agilent technologies Series 1100 instrument), equipped with UV-vis detector (Agilent, USA), using a Agilent HC-C18 (250 mm x 4.6 mm, 5 µm) reversed-phase column from Aligent, USA. A 10 µl volume of the proper dilution of the reaction mixture was injected. The eluant, ethanol (90:10, v/v), was used at 50°C with a flow rate of 1 ml/min. The wavelength of UV-vis detector was 230 nm. Retention times were: diol 1, 8.6 min; monoester product, 11.4 min. The conversion rate was calculated as $(1\text{-}S_1/S) \times 100\%$, where S_1 and S were the chromatographic peak areas of the monoester product of the experiment and the control test, respectively. Monoester product was: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.94 (s, 3H), 0.95 (s, 3H), 1.55-1.58 (m, 2H), 1.40-1.42 (m, 2H), 1.68-1.69 (m, 3H), 1.83 (s, 3H), 1.96 (t, J = 6.0 Hz, 2H), 2.04 (s, 1H), 2.72 (t, J = 4.7 Hz, 2H), 4.50-4.62 (m, 3H), 5.27-5.30 (m, 1H), 5.45-5.48 (m, 1H), 5.62 (dd, J = 11.6, 9.2 Hz, 1H) and 5.96 (d, J = 11.5 Hz, 1H).

Study of lipase stability

After the completion of a monoacylating reaction (selectivity >99%), the lipase was filtered off, washed with vinyl acetate, dried at room temperature under vacuum for 10 min. The lipase was subsequently used as a catalyst to the batch reactor as previously described.

RESULTS AND DISCUSSION

Effect of organic solvents

It is well know that the type of organic solvent strongly

Table 1. Effect of the organic solvent on the conversion and selectivity[a].

Organic solvent	Log *P* [b]	Conversion (%)	Selective (%)
n-Hexane	3.5	95.3	≥99
Cyclohexane	3.2	53.3	≥98
n-Butyl alcohol	1.7	42.6	≥98
Tetrahydrofuran	0.49	21.2	≥98
Acetone	-0.23	47.8	≥98

[a] The reaction condition was carried out at 50°C by adding 20 mmol/L diol 1, 60 mmol/L vinyl acetate and 5 mg/ml lipase into 5 ml n-hexane for 90 min. [b]Log P values of the solvents were obtained from the literature (Laane et al., 1987).

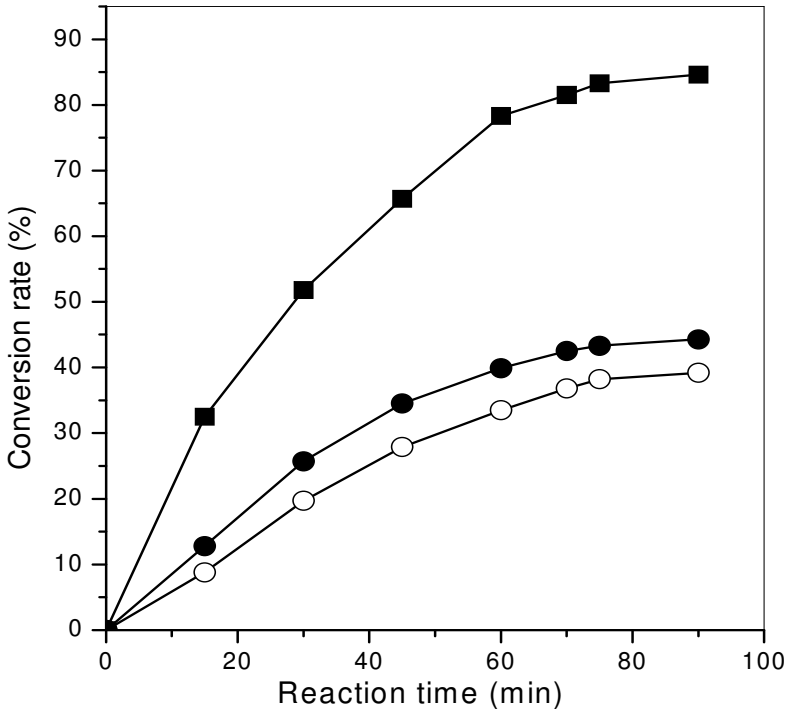

Figure 3. Comparison of acetylating agent in the lipase B catalyzed mono-acylation of diol 1. The reaction condition was carried out at 50°C by adding 20 mmol/L diol retinol, 60 mmol/L vinyl acetate and 5 mg/ml lipase into 5 ml n-hexane for 90 min. (■): Vinyl acetate; (●): ethyl acetate; (○): acetate.

affects enzymatic activity and enantioselectivity in organic solvent (Anthonsen and Hoff, 1998). Therefore, the effect of organic solvent on the conversion of monoester product was investigated in this study. Reactions were carried out in a wide variety of solvents with log *P* values of -0.29 to 3.5. All solvents were dried with 3 Å molecular sieves before use. The different conversions were obtained depending on the nature of solvent that plays a crucial role in the solubilisation of the diol 1 (Table 1). The conversion rate increased with the increase of log *P* values of the solvent with the exception of acetone. N-hexane displayed the highest conversion rate as an organic solvent as compared to other solvents. Therefore, the n-hexane was selected as the optimal organic solvent for monoacylating reaction in this study.

Effect of acyl donor

The process in which lipase B catalyzed esterification and transesterification in organic solvents is often of very slow conversion rate due to reversible reaction (Chen et al., 1987). This problem can be overcome be selecting suitable acyl donor to make reaction process irreversible. Various acyl donors was screened for the lipase B-catalyzed acylation of diol 1 in *n*-hexane at 50°C and the result is shown in Figure 3. Vinyl acetate displayed the highest conversion rate as an acyl donor as compared to

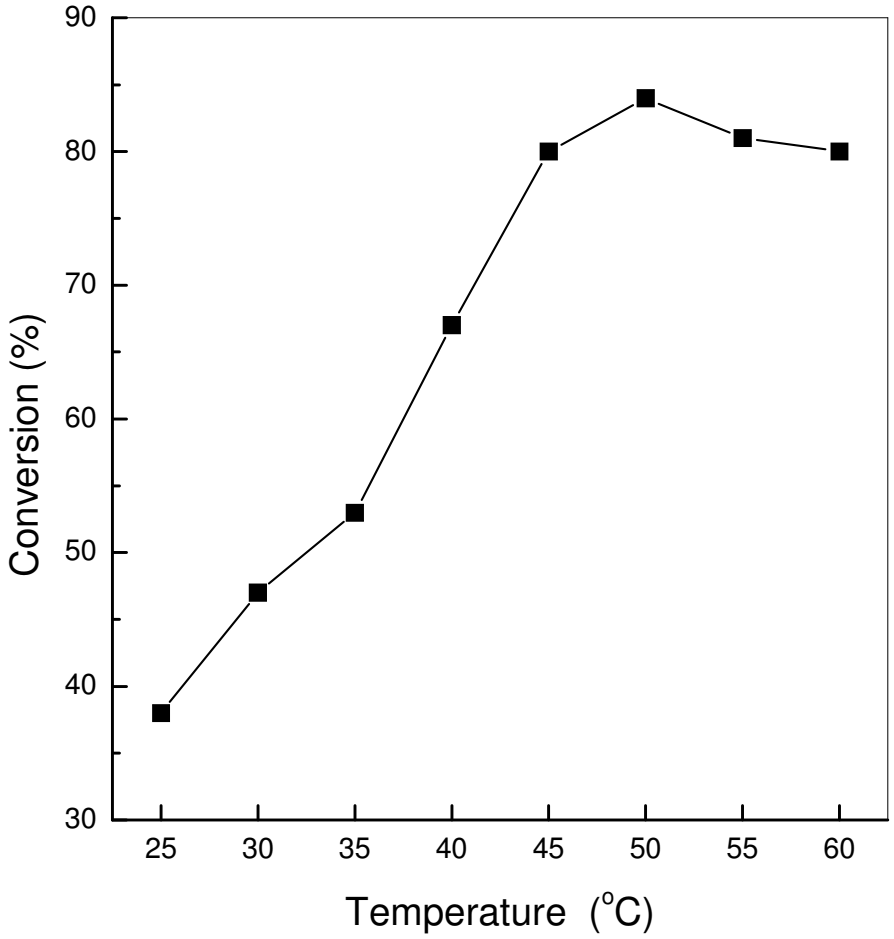

Figure 4. Effect of temperature on conversion rate. The reaction was carried out with 20 mmol/L diol 1, 60 mmol/L vinyl acetate and 5 mg/ml lipase in 5 ml n-hexane at different temperature for 90 min.

other acetates. It is explained that vinyl acetate as acyl donor makes the reaction irreversible since it converse into acetaldehyde.

Effect of reaction temperature

Temperature influences the activity, regioselectivity and stability of a biocatalyst as well as the equilibrium of a reaction (Philip, 1996). As shown in Figure 4, it was found that the conversion rate increased with the temperature rising up to 50°C. When the temperature further increased, the conversion rate dropped. It was explained that the stability of diol 1 and activity of lipase B would decrease with the temperature above 50°C. In this study, the optimal temperature was considered to be 50°C in monoacylating reaction.

Effect of rotation speed

Rotation speed influences the diffusion and partition of the substrate and the production. In this study, it was found that the initial reaction rate obviously increased with the increase of shaking speed from 50 to 200 rpm (Table 2). Little change in the initial reaction rate was observed with further increase of shaking speed above 200 rpm. Therefore, the optimal rotation speed was considered to be 200 rpm.

Effect of molar ratio of diol 1 to vinyl acetate

A study was carried out to determine the optimal ration of reactants. The molar ratio of reactant is an important parameter affecting the equilibrium of the reaction. The excess acetylating agent of vinyl acetate can shift the equilibrium in favour of synthesis. The diol 1 was fixed at 10 mmol/L, while vinyl acetate concentration was varied from 10 to 60 mmol/L. As shown in Figure 5, it was observed that the conversion rate of monoester synthesis increased with the decrease of the molar ratio of the diol 1 to vinyl acetate, and reached a maximum value at a

Table 2. The effect of rotation speed on the reaction initial rate[a].

Rotation speed (rpm min^{-1})	Initial rate (mmol L^{-1} h^{-1})
50	28.35
100	29.12
150	29.34
200	32.12
250	32.46

[a]The reaction was carried out at 50°C by adding 20 mmol/L diol 1, 60 mmol/L vinyl acetate and 5 mg lipase into 5 ml n-hexane with different rotation speed.

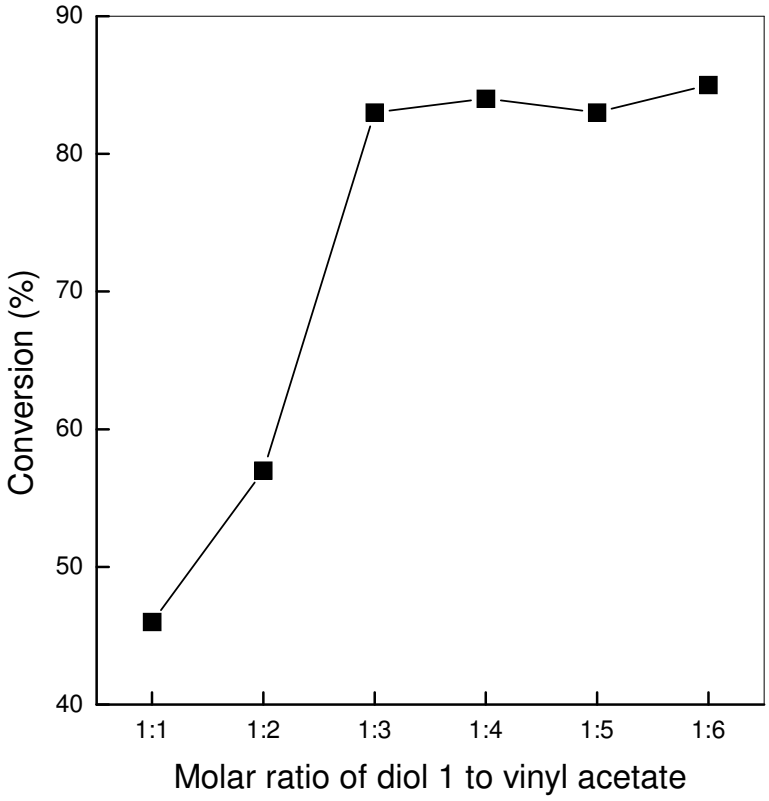

Figure 5. Effect of molar ratio of diol 1 to vinyl acetate on conversion rate. The reaction was carried out at 50°C by adding 5 mg/ml lipase into 5 ml *n*-hexane for 90 min, with different molar ratio of dio 1 to vinyl acetate.

critical molar ratio of 1:3. With further decrease of the molar ratio, the conversion rate remained steady. Therefore, molar ratio of 1:3 was considered to be the optimal ratio of reactant in this study.

Effect of substrate concentration

It is well known that acyl-transfer reactions catalyzed by lipase B from *C. antarctica*) follow bi-bi ping-pong mechanism, and the alcohol used as acyl acceptor display competitive substrate inhibition (Martinelle and Huit, 1995). High concentration of the diol 1 may inactivate the activity of lipase B, so substrate load in the organic solvent must be optimized. The substrates (diol 1) concentration was explored from 5 to 40 mmol/L, maintaining 1:3 molar ratio of the diol 1 to vinyl acetate in *n*-hexane. The effect of this parameter on initial reaction rate is shown in Figure 6. In this case, initial reaction rate first raised and then decreased with the increase of diol concentration. The initial rate reaches a maximum value when the concentration of the diol increases to 25 mmol/L. The decrease of initial reaction rate at high diol 1 concentrations could be explained thus: the free amount

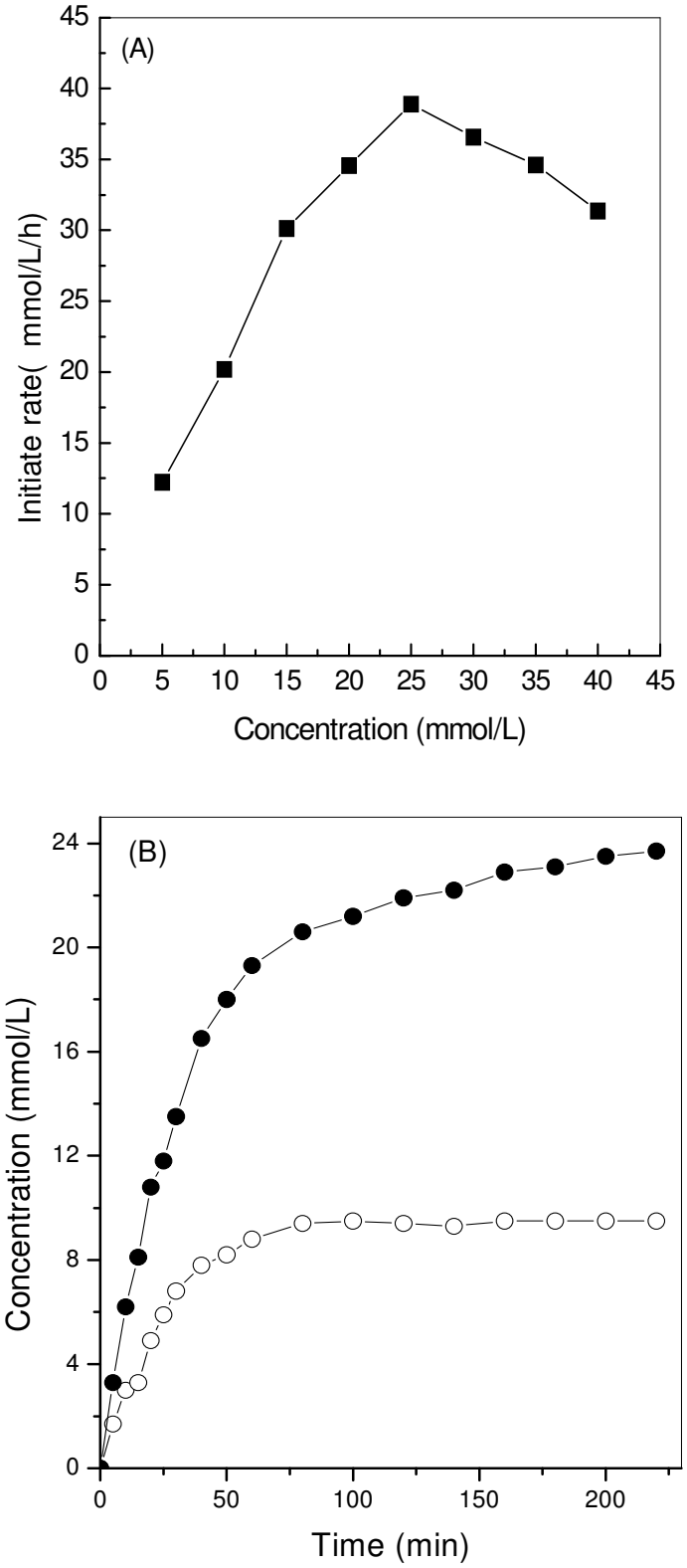

Figure 6. Time course of mono-acylation with lipase B in different substrate concentration. (A): The reaction was carried out at 50℃ with 5 mg lipase in 5 ml n-hexane for 90 min, with diol concentration varying from 5 to 45 mmol/L. (B): The reaction was carried out at 50℃, adding 25 mmol/L diol (●), 75 mmol/L vinyl acetate and 10 mmol/L (○) diol, 30 mmol/L vinyl acetate into 5 ml n-hexane.

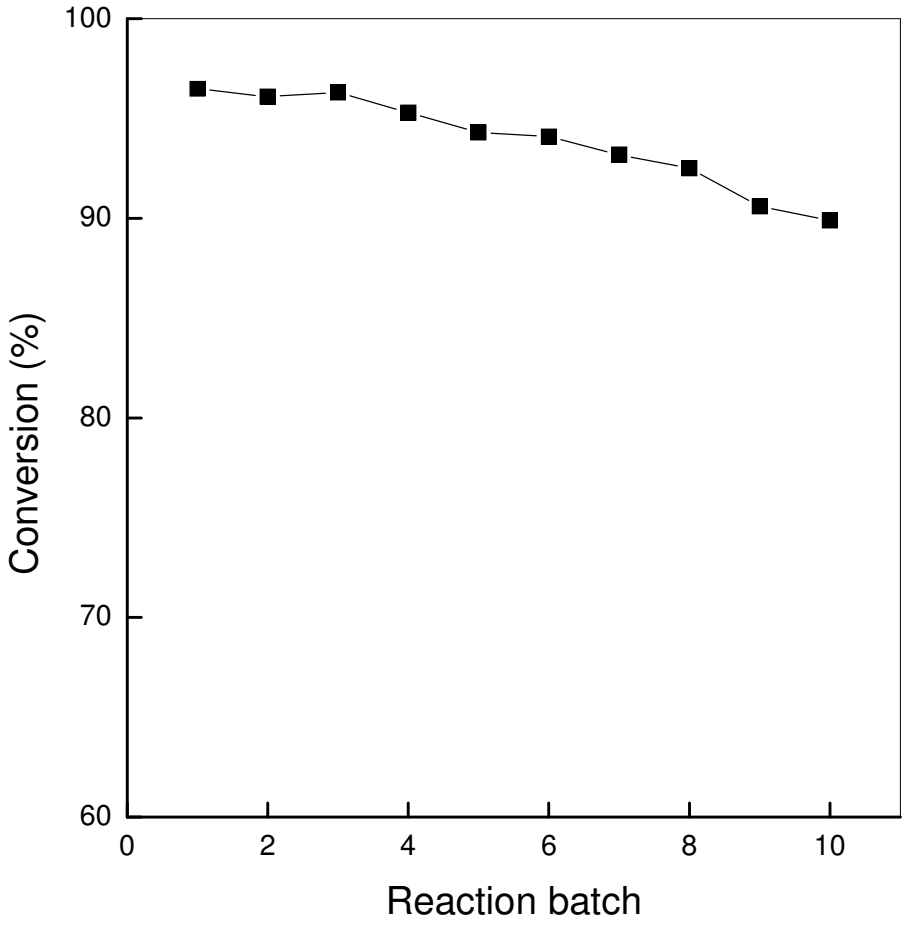

Figure 7. Effect of lipase B stability. The reaction was carried out at 50°C by adding 20 mmol/L diol 1, 60 mmol/L vinyl acetate and 5 mg lipase into 5 ml n-hexane for 90 min.

of substrate in medium caused lipase B deactivation. Terminal inhibition by alcohol for lipase B catalysis was previously reported (Chowdary et al., 2000). Under the optimum conditions, the selectivity of primary hydroxyl group of 1, 6-diol reached 99%, and the conversion rate reached 97.5% (Figure 6).

Effect of lipase stability

Reusability study was carried out to test the stability of the immobilized enzyme. Figure 7 shows that the immobilized lipase B still possessed over 90% acetylating activity, even 10 batch reaction in *n*-hexane solvent. This proved that the immobilized lipase B has good stability and could be applied for continuous reaction.

Conclusion

This work was done to develop a method for mono-acetylation of primary hydroxyl group of 1, 6-diol (vitamin A precursor) by enzymatic catalysis in *n*-hexane. The commercial lipase, Novozym 435, was very efficient in catalyzing the monoester of 1, 6-diol. Its conversion rate increased with temperature up to 50°C, which was near the boiling point of the mixture. Vinyl acetate was a better acyl donor than acetic acid since it made acylation reaction irreversible. 1:3 molar ratio of diol 1 to vinyl acetate was the optimum ratio for maximizing monoester yield. The diol 1 concentration should be limited less than 25 mmol/L because high concentration diol 1 provoked enzyme inactivation. To improve initial reaction rate, the rotation speed should increase to 200 rpm. The Novozym 435 showed high activity and stability, even 10 batch acylation reaction. Under the optimal reaction condition, the monoester conversion rate and mono-acetylation selectivity were 97.5 and 99%, respectively.

The lipase B-catalyzed acetylation of primary hydroxyl group of 1, 6-diol show good potential for production of monoester of vitamin A precursor. In consideration of environmental, technical and economical aspects of modern industrial synthesis, this process is superior to classical acetylating procedures.

REFERENCES

Akita H, Chen CY, Nagumo S (1994). A highly stereoselective synthesis of the versatile chiral synthons possessing two stereogenic centers, the formal total syntheses of (–)-oudemansins A, B, and X. Tetrahedron: Asymmetry, 5: 1207-1210.

Anthonsen T, Hoff BH (1998). Resolution of derivatives of 1,2-propanediol with lipase B from *Candida antarctica*: Effect of substrate structure, medium, water activity and acyl donor on enantiomeric ratio. Chem. Phys. Lipids, 93: 199-207.

Berger M, Laumen K, Scheider PM (1992). Lipase-catalyzed esterification of hydrophilic diols in organic solvents. Biotechnol. Lett. 14: 533-588.

Chen CS, Wu SH, Gridaukas G, Sih CJ (1987). J. Am. Chem. Soc. 109: 2812-2817.

Chowdary GV, Ramesh MN, Prapulla SG (2000). Enzymatic synthesis of isoamyl isovalerate using immobilized lipase from Rhizomucor miehi: a multivariable analysis. Process Biochem. 36: 331-339.

Hari Krishna S, Divakar S, Prapulla SG, Karanth NG (2001). Enzymatic synthesis of isoamyl acetate using immobilized lipase from *Rhizomucor miehei*. J. Biotechnol. 87: 193-201.

Hsiao KF, Yang FL, Wu SH (1996). Selective monoacetylation of diol compounds by *aspergillus niger* lipase. Biothchnol. Lett. 18: 1277-1282.

John M, Hähnlein W (1996). Ullmann's Encyclopedia of Industrial Chemistry, vol. A27, VCH, Weinheim. pp. 453-469.

Laane C, Boeren S, Vos K, Veeger C (1987). Rules for the optimization of biocatalysis in organic solvents. Biotechol. Bioeng. 30: 81-87.

Marks J (1985). The Vitamins, Their Role in Medical Practice, MTP Press Ltd. Lancaster. pp. 25-87.

Martinelle M, Hult K (1995). Kinetics of acyl transfer reactions in organic media catalysed by *Candida antarctica* lipase B. BBA-Protein Struct M. 1251: 191-197.

Molinari F, Gandolfi R, Converti A, Zilli M (2000). Mycelium-bound carboxyl esterase from *Aspergillus oryze* : an efficient catalyst for acetylation in organic solvent. Enzyme Microb. Technol. 27: 626-630.

Orsat B, Spurr P, Wirz B (2001). Enzyme acylation of a retinol derivative. EP patient EP0802261 A1.

Philips RS (1996). Temperature modulation of the stereochemistry of enzymatic catalysis: prospects for exploitation. Trends Biotechnol. 14: 13-6.

Romero MD, Calvo L, Albo C, Daneshfar A, Ghziaskar HS (2005). Enzymatic synthesis of isoamyl acetate with immobilized *Candida Antarctica* lipase in *n*-hexane. Enzyme Microb. Technol. 37: 42-48.

Torres P, Reyes-Duarte D, Lopez-Cortes N, Ferrer M, Ballesteros AJ, Plou F (2008). Acetylation of vitamin E by *Candida antarctia* lipase B immoblized on different carriers. Process Biochem. 43: 145-153.