Chemical Engineering Journal 99 (2004) 15–22

Chemical Engineering Journal

www.elsevier.com/locate/cej

Biocatalytic esterification of β-methylglucoside for synthesis of biocompatible sugar-containing vinyl esters

Jiseok Kim [a], Seungjoo Haam [a,*], Dae-Won Park [a], Ik-Sung Ahn [a], Tai Gyu Lee [a], Hae-Sung Kim [b], Woo-Sik Kim [a]

[a] *Department of Chemical Engineering, College of Engineering, Yonsei University, Seoul 120-749, South Korea*
[b] *Department of Chemical Engineering, College of Engineering, Myongji University, Kyonggi-do 449-728, South Korea*

Received 29 May 2003; accepted 15 September 2003

Abstract

Biocatalytic synthesis of β-methylglucoside methacrylate/acrylate by the esterification of β-methylglucoside with vinyl methacrylate/acrylate was carried out using an immobilized lipase from *Candida antarctica* (Novozym 435) in organic solvents. Among five model solvents including *t*-butanol, *t*-amyl alcohol, acetone, acetonitrile and 1,4-dioxane, *t*-butanol was found to be an optimal solvent. The amount of 5% (w/v) Novozym 435 resulted in the highest conversion after 48 h, and 1:3 molar ratio of β-methylglucoside to vinyl methacrylate showed 100% final conversion after 24 h. The optimal initial concentration of β-methylglucoside and temperature determined from the initial rate and final conversion were observed at 60 g/l and 50 °C, respectively. The productivity of sugar esters could be enhanced by the heterogeneous reaction mixture and the addition of β-methylglucoside after one cycle of reaction in homogeneous reaction. Based on the over 95% of cell viability by MTT assay, produced sugar esters were found to be highly biocompatible. Structural analysis of products was performed by FT-IR and ^{13}C NMR.

Keywords: Novozym 435; β-Methylglucoside; MTT assay; Vinyl methacrylate; Vinyl acrylate

1. Introduction

The use of lipase, a biocatalyst, in organic media has been increased dramatically in recent years. In general, lipase is divided into either 1,3-specific lipase with regioselectivity at 1,3-carbons or non-specific lipase without regioselectivity during the hydrolysis of triacylglyceride [1].

In particular, Novozym 435 from *Candida antarctica* is known as a non-specific lipase. It promotes the reaction between a wide range of alcohols and vinyl ester [2], and is a very heat-tolerant enzyme indicating maximum activity in the range 70–90 °C [3]. Several works have been reported on Novozym 435-catalyzed esterification in solvent-free process as well as in solvent process [4–6].

Enzymatic esterification of sugars offers an alternative to poor selectivity of chemical synthesis [7]. Sugars used in esterifications are biocompatible and biodegradable and reveal antimicrobial activity. Therefore, sugar-containing esters have been used as not only the main starting materials for industrial products of cosmetics and detergents but also the monomers of polymeric materials for biomedical and optical applications, i.e. a drug carrier and contact lens [8,9].

In general, sugars have poor solubility in most organic solvents. In our previous article, we found that *t*-butanol, which could solubilize desired amounts of sugars, was a more efficient solvent for the synthesis of β-methylglucoside ester with α-hydroxy acids than other solvents [10].

In this study, we performed optimizations of reaction parameters such as amount of enzyme, molar ratio of substrates, initial concentration of substrate and reaction temperature to increase productivity as well as to reduce reaction time. Moreover, the heterogeneous reaction system containing an excess initial concentration of β-methylglucoside, and the addition effect of β-methylglucoside after one cycle of reaction were also studied to enhance the amount of product. In vitro cytotoxicity tests of products were performed using MTT assay to verify the biocompatibility. The product, β-methylglucoside acrylate (or methacrylate), was characterized by structural analyses using FT-IR and ^{13}C NMR.

* Corresponding author. Present address: 134 Shinchon-dong, Seodaemoon-ku, Seoul 120-749, South Korea. Tel.: +82-2-2123-2751; fax: +82-2-312-6401.
E-mail address: haam@yonsei.ac.kr (S. Haam).

1385-8947/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.cej.2003.09.001

2. Experimental

2.1. Materials

Novozym 435 (Lipase B from *C. antarctica*, a non-specific lipase immobilized on a macroporous acrylic resin, 10,000 PLU (propyl laurate units)/g) was purchased from Novo Nordisk A/S (Bagsvaerd, Denmark). β-Methylglucoside (β-MG) and molecular sieves (3 Å, powder form) were purchased from Sigma (St. Louis, MO). Vinyl acrylate (VA) and vinyl methacrylate (VMA) were purchased from TCI (Japan). *t*-Butanol and *t*-amyl alcohol were purchased from Fluka (S^{t}Quentin-Fallavier, Switzerland). Acetone, acetonitrile, and 1,4-dioxane were supplied by Junsei (Japan). All other chemicals were of analytical grade, and the solvents were dried with molecular sieves prior to use for 24 h.

2.2. Enzymatic esterification

Effect of solvents: β-MG and VMA, which were prepared in molar ratio of 1:2, were added in 20 ml screw capped test tubes containing 10 ml reaction media (acetone, acetonitrile, *t*-amyl alcohol, *t*-butanol, and 1,4-dioxane) and 1% (w/v) of Novozym 435. The media was stirred magnetically, and the reactions were performed at 50 °C for 24 h.

Effect of enzyme content: The molar ratio of β-MG and VMA was 1:2. Various concentrations, 1, 3, and 5% (w/v), of Novozym 435 were added in 10 ml of a selected optimal solvent to start esterifications at 50 °C.

Effect of molar ratio: The amount of Novozym 435 was kept constant at 5% (w/v). The molar ratios of β-MG to VMA were 1:1, 1:2 and 1:3. Reactions were performed in 10 ml of a selected optimal solvent at 50 °C.

Effect of reaction temperature: The amount of Novozym 435, and the molar ratio of β-MG and VMA were 5% (w/v) and 1:3, respectively. The reaction temperatures were 45, 50, 55, and 60 °C.

Effect of initial concentration of β-MG: The amount of Novozym 435, and the molar ratio of β-MG and VMA, and reaction temperature were 5% (w/v), 1:3, and 50 °C, respectively. The initial concentrations of β-MG were 30, 40, 50, and 60 g/l.

Synthesis of β-methylglucoside acrylate (MGA) and β-methylglucoside methacrylate (MGMA) at optimal conditions: The initial concentration of β-MG, the molar ratio of β-MG to VMA (or VA), the amount of enzyme and temperature were 60 g/l, 1:3, 5% (w/v), and 50 °C, respectively.

Enhancement of productivity in homogeneous and heterogeneous reaction mixtures: In homogeneous reaction mixture, after one cycle of reaction, 60 g/l of β-MG was added in reactor, and there was no addition of VA and VMA.

In addition, the heterogeneous reaction system in which β-MG remained as a powder form in a solvent was also studied for higher productivity using 100 and 150 g/l of excess initial concentrations of β-MG with the 1:3 molar ratio of β-MG to VA (or VMA).

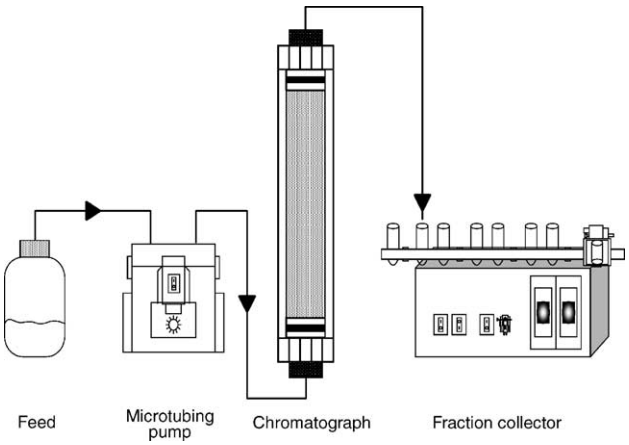

Fig. 1. Schematic representation of a liquid chromatographic setup.

2.3. Purification

After the reaction, the mixture was filtered to remove enzyme and dried under reduced pressure at 40 °C. Equivolume of chloroform was added to the reaction mixture. The mixture was mixed vigorously for 2 h and filtered to eliminate β-MG remaining as a solid phase in chloroform. Then the filtered chloroform was evaporated at 40 °C under reduced pressure.

To separate the reaction mixture including β-MG, MGA and MGMA, liquid chromatography was used with carbohydrate column (Nova-Pak, 4.6 mm × 250 mm) and auto fraction collector with 30 s of time interval (Fig. 1). Reaction mixture was dried to remove solvent under reduced pressure and dissolved in methanol (50%, v/v). An acetonitrile/methanol/water mixture (50:30:20, v/v) was used as the mobile phase. The flow rate was 0.5 ml/min, and 0.5 ml volume of the sample was injected into the chromatograph.

2.4. Analysis

Enzymatic reactions were monitored by analysis of the conversion rate of β-MG using HPLC (Waters) consisting of 600E isocratic pump, a carbohydrate column (Nova-Pak, 4.6 mm × 250 mm), a R401 refractive index detector, and a M746 integrator. The injection volume was 20 μl and the column temperature was kept at 35 °C. The eluent used for quantification of β-MG was acetonitrile/water (85:15, v/v). The retention time for β-MG was about 5.1 min at a flow rate of 1.2 ml/min. Results are expressed as the mean value of at least two independent measurements, and β-MG was used as an inner standard for calibrations. For the measurement of MGA/MGMA, C18 column (Bondapak, 250 mm × 4.6 mm, Waters) was used with acetonitrile/methanol/water (50:45:5) as a mobile phase, and the same detector described above at a flow rate of 1 ml/min.

FT-IR (Tensor 27, Bruker) analysis of MGA and MGMA was performed to observe the presence of specific groups of ester. FT-IR spectra were obtained at 4 cm^{-1} resolution with 32 scans. The spectrometer was equipped with 6 mm

DTGS detector. The solid samples used for the FT-IR studies were compression-molded with KBr powders and the liquid sample was spread over the KBr window.

^{13}C NMR spectra were recorded on a Varian 300 MHz instrument with D_2O and $CDCl_3$ as the solvent.

2.5. Estimation of enzyme activity

Enzyme activity was estimated by our method to fit to this work, and the conditions were as follows: 50 g/l of initial concentration of β-MG, 1:1 molar ratio of β-MG to VMA (or VA), 0.5 g of enzyme, 10 ml of *t*-butanol, and 50 °C. One unit was defined based on the produced gram sugar esters for 30 min. Results are expressed as the mean value of at least three independent measurements.

2.6. Cytotoxicity test

In vitro cytotoxicity tests were carried out by 3-(4,5-dimethylthiazol-2-y)-2,5-diphenyltetrazolium bromide (MTT) assay.

Cell culture: Mouse fibroblast cell line NIH3T3 (ATCC, Manassas, VA) was used for cytotoxicity test. The cells were cultured in indicator-free Dulbecco's modified Eagle's medium (DMEM) (Sigma Chemical Co., St. Louis, MO) supplemented with 10% fetal bovine serum (Gibco, Gaithersburg, MD), Fungizone (Gibco), penicillin/streptomycin/neomycin (PSN) antibiotic mixture (Gibco), and 25 mM HEPES buffer (Sigma, St. Louis, MO) at 37 °C for 1 day under 5% CO_2 atmosphere. After incubation, trypsin (Gibco) was used to harvest the cells. Then NIH3T3 cells were seeded in 96-well plates at a density of 1×10^4 cells per well, and then incubated at 37 °C for 1 day under 5% CO_2 atmosphere. After incubation, the cells were recovered, and replaced on fresh plates containing MGA or MGMA solutions prepared with 5 g/l of concentration. After 1 week of incubation, cell viability was determined by MTT assay.

MTT assay: This technique is a measure of the activity of cellular mitochondria and has been described as a quantitative technique. A stock solution of MTT was made with phosphate-buffered saline (PBS) at a concentration of 5 mg/ml. Sterile aliquots of this solution at a ratio of 20 μl/200 μl of media were added to wells. The plates were then incubated for 4 h at 37 °C. After removing the media, 100 μl of DMSO was added to each well to lyse the cells and dissolve the dye. The samples were then placed into cuvettes, and absorbances were measured on a Hewlett Packard spectrophotometer at a wavelength of 570 nm.

3. Results and discussion

3.1. Effect of solvents

The solvent in reactions is a key factor to determine the enzymatic activity. Table 1 indicates that the esterification of β-MG with VMA showed higher conversion in more hydrophobic solvents, which have a higher log *P* value. Although there was no linear dependence of conversion on log *P* values, the use of solvents with positive log *P* values (0.80 and 1.30) resulted in two to five times higher conversion than that of solvents with negative log *P* values (−1.10 and −0.33). Especially, no reaction was observed in acetone; the conversion of β-MG after 12 h was 0%. These results were good indication that the hydrophobic solvents rather than hydrophilic ones were more favorable to the enzymatic esterification of β-MG with VMA. Among five solvents, *t*-butanol, a non-denaturing solvent for lipases [11], showed the highest conversion after 12 h, and hence was found to be the most efficient for the esterification of β-MG with VMA. The polarity of the organic solvents employed for the esterification reaction is known to affect the enzyme activity [12]. The value of log *P*, a partition coefficient of solvents between water and octanol, was known as not only the widely used parameter to describe solvent polarity but also a good indicator for enzymatic synthesis [13]. In general, solvents with lower log *P* values results in unfavorable effect on the biocatalytic activity due to their hydrophilic characteristic [14]. The use of solvents with log *P* value over 3.5 was successful for the esterification of primary terpene alcohol with vinyl esters [15]. Even the addition of more hydrophobic solvents to the original solvent to enhance the reactivity was reported [16]. The present study also showed that the highest conversion was observed at *t*-butanol with relatively higher value of log *P*.

Table 1
Esterification of β-MG with VMA in various organic solvents

Solvent	log *P*	Conversion (%) of β-MG
1,4-Dioxane	−1.10	10.68
Acetonitrile	−0.33	7.77
Acetone	−0.23	0
t-Butanol	0.80	37.9
t-Amyl alcohol	1.30	26.3

3.2. Effect of enzyme content

Although enzymes have regioselectivity to simplify reaction steps, which makes biocatalytic processes more competitive than chemical processes, determination of the amount of enzyme is important for shorter reaction time as well as higher yields. Fig. 2 shows the conversion profiles of β-MG at predetermined time intervals. The final conversion as well as the initial rate was increased with the amount of enzyme. The final conversions at each amount of enzyme, 1, 3, and 5% (w/v), after 48 h were 78, 92, and 98%, respectively. The amount of enzyme over 5% (w/v) was not used since the final conversion at 5% (w/v) reached about 100%. Therefore, 5% (w/v) of enzyme was chosen as an optimal amount for the esterification of β-MG with VMA.

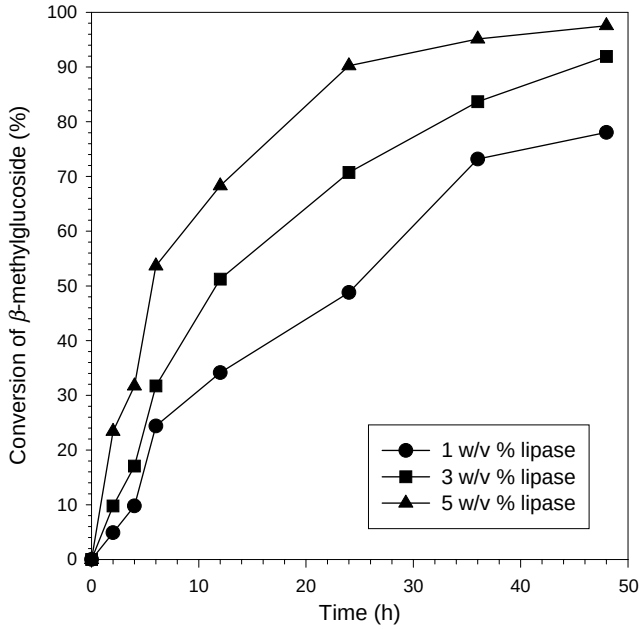

Fig. 2. Effect of enzyme concentrations on the esterification of β-MG with VMA.

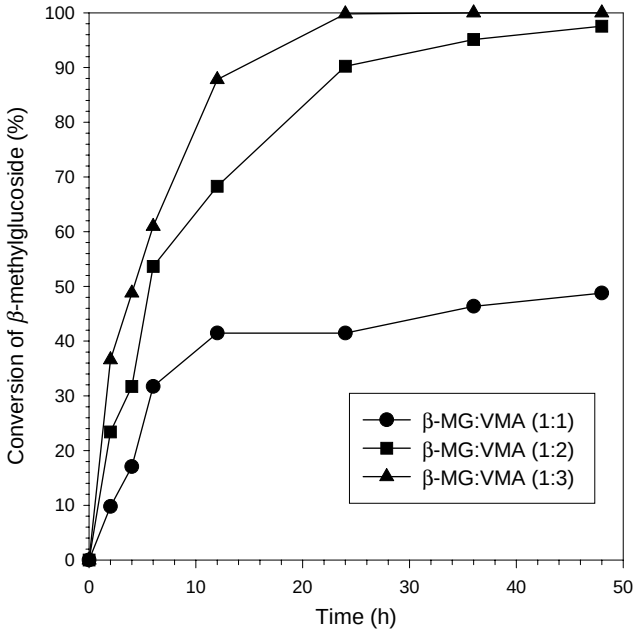

Fig. 3. Effect of molar ratios on the esterification of β-MG with VMA.

3.3. Effect of molar ratio

In order to achieve the highest product yield and the lowest side reaction, the molar ratio of substrates should be determined. The amounts of Novozym 435 and β-MG were held constant, whereas the quantity of VMA was varied to show the effect of the amount of acyl donor on the reaction rate. In the presence of excess amounts of VMA, the highest conversion, 100%, of β-MG was obtained at a 1:3 molar ratio of β-MG to VMA (Fig. 3). At the molar ratio of 1:1, however, a slight increase in the conversion of β-MG was observed after 12 h. Moreover, the final conversion was over two times lower than that of 1:2 and 1:3. Therefore, the use of equimolar ratio was found to be unfavorable to the esterification of β-MG. The use of excess amounts of acyl donor was reported to enhance the product yield. The study by Carlos et al. [17] found that 1:8 of molar ratio of octyl-β-D-glucopyranoside to ethyl lactate led to the highest conversion (75%) of octyl-β-D-glucopyranoside for the synthesis of lactic acid derivatives. Moreover, Thierry et al. [18] reported that the most advantageous molar ratio of sorbitol to methyl salicylate for the synthesis of water-soluble salicylic acid derivatives was 1:10. However, we found that β-MG was completely conversed to the MGMA with the only 1:3 of molar ratio of β-MG to VMA. Moreover, the conversion of over 95% could be obtained even with 1:2 molar ratio. Increased molar ratio may have promoted a shift of the reaction equilibrium toward the ester formation. However, the higher the molar ratio, the higher the residual concentration of VMA, and it may lead to multiple steps for ester purification.

3.4. Effect of reaction temperature

The reaction temperature has a great influence on the rate of esterification. The elevated temperature is one of the most common causes for the inactivation of enzymes, which results in the loss of the catalytically competent conformation. The effect of temperature on sugar ester formation from β-MG by Novozym 435 is shown in Fig. 4. The increase in the conversion of β-MG within 10 h was observed when

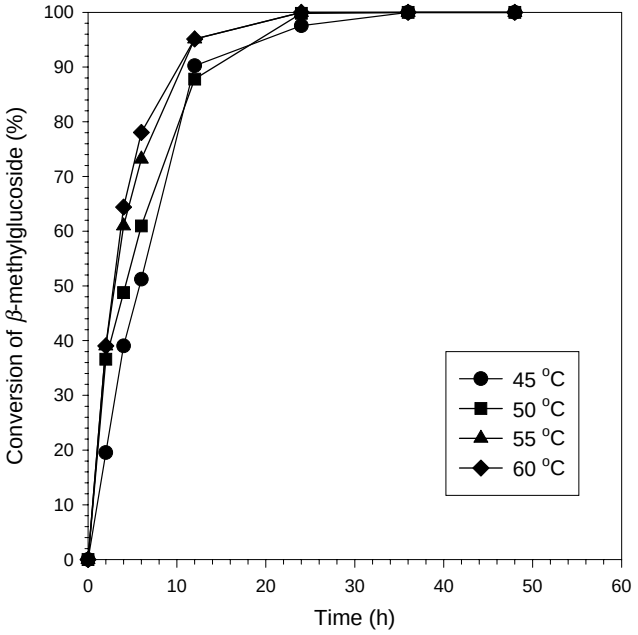

Fig. 4. Effect of reaction temperature on the esterification of β-MG with VMA.

reaction temperature was increased, and then reached 100% after 24 h except at 45 °C. No significant decrease in enzymatic activity of Novozym 435 was observed in this range of temperature, 45–60 °C. The initial rates at 45, 50, 55, and 60 °C were 343, 631, 672, and 672 μmol/l min, respectively. The relatively low reaction rate at 45 °C was probably due to the existence of acetaldehyde (CH_3CHO) which was not evaporated instantly at lower temperature. The esterification of β-MG with VMA is the reaction that produces another ester and vinyl alcohol as a by-product, and it can be represented as follows:

$$\mathrm{RCOOR'} + \mathrm{R''OH} \rightarrow \mathrm{RCOOR''} + \mathrm{R'OH} \uparrow \quad (1)$$

where RCOOR′, R″OH, RCOOR″, and R′OH are VMA, β-MG, β-MG ester, and vinyl alcohol (CH_2=CHOH), respectively. During the reaction, the vinyl alcohol leaving group tautomerized to acetaldehyde, and hence led to the irreversible reaction [15]. Although acetaldehyde is known to deactivate many enzymes [19], it did not seem to affect the reaction because of the instant evaporation of acetaldehyde over 50 °C. Although a higher temperature was required for the instant evaporation of acetaldehyde during the reaction, the upper limit of temperature was fixed at 60 °C to avoid denaturation of enzyme as well as to protect browning reaction of β-MG. Under the same conversion, a lower reaction temperature may not only lead to the easy operation of the process but also protect the caramelization of β-MG. Therefore, all subsequent experiments were performed at 50 °C based on 100% conversion and relatively fast initial rate.

3.5. Effect of initial concentration of β-MG

The initial concentration in reactions is one of the key factors to determine the reaction rate as well as product yields. With the goal of increasing the reaction rate and yield in *n*-hexane without adverse effects, Carlos and Cristina [20] reported the effects of using large amounts of reactants. According to Krishna et al. [21], increase in substrate concentration was found to dramatically decrease the esterification for the synthesis of isoamyl acetate. We found that the homogeneous phase could be obtained with the reaction mixture including β-MG up to 60 g/l. In addition, we could achieve complete conversion of β-MG regardless of its concentration. Fig. 5 shows that the order of initial conversions within 2 h is 40 > 50 > 60 > 30 g/l. However, 100% final conversion was obtained at all initial concentrations after 36 h, and no significant decrease in enzyme activity was observed. In order to determine the initial concentration of substrates, both the final conversion and the initial rate should be considered simultaneously. The initial rates at 30, 40, 50 and 60 g/l were 206, 618, 708 and 824 μmol/l min, respectively. From the results of the highest initial rate and 100% conversion after 24 h, 60 g/l of initial concentration of β-MG was found to be an optimal initial concentration in homogeneous phase of reactants.

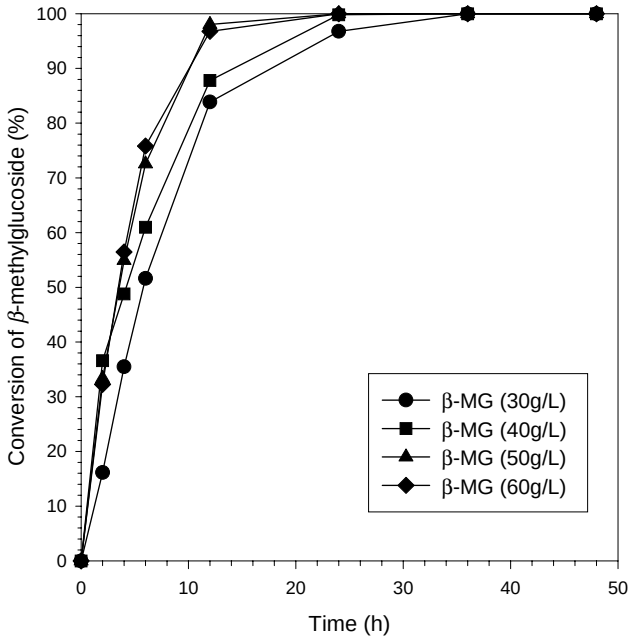

Fig. 5. Effect of initial concentration on the esterification of β-MG with VMA.

3.6. Synthesis of MGA and MGMA at optimal conditions

The type of the acyl donors plays an important role in the reaction kinetics when enzymes are used as biocatalyst. In our study, two acyl donors, VA and VMA, were compared. Fig. 6 shows the reaction profiles for the esterification of β-MG with VA and VMA at optimal conditions; 60 g/l of β-MG, 1:3 molar ratio of β-MG to VMA (or VA), 5% (w/v)

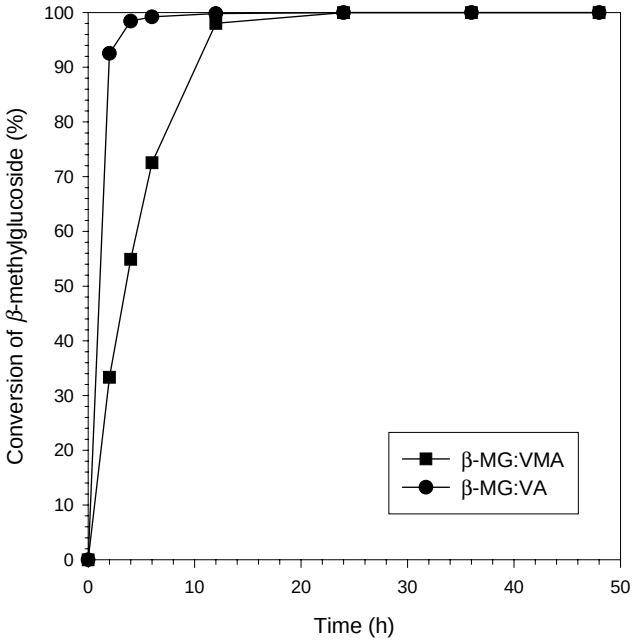

Fig. 6. Comparison of esterification between β-MG with VA and β-MG with VMA at optimal conditions.

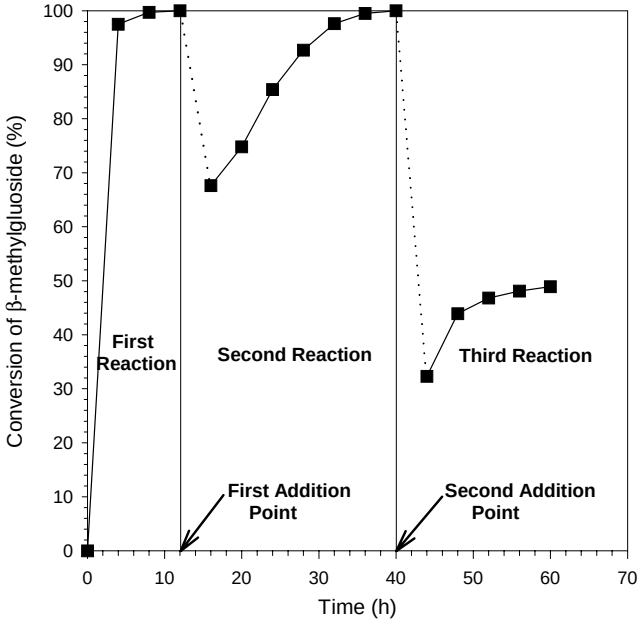

Fig. 7. The addition effect of β-MG on the esterification with VA.

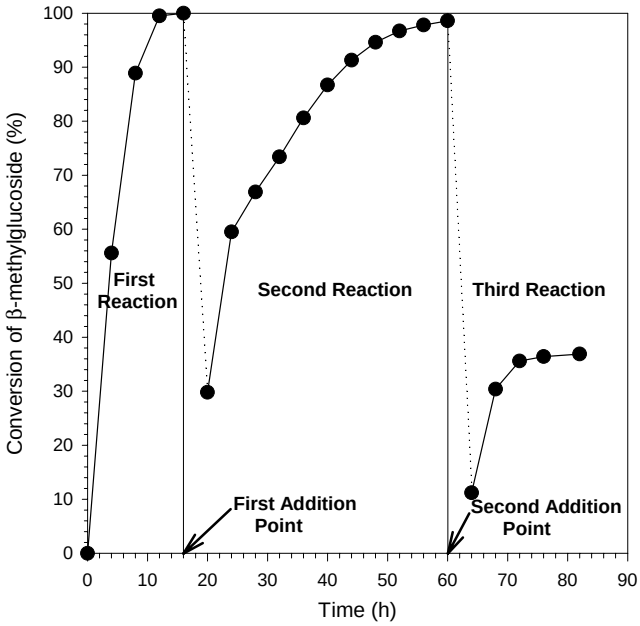

Fig. 8. The addition effect of β-MG on the esterification with VMA.

of Novozym 435, and 50 °C. The conversions of β-MG with both VA and VMA after 24 h reached 100%. However, much faster initial rate was observed at the esterification between β-MG and VA: 2368 μmol/l min for esterification with VA and 824 μmol/l min with VMA. This observation explained well that optimal conditions, which were determined on the basis of the esterification between β-MG and VMA, would be efficient for the esterification between β-MG and VA as well.

3.7. Enhancement of productivity in homogeneous and heterogeneous reaction mixture

Figs. 7 and 8 show the addition effect of β-MG on the esterification with VA and VMA, respectively, in homogeneous reaction mixture. In both cases, the addition of β-MG after one cycle of reaction resulted in the further consumption of reactant. A longer period of time was needed to reach equilibrium in the second reaction, and the significant decrease in the conversion was observed in the third reaction. The prolonged reaction time in the second reaction and lower conversion rate in the third reaction might result from the decrease in the molar ratio of β-MG to vinyl esters, since there was no addition of vinyl esters. Moreover, according to the enzyme activity before (2.90 U for β-MG with VA, 1.76 U for β-MG with VMA) the first reaction, and after (2.72 U for β-MG with VA, 1.70 U for β-MG with VMA) the third reaction, no significant decrease in the enzyme activity was observed. Therefore, the decrease in the molar ratio was found to be the main factor of the reaction behavior in the second and the third reaction. Additional experiments were performed to test the production of larger amounts of MGA and MGMA in heterogeneous reaction mixture using excess initial concentration of β-MG, which remained as a solid phase early in the reaction. Although the initial rate of conversion at 100 g/l of β-MG was higher than that at 150 g/l, both 100 and 150 g/l of β-MG were completely converted to MGA and MGMA after 36 h (Fig. 9), and the initial rates at 100 and 150 g/l of β-MG were 1239 and 1728 μmol/l min with VA, 644 and 837 μmol/l min with VMA, respectively. Consequently, the excess amount of β-MG was found to be applicable for the formation of larger amount of MGA and

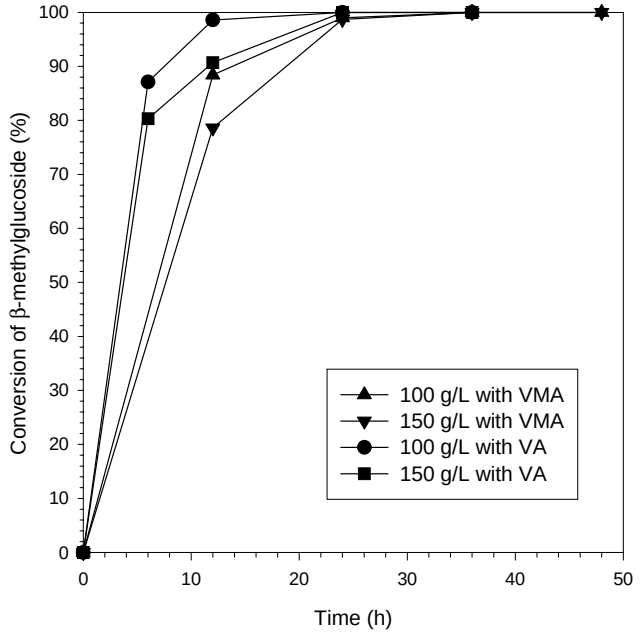

Fig. 9. Effect of excess initial concentration of β-MG on the esterification with VA and VMA.

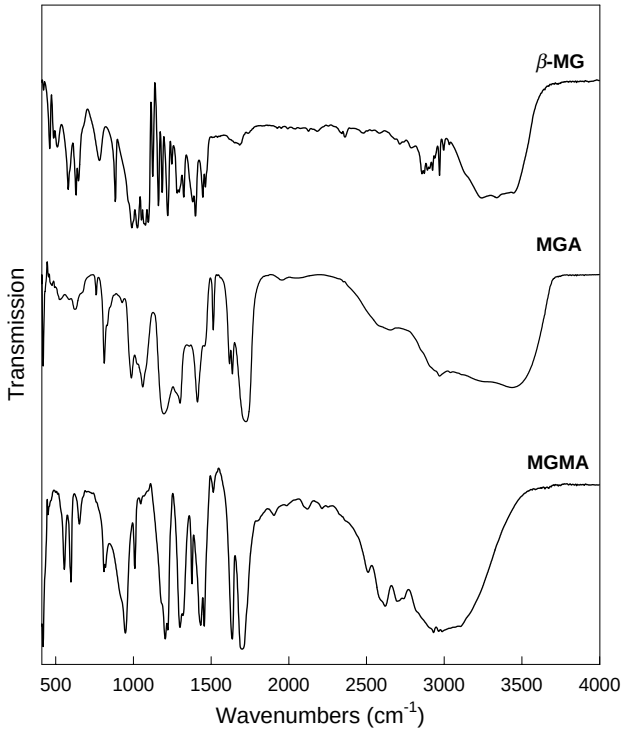

Fig. 10. FT-IR spectra of β-MG, MGA and MGMA.

MGMA even though it remained as solid phase in early period of reaction, and 150 g/l was considered to be more efficient based on the initial rate and 100% final conversion.

3.8. Structural analysis

3.8.1. FT-IR

FT-IR spectra of β-MG, MGA and MGMA are shown in Fig. 10. The successful incorporation of the acrylate/methacrylate group into β-MG was demonstrated by the presence of ester FT-IR band (1724 cm^{-1} for MGA, 1698 cm^{-1} for MGMA). The presence of pendant vinyl groups in MGA/MGMA was confirmed by the FT-IR bands at 1637/1636 cm^{-1} (C=C) and 812/810 cm^{-1} (C=C–H). The band for C–O stretching of secondary alcohols appeared at 1200–1300 cm^{-1}, and O–H stretching gave a strong as well as a broad band at 2800–3500 cm^{-1}. These were due to the presence of β-MG moiety.

3.8.2. ^{13}C NMR

Carbon 13 nuclear magnetic resonance (^{13}C NMR) spectrum of MGA and MGMA (Fig. 11) was recorded using a Varian 300 MHz spectrometer. ^{13}C NMR/$CDCl_3$ (δ in ppm) for MGA: 59.90 (OCH_3, 1), 63.79 (C-7), 69.70 (C-6), 73.17 (C-4), 75.82 (C-5), 75.99 (C-3), 103.31 (C-2), 128.13 (–C=CH_2, 9), 131.76 (–C=CH_2, 10), 166.34 (C=O, 8). ^{13}C NMR/$CDCl_3$ (δ in ppm) for MGMA: 18.31 (–CH_3, 9), 56.96 (OCH_3, 1), 64.37 (C-7), 70.58 (C-6), 73.37 (C-4), 73.75 (C-5), 76.32 (C-3), 103.40 (C-2), 126.46 (–C=CH_2, 10), 135.96 (–C=CH_2, 11), 167.81 (C=O, 8).

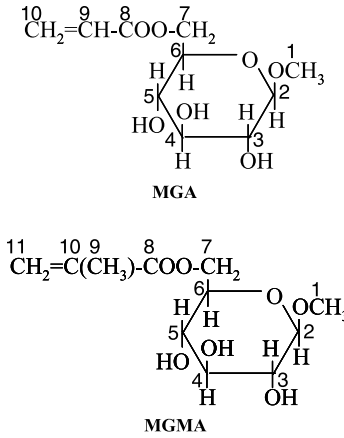

Fig. 11. Structures and carbon number of MGA and MGMA.

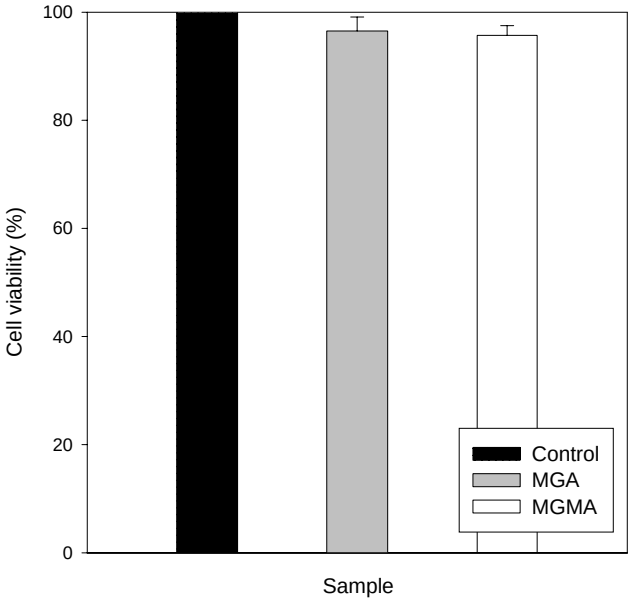

Fig. 12. Viability of cells incubated with MGA and MGMA.

3.9. Cytotoxicity test

In order to verify the biocompatibility of produced sugar esters, we introduced MTT assay to examine the cell viability. Fig. 12 shows the cell viability after 1 week of incubation with MGA and MGMA. In both incubations, over 95% of cells could survive under the sugar ester environment. Based on this result, we found that produced sugar esters were highly biocompatible. Their biocompatibility would be expected to play an important role when used as monomers of polymeric materials for biomedical applications, especially drug carriers and wound dressing agents.

4. Conclusion

The synthesis of sugar-containing vinyl esters was carried out by the esterification of β-MG with VA and VMA

catalyzed by Novozym 435. Solvents with a higher log *P* resulted in better reaction rate, and *t*-butanol was determined as an optimal solvent for all subsequent experiments. Hundred percent conversion of β-MG could be obtained at 1:3 molar ratio, 5% (w/v) Novozym 435, 60 g/l of β-MG, and 50 °C. The addition of β-MG after one cycle of reaction in homogeneous reaction mixture, and the use of excess initial concentration of β-MG in heterogeneous reaction mixture led to the enhancement of productivity. By in vitro cytotoxicity tests, highly biocompatible property of both MGA and MGMA was verified.

Acknowledgements

This work was supported by Advanced Environmental Biology Research Center (R11-2003-006).

References

[1] B. Atkinson, F. Mavituna, Biochemical Engineering and Biotechnology Handbook, 2nd ed., Stockton Press, New York, 1991.
[2] C. Vitro, P. Adlercreutz, Lysophosphatidylcholine synthesis with *Candida antarctica* lipase B (Novozym 435), Enzyme Microb. Technol. 26 (2000) 630–635.
[3] P.W. Owen, F. Jianwen, L. Zuyi, Lipase-catalyzed synthesis of a sugar ester containing arachidonic acid, Enzyme Microb. Technol. 20 (1997) 52–56.
[4] M.P. Bousquet, R.M. Willemot, P. Monsan, E. Boures, Enzymatic synthesis of α-butylglucoside lactate: a new α-hydroxy acid derivative, Biotechnol. Bioeng. 62 (1999) 225–234.
[5] M.P. Bousquet, R.M. Willemot, P. Monsan, E. Boures, Enzymatic synthesis of unsaturated fatty acid glucoside esters for dermocosmetic applications, Biotechnol. Bioeng. 63 (1999) 730–736.
[6] T. Carlos, O. Cristina, Enzymatic synthesis of lactate and glycolate esters of fatty alcohols, Part I, Enzyme Microb. Technol. 25 (1999) 745–752.
[7] V. Dossat, D. Combes, A. Marty, Lipase-catalyzed transesterification of high oleic sunflower oil, Enzyme Microb. Technol. 30 (2002) 90–94.
[8] X. Chen, B.D. Martin, T.K. Neubauer, R.F. Linhardt, J.S. Dordick, D.G. Rethwisch, Enzymatic and chemoenzymatic approaches to synthesis of sugar-based polymer and hydrogel, Carbohyd. Polym. 28 (1995) 15–21.
[9] N.S. Patil, J.S. Dordick, D.G. Rethwisch, Macroporous poly(sugar acrylate) hydrogel for controlled release of macromolecules, Biomaterials 17 (1996) 2343–2350.
[10] D.W. Park, J.S. Kim, S. Haam, H.S. Kim, W.S. Kim, Lipase-catalyzed synthesis of β-methylglucoside esters containing an α-hydroxy acid, Biotechnol. Lett. 23 (2001) 1947–1952.
[11] L. Cao, U.T. Bornscheuer, R.D. Schmid, Lipase-catalyzed solid-phase synthesis of sugar esters. Influence of immobilization on productivity and stability of the enzyme, J. Mol. Catal. B: Enzymatic 6 (1999) 279–285.
[12] C. Laane, Boeren, K. Vos, C. Veeger, Rules for optimization of biocatalysis in organic solvent, Biotechnol. Bioeng. 30 (1987) 81–87.
[13] A.R.M. Yahya, W.A. Anderson, M. Moo-Young, Ester synthesis in lipase-catalyzed reactions, Enzyme Microb. Technol. 23 (1998) 438–450.
[14] P. Degn, W. Zimmermann, Optimization of carbohydrate fatty acid ester synthesis in organic media by a lipase from *Candida antarctica*, Biotechnol. Bioeng. 74 (2001) 483–491.
[15] C.A. Casimir, N.Y. Lisa, Lipase-catalyzed transesterification of primary terpene alcohols with vinyl esters in organic media, J. Mol. Catal. B: Enzymatic 4 (1998) 149–153.
[16] C. Sophie, J.T. Russell, S.C. Jean, M. Alain, Water activity control: a way to improve the efficiency of continuous lipase esterification, Biotechnol. Bioeng. 60 (1998) 362–368.
[17] T. Carlos, B. Manuel, O. Cristina, Enzymatic synthesis of lactic acid derivatives with emulsifying properties, Biotechnol. Lett. 22 (2000) 331–334.
[18] M. Thierry, B. Melanie, R. Barbara, D.L. Marie, Enzymatic synthesis of water-soluble derivatives of salicylic acid in organic media, Biotechnol. Lett. 23 (2001) 989–993.
[19] B. Berger, K. Faber, Immunization of lipase against acetaldehyde emerging in acyl transfer reaction from vinyl acetate, J. Chem. Soc., Chem. Commun. 17 (1991) 1200–1998.
[20] T. Carlos, O. Cristina, Direct enzymatic esterification of lactic acid with fatty acids, Part III, Enzyme Microb. Technol. 29 (2001) 3–12.
[21] S.H. Krishna, S. Divakar, S.G. Prapulla, N.G. Karanth, Enzymatic synthesis of isoamyl acetate using immobilized lipase from *Rhizomucor miehei*, J. Biotechnol. 87 (2001) 193–201.