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Enzymatic synthesis of ethyl glucoside lactate in non-aqueous system

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Abstract

Ethyl glucoside lactate, a novel α-hydroxy acid derivative, was synthesized by transesterification in non-aqueous phase using immobilized lipase as biocatalyst. Parameters such as solvent type, substrate concentration, reaction temperature, and enzyme concentration were investigated to optimize the lipase-catalyzed transesterification. In solvent-free system with butyl lactate as both acyl donor and solvent, a 71% conversion was achieved. In order to investigate the effect of initial water content, the reactions were carried out in the mediums treated with molecular sieves. The results showed that conversion and initial rate decreased with the increase of water content. The conversion and initial rate reached to 95% and 67.4 mM/h, respectively, by carrying out the reaction under reduced pressure, which was employed to eliminate butanol and the initial water.

Keywords: Ethyl glucoside; α-Hydroxy acid; Lipase; Transesterification; Conversion

1. Introduction

α-Hydroxy acids (AHAs) constitute a class of organic acids generally extracted from fruit and sugarcane. The AHAs are used in cosmetics to reduce fine winkles and eliminate keratosis and chloasma [1,2]. AHAs with short chain such as lactic acid are far more active in regulating the rate of regeneration of the stratum corneum and improving skin dryness [3]. However, AHAs can penetrate the stratum corneum quickly (<1 min) and reach a peak concentration in the viable epidermis, bringing out irritant effects on the skin for its low pH value (pH <2) [4,5]. Moreover, AHAs can also stimulate the sensory nerves and make the skin more sensitive to the sun [6].

To overcome these drawbacks, AHAs could be grafted to amphiphilic molecules including alkyl glucosides that contain several reactive hydroxyl groups, which to some extent neutralizes the AHAs [7]. As ester bond can be easily hydrolyzed by epiderm esterases, alkyl glucoside lactate becomes a controlled release form of free lactic acid. Ethyl glucoside itself also has a function of sun protection and compromises of the side-effect of lactic acid [8]. Consequently, ethyl glucoside lactate might be a product of interest in cosmetic industry.

The glycoside lactate can be synthesized by transesterification of ethyl glucoside and lactate in non-aqueous phase using immobilized lipase as biocatalyst [9]. Lipases have been shown to remain active in predominately organic reaction systems containing

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very low levels of water [9,10]. The thermodynamic equilibrium of the reactions is shifted towards product synthesis of transesterification instead of transesterification. Many studies also suggest that bulk water is not crucial for enzymatic hydration in nearly anhydrous organic solvents, whereas, tightly bound structure water does play an important role in catalysis [11,12]. An excess of water can decrease the enzyme activity and favor the hydrolysis of the ester product. As transesterification is reversible, the by-product is thermodynamically unfavorable to ester synthesis. It is necessary to remove it from the medium to achieve a high conversion.

In this paper, factors including solvent, substrate concentration, enzyme concentration and reaction temperature under atmospheric pressure are investigated to evaluate their effect on initial rate and conversion of ethyl glucoside lactate. Furthermore, effects of initial water content and the butanol were investigated. The reaction under reduced pressure was carried out to examine the change of reaction conversion, which could shift the equilibrium towards the synthesis of the new product.

2. Materials and methods

2.1. Materials

Novozym® 435 (type B lipase from *Candida Antarctica* absorbed on an acrylic resin), having an activity of 7000 propyl laurate unit per gram (PLU/g), was gift from Novo Nordisk Industries (Denmark). Molecular sieves (4 Å) were purchased from Shanghai Hengye Company (China). Lactic acid, ethyllactate, buthyllactate, hexane, heptane, 2-methyl-2-butanol were purchased from Shanghai Chemical Company (China). All other chemicals used in this work were of analytical grade. Elution solvents used in chromatography were of HPLC grade.

Ethyl glucoside was produced and purified as described by Tu and Wei [13].

2.2. Methods

2.2.1. Enzymatic synthesis of ethyl glucoside lactate under atmospheric pressure

Enzymatic synthesis was carried out in 4 ml butyl lactate containing ethyl glucoside initiated by the addition of enzyme at a designed concentration on a stopper shaken at 200 rpm. The reaction temperature was varied in the range of 40–80 °C. Samples were drawn at intervals and analyzed by HPLC methods. A Shimadzu HPLC (Scl-10Avp, equipped with a RID-10A refractive detector), linked with a data system (Shimadzu Lc workstation Class-VP) was employed for data acquisition and storage. The detection was carried out on a Waters spherisorb S5 NH_2 column (150 mm × 4.6 mm) using a elution of acetonitrile/water (85/15) with a flow rate of 1 ml/min. Water content of the reaction system was determined by the Karl Fischer titration method on a Mettler Toledo DL 35 apparatus.

2.2.2. Enzymatic synthesis of ethyl glucoside lactate under reduced pressure

Ethyl glucoside was dissolved in 15 ml butyl lactate at 0.01 MPa in the presence of enzyme (80 g/l). The temperature was usually 60 °C. The mixture was incubated under agitation on a rotate apparatus. Samples were taken at intervals.

2.2.3. Separation and purification of ethyl glucoside lactate

At the end of the enzymatic reaction, reaction medium was collected and butyl lactate can be removed using water–hexane two-phase extraction. One volume of reaction medium was mixed with water and hexane (1:2 (v/v)), and then vigorously shaken for 30 min and placed the mixture until organic and aqueous phases separated. The bottom (aqueous phase) was recovered and evaporated at 60 °C under reduced pressure. Then hexane was added to the mixture at a voluminal ratio of approximately 10:1 to remove the residue water. After intensive mixing, the upper layer (organic phase) was taken off and residual hexane was evaporated at 40 °C under reduced pressure.

2.2.4. Product analysis

Infrared spectrum data of purified component were obtained on Nicolet Magna-IR550 (solvent, MeOH). The structure of the product was established by ^1H NMR using Brücker AM 500 spectrometer performed in D_2O. Mass spectrometry data were obtained on Perkin-Elmer SCIEX API 100 for ESI measurements (flow rate, 10 Ul/min; solvent, MeOH).

3. Results and discussion

3.1. Effect of solvent

The choice of solvent is critical for high conversion of transesterification reaction. Synthesis of ethyl glucoside lactate was investigated either in various solvent phase (hexane, heptane, 2-methyl-2-butanol) or in solvent-free medium (lactic acid, butyl lactate). The reactions were carried out at 50 °C and prolonged about 30 h. Conversion was defined as the molar ratio of converted ethyl glucoside to initial ethyl glucoside.

As shown in Table 1, the conversion in non-polar solvent medium (hexane or heptane) was very low. This may be caused by the low solubility of ethyl glucoside rather than lipase denaturation in these solvent mediums. Torres and Otero [14] also reported that the hydrophobic solvent such as hexane ($\log P > 3.5$, partition coefficient between *n*-octanol and water) significantly decreased the fraction of a polar substrate that initially dissolves. No products can be detected in lactic acid in this study. It is probably due to the low $\log P$ value, high dielectric constant and high $S_{W/O}$ (the molar solubility of water parameter) value [15]. As a more polar solvent, lactic acid could disrupt the enzyme-bound water and change the protein ionization state, resulting in enzyme de-action. As known, tightly bound structural water does play an important role in catalysis, which can maintain the catalytically active enzyme conformation [16]. Furthermore, the direct esterification can produce H_2O, which can enhance the hydrolysis reaction instead of the synthesis of ethyl glucoside lactate [17]. It seems that butyl lactate is more suitable, whose conversion can reach 30% in the investigated reaction system.

Table 1
Effect of solvent on the conversion of ethyl glucoside lactate[a]

	Butyl lactate	Conversion (%)
Reaction in the presence of solvent	Hexane	8
	Heptane	5
	2-Methyl-2-butanol	12
Reaction in solvent-free medium	Lactic acid	0
	Butyl lactate	30

[a] All the reactions were performed using ethyl glucoside (50 mM) and enzyme (25 g/l) in 3 ml solvent. When the reaction was carried out in the presence of solvent (hexane, heptane or 2-methyl-2-butanol), butyl lactate was fixed at 200 mM. The reaction time and temperature were, respectively, 30 h and 50 °C.

The above results suggested that the ideal solvent of enzymatic reactions in organic systems should conform to the following considerations. (1) Solubilizing all the substrate during the reaction. (2) Less negative effect on the enzyme activity. (3) Nontoxic when used in pharmaceutical, food or cosmetic industries. When butyl lactate was used not only as substrate but also as solvent, the best result was obtained and further study of enzymatic synthesis will be focused on such solvent-free system.

3.2. Effect of initial ethyl glucoside concentration

The test was carried out using the ethyl glucoside concentration in the range from 0.2 to 0.73 M. As shown in Fig. 1, the initial rate increased with the increase of the ethyl glucoside concentration (from 0.2 to 0.65 M), but a subsequent increase of the ethyl glucoside concentration can decrease the initial rate. It can be inferred that ethyl glucoside is an inhibitor of the enzyme for the lipase-catalyzed transesterification. The amount of initial water that leads to hydrolysis of esters may be increased with the increased concentration of ethyl glucoside [18]. Lactic acid and butanol release is likely to induce lower reaction efficiency.

3.3. Effect of enzyme concentration

From an economic view, if the cost of enzyme is most important, the reaction can be carried out with less enzyme and for longer reaction time. On the other hand, if the reaction time is first considered, it would be better to use more enzymes. The tests were conducted with 0.43 M ethyl glucoside at 70 °C using six amount levels of enzyme, and the initial rate and conversion were both detailed.

As shown in Fig. 2, the initial rate was drastically raised with the increase of the amount of enzyme. However, when the enzyme concentration was more than 100 g/l, the initial rate was not proportionally increased. It may be due to possible diffusional problems in reaction mixtures containing the higher amounts of immobilized biocatalyst that have an inability to contact with the solution [14,19]. Moreover, the high enzyme loading will enhance possibilities for agglomeration of the products in locations that may

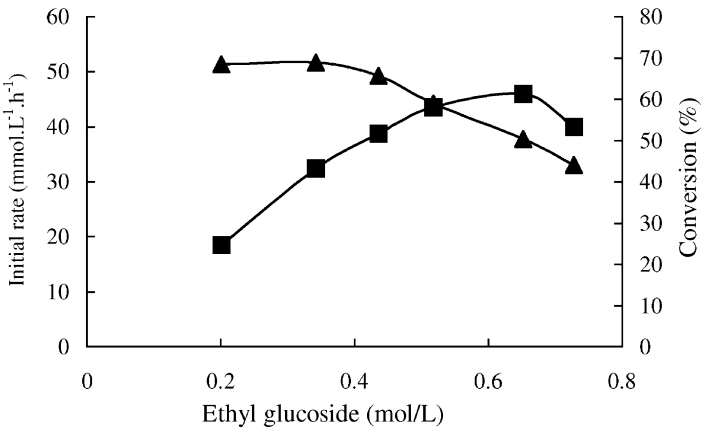

Fig. 1. Influence of ethyl glucoside concentration on initial rates (■) and conversion (▲). The reactions were carried out in 4 ml butyl lactate, using enzyme 50 g/l, ethyl glucoside (different concentration) at 70 °C with magnetic stirring until steady state.

render the immobilized enzyme less accessible [19]. Table 2 shows enzyme concentration cannot affect the equilibrium of the reaction, as long as the latter can be reached (for initial enzyme concentration above 75 g/l). The best results were obtained with 100 g/l enzyme, which produced the high conversion of the product in a relatively short time.

3.4. Effect of initial water content on conversion of ethyl glucoside lactate

As we known, there are two reactions existing in the system: transesterification and hydrolysis.

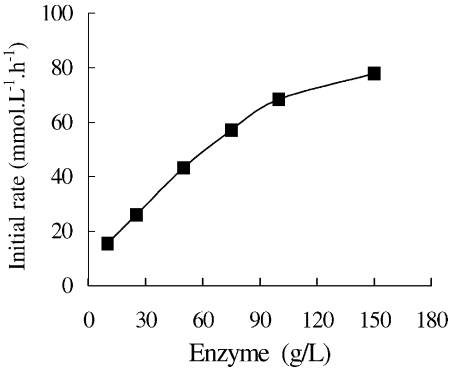

Fig. 2. Effect of enzyme concentration on initial rates. The reactions were performed in 4 ml butyl lactate using 0.43 M ethyl glucoside with magnetic stirring until steady state (except for concentrations of 10, 25 and 50 g/l) at 70 °C.

$$\text{butyl lactate} + \text{ethyl glucoside} \overset{\text{Transesterification}}{\rightleftharpoons} \text{ethyl glucoside lactate} + \text{butanol}$$

$$\text{butyl lactate} + H_2O \overset{\text{Hydrolysis}}{\rightleftharpoons} \text{lactic acid} + \text{butanol}$$

In order to investigate the effect of initial water content, the reaction medium was dried with molecular sieves. The water contents of water saturated medium and dried medium were 0.24 and 0.16%, respectively. When vacuum was applied during the reaction, the water content in the reaction medium was reduced to 0.08%.

As shown in Fig. 3, the lower the water content value was, the higher was the conversion of ethyl glucoside lactate. The initial rate was also increased with

Table 2
Effect of enzyme concentration on conversion[a]

Enzyme (g/l)	Conversion (%)	Reaction time (h)
10	44	125
25	62	125
50	66	125
75	72	66
100	73	55
150	72	50

[a] All the reactions were performed in 4 ml butyl lactate, using 0.43 M ethyl glucoside with magnetic stirring until steady state (except for concentrations of 10, 25 and 50 g/l). The temperature was 70 °C.

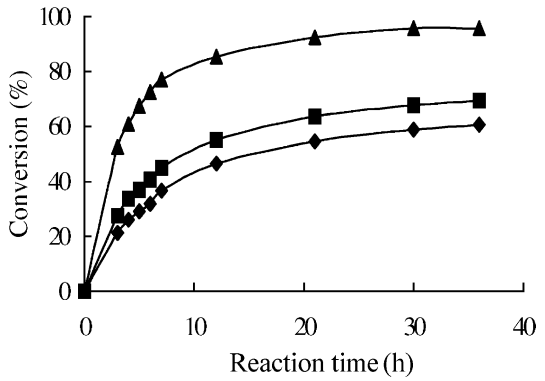

Fig. 3. Effect of initial water content on conversion. The reactions were performed in 4 ml butyl lactate dried by molecular sieves (■) or not (◆) using 0.32 M ethyl glucoside and 80 g/l enzyme at atmospheric pressure for 36 h. The temperature was 70 °C. The reactions under reduced pressure (▲) were performed in 15 ml butyl lactate at 60 °C, in the presence of 80 g/l enzyme and 0.32 M ethyllactate for 36 h.

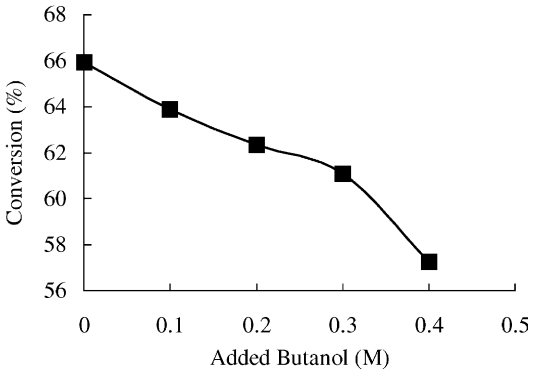

Fig. 4. Effect of butanol concentration on conversion. The reactions were performed in 3 ml butyl lactate, using enzyme 75 g/l, ethyl glucoside 0.2 M, and butanol (different concentration) at 70 °C with magnetic stirring and stopped after 65 h.

the decreased water content. However, the water in the reaction medium could not be removed completely by the 4 Å molecular sieve due to the hydrophilic property of butyl lactate. The butanol and water boiling point at atmospheric pressure is 118 and 100 °C, respectively, which are lower than that of butyl lactate (186 °C). As shown in Fig. 3, the butanol concentration in the reaction system under reduced pressure was lowest due to the butanol evaporation. So reduced pressure (0.01 MPa) was employed to eliminate butanol and the initial water, with conversion and initial rate reaching 95% and 67.4 mM/h, respectively. Consequently, a single purification step, removing butyl lactate, was sufficient for recovering ethyl glucoside lactate.

3.5. Effect of butanol on conversion of ethyl glucoside lactate

In the series of experiments, different amounts of butanol was added to the reaction mediums and the conversions of ethyl glucoside lactate were measured after 65 h. Fig. 4 shows that the conversion is decreased with the butanol concentration increase in the system, which conforms the butanol side-effect on the reaction. To improve the progress, it is necessary to apply vacuum during the reaction.

3.6. Effect of reaction temperature

In equilibrium-controlled synthesis, reaction temperature is the most critical factor to determine the conversion. As shown in Fig. 5, the initial rates increased with the increase of temperature even up to 80 °C, confirming that enzyme thermoresistant was very good [20] and also showing that the investigated reaction followed the equilibrium-controlled mechanism. The stability of enzyme might be benefited from immobilization. Higher initial rates were achieved at higher temperatures, which may be due to the higher energy state of the molecules resulting in more fruitful collisions [21]. Moreover, the conversion increased

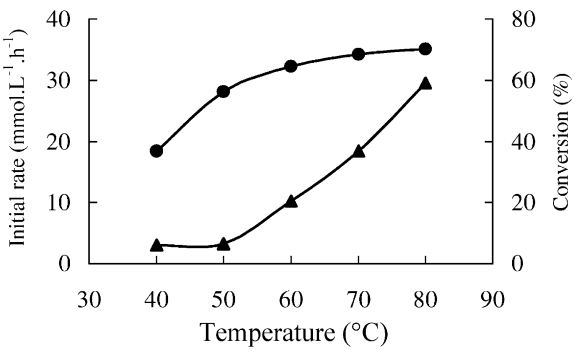

Fig. 5. Effect of temperature on initial rate (▲) and conversion (●). The reactions were performed in 4 ml butyl lactate using 0.2 M ethyl glucoside and 50 g/l enzyme for 48 h. The temperature was from 50 to 80 °C.

Table 3
Chemical shifts of ^{1}H NMR

Proton	δ (ppm)
H_1	4.80
H_2	3.59
H_3	3.65
H_4	3.45
H_5	3.80
H_6	4.29
H'_6	4.37
CH_3 (a)	1.104
CH_2 (b)	3.15–3.35
CH (c)	4.33
CH_3 (d)	1.32

with the temperature raised, as butanol and water may be partially evaporated under the higher temperature. Then butanol and the initial water can be eliminated continuously so as to displace the reaction equilibrium towards synthesis, which makes the conversion high.

3.7. Product analysis

After ethyl glucoside lactate was purified by extraction, a single product was obtained at purity above 90%.

Ethyl glucoside contains several reactive hydroxyl groups, therefore, the reaction can perhaps produce mono-, di-, or tri-esters. However, regioselective acylation of lipase have been proved in the enzymatic production of ethyl glucoside at the 6-position [22,23]. The transesterification occurs at the 6-position of the ethyl glucoside identified by means of ^{1}H NMR (Table 3). Mass spectrometry data also gave a molecular ion at $m/z = 303.05$ $[M_1 + Na]^+$, M_1 is corresponding exactly to molecular mass of ethyl glucoside monolactate.

4. Conclusions

In this paper, ethyl glucoside lactate was synthesized by transesterification in non-aqueous phase using immobilized lipase as biocatalyst and butyl lactate as both acyl donor and solvent. The conversion was enhanced up to 95% by the removal of water and the co-product butanol under reduced pressure for the change of the reaction equilibrium towards synthesis of the ester. The results obtained show that it is possible to produce high quality glucoside lactate easily through biocatalysis under reduced pressure.

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