Regioselective acylation of resveratrol catalyzed by lipase under microwave

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**ABSTRACT**

The enzymatic regioselective acylation of resveratrol was achieved in organic solvents catalyzed by the lipase from *Candida sp.* 99–125 (CSL) under microwave irradiation. Influences of various reaction conditions have been studied. After selecting the optimum conditions (MTBE (20 ml, \(a_w = 0.38\)), resveratrol (0.1 mmol), vinyl acetate (1.0 mmol) and CSL (100.0 mg) under microwave irradiation (35°C, 400 W)), CSL exhibited a satisfied enzyme activity (281 ± 11 \(\mu\)mol/g/h) and yield of 75% for 4′-O-acetyl-resveratrol could be obtained in about 4 h when performing the reaction on a 25-fold-larger scale.

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**Introduction**

As an important polyphenolic phytochemical, resveratrol (3, 4′, 5-trihydroxystilbene) can be found in many natural plants (grapes, berries and peanuts, etc.) (1,2). Previous reports have demonstrated that resveratrol has many beneficial effects on health (3–6). However, its fast metabolism has impaired its bioavailability (7,8) and seriously limited its application. Acyl modification can solve this problem (9–11). The chemical process and enzymatic synthesis have been applied for this purpose. Among the two routes, enzymatic method is more efficient and highly regioselective. In the past few years, the regioselective acylation of resveratrol catalyzed by lipase has already been reported (12–17). For example, the immobilized lipase QLG (lipase from *Alcaligenes* sp.) has been successfully used for the synthesis of 3-O-acyl-resveratrol by transesterification (13). CAL-B (*Candida antarctica* lipase B) and its commercially available immobilized enzyme (Novozyme 435) have achieved the regioselective acylation of resveratrol affording 4′-O-acyl-resveratrol (12, 15, 17). However, almost all these lipases need long reaction time to obtain a satisfied yield due to their poor enzyme activity.

Microwave irradiation (MW) is a rapid developing green technology and has been successfully employed to accelerate the enzymatic reaction (18–20). In this study, we mined out a new commercially available lipase, *Candida sp.* 99–125 lipase (CSL), for the regioselective acylation of resveratrol at 4′-OH (Scheme 1). Microwave irradiation has been used to accelerate the regioselective transesterification and the reaction conditions have been optimized.

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Experimental

Enzymes and chemicals

Resveratrol was provided by Shanghai Korey Pharm. Co., Ltd. (Shanghai, China). Organic solvents were from Jilin Chemical Reagent Company (Jilin, China). CAL-B (lipase B from Candida antarctica) was from Novo (Bagsværd, Denmark). PSL (Pseudomonas sp. lipase) was purchased from Amano Pharmaceutical Co., Ltd. (Tokyo, Japan). AKL (Achromobacter sp. alkaline lipase) was from Dadi Kangen Biotech. Co., Ltd. (Shenzhen, China). CSL (Candida sp. 99–125 lipase) was purchased from Beijing CTA New Century Biotechnology Co., Ltd. (Beijing, China).

Water activity setting

The reaction reagents were dried in a vacuum of 1 mm Hg for 12 h before use. Micro-amount of water was added to the dried reagents to obtain a specific water activity ($a_w$). The resulting reagent was pre-equilibrated in a sealed vial for 24 h before $a_w$ measurement by a Hygrolab Humidity Detector (Rotronic, Switzerland).

Regioselective acylation of resveratrol

Resveratrol (22.8 mg, 0.1 mmol), vinyl acetate (92.5 $\mu$L, 1.0 mmol) and CSL (100 mg) were mixed into tert-butyl methyl ether (MTBE, 20 ml, $a_w = 0.38$). The acylation was performed in MCR-3 microwave reactor (Shanghai JieSi Microwave Chemistry Corporation, Shanghai, China). The microwave parameters were set as 160 rpm, 35°C and 400 W. The reaction mixture was filtered by 0.22 $\mu$m PDVF filters. Concentration and chromatography gave a white solid, which was identified by NMR to be 4′-O-acetyl-resveratrol. $^1$H NMR of 4′-O-acetyl-resveratrol (Acetone-$_d_6$, 500 MHz): $\delta$ 8.25 (s, 2H), 7.60 (d, $J = 8.6$ Hz, 2H), 7.10 (dd, $J = 15.1, 9.0$ Hz, 4H), 6.58 (s, 2H), 6.31 (s, 1H), 6.31 (s, 1H), 2.26 (s, 3H).

HPLC procedure

HPLC (SPD-M20A, Kyoto, Japan) was used to monitor the acylation of resveratrol. The mobile phase (1.0 ml/min) was a solution of methanol (A) in water (containing 0.1% of acetic acid, v/v). The gradient program was: 30% A for 5 min, 30–50% A over 5 min, 50% A for 40 min. The samples were measured at 310 nm with a Thermo C18 column (4.6 mm × 250 mm × 5 $\mu$m, USA). The retention time of resveratrol, 4′-O-acetyl-resveratrol, 3-O-acetyl-resveratrol and 3, 4′-diester of resveratrol was 7.293, 14.690, 16.019 and 33.813 min, respectively. One unit of enzyme activity ($\mu$mol/g/h) referred to the amount ($\mu$mol) of resveratrol ester produced per gram of enzyme per hour. Each experiment has been performed triplicate to obtain the average value.

Results and discussion

Enzyme origin

In this study, we selected four commercial lipases for the acylation. The results listed in Table 1 confirmed that all

Table 1. Effect of lipase origin on the regioselective acylation of resveratrol.

| Lipasea | Enzyme activity ($\mu$mol/g/h) | Conversion (%) | 4′-O-acetyl-resveratrol | 3-O-acetyl-resveratrol | diester |
|---------|-------------------------------|----------------|------------------------|-----------------------|--------|
| PSL     | Conventional heatingb         | 25 ± 4         | 10.2 ± 1.3             | 27.2 ± 0.3            | 52.4 ± 0.6 | 20.4 ± 0.1 |
| CSL     | Conventional heatingb         | 147 ± 13       | 54.9 ± 1.5             | 25.6 ± 0.5            | 52.1 ± 0.7 | 22.3 ± 0.6 |
| CSL     | Microwavec                    | 37 ± 3         | 14.9 ± 1.1             | 93.1 ± 0.9            | n.d.    | 6.9 ± 0.5 |
| AKL     | Conventional heatingb         | 11 ± 2         | 4.6 ± 0.7              | 31.1 ± 0.7            | 30.4 ± 0.3 | 38.5 ± 0.7 |
| AKL     | Microwavec                    | 69 ± 7         | 27.5 ± 1.5             | 30.7 ± 0.3            | 29.5 ± 0.8 | 39.8 ± 0.5 |
| CAL-B   | Conventional heatingb         | 23 ± 3         | 9.4 ± 1.2              | 85.8 ± 1.1            | 2.9 ± 0.4 | 11.3 ± 0.3 |
| CAL-B   | Microwavec                    | 136 ± 15       | 53.9 ± 1.9             | 84.4 ± 1.4            | 3.0 ± 0.1 | 12.6 ± 0.2 |

aPSL: Pseudomonas sp. Lipase; CSL: Candida sp. 99–125 lipase; AKL: Achromobacter sp. alkaline lipase; CAL-B: lipase B from Candida antarctica.
bConditions: Reactions were carried out in MTBE (20 ml, $a_w = 0.38$) containing resveratrol (0.1 mmol), vinyl acetate (1.0 mmol) and lipase (100.0 mg) under magnetic stirring (160 rpm, 35°C) for 4 h.
cConditions: Reactions were carried out in MTBE (20 ml, $a_w = 0.38$) containing resveratrol (0.1 mmol), vinyl acetate (1.0 mmol) and lipase (100.0 mg) under microwave irradiation (160 rpm, 35°C, 400 W) for 4 h.
dNot detected.
the used lipases can carry out the acylation and the regioselectivity greatly depended on the lipase origin. Among the selected lipases, PSL mainly catalyzed the acylation of the phenolic group at 3-OH and yielded 3'-O-acetyl-resveratrol. AKL afforded a nearly equi-molar mixture of 3'-O-acetyl-resveratrol and 4′-O-acetyl-resveratrol. Both CSL and CAL-B were highly specific at 4′-OH of resveratrol (confirmed by NMR).

The results in Table 1 also demonstrated that microwave could not change the regioselectivity of enzyme and the enzyme activity under microwave is much higher than that of conventional heating. The substrates can absorb the microwave energy, which means their functional groups under microwave have much higher reactivity than those under conventional heating at the same temperature (21). So, we can take the advantage of the microwave to improve the catalytic activity of enzyme. In this study, CSL exhibited the highest enzyme activity (281 ± 11 μmol/g/h) under microwave and has been chosen as the optimum enzyme.

**Reaction media**

Table 2 displayed the catalytic performance of CSL obtained in different organic solvents. Organic media can change the solvation of the substrate. It can also affect the enzyme conformation and then influence the enzyme performance. The maximum enzyme activity (281 ± 11 μmol/g/h) was obtained in MTBE. Thus, MTBE was selected for further study.

**Water activity**

Besides the nature of the solvent, water activity is another significant parameter that may affect the catalytic performance of the lipase suspended in organic solvents (22). The influence of water activity on the acylation of resveratrol is demonstrated in Figure 1. CSL exhibited an obvious high enzyme activity with the water activity increasing and the maximum enzyme activity was obtained at αw = 0.38. Further increasing the water activity will decrease the enzyme activity. The results we observed may suggest that CSL need a suitable micro-amount water to keep its optimum conformation and exhibit its best catalytic performance in organic solvents (23,24). In this study, the optimum water activity was 0.38 and has been selected for further study.

**Temperature**

Temperature is a vital parameter affecting the catalytic activity of lipase under microwave irradiation. Figure 2 demonstrated its influence on CSL in the range of 20 to 50°C. It could be found that the increasing temperature can enhance the enzyme activity of CSL and the highest enzyme activity was observed at 35°C. The activity significantly decreased at higher temperatures, which might be due to the denaturation of the enzyme (25).

**Microwave power**

In order to lower the energy consumption, the effect of microwave power has been studied and the results were presented in Figure 3. A bell-shaped curve could be observed when microwave power changed from 0
to 800 W and the highest enzyme activity was obtained at 400 W. Microwave with higher power can increase the reactivity of the reactants and then enhance the enzyme activity (26). However, if the microwave power was enhanced further, the enzyme activity will decline heavily as the result of the denaturation of the enzyme (27). Hence, we chose 400 W for this reaction.

The effects of molar ratio of substrates and enzyme dosage have also been investigated (data not shown). We found that the optimum molar ratio (vinyl acetate/resveratrol) was about 10:1 and the optimum enzyme dosage was 100.0 mg. By increasing the enzyme dosage, more lipases would take part in the reaction and then increase the conversion. However, high enzyme dosage may cause the aggregation of enzyme, which may have some negative effects on the enzymatic reaction process.

**Time course of the reaction**

We scaled up the enzymatic reaction on a 25-fold-larger scale under the optimal conditions and monitored the reaction process by HPLC (Figure 4). When the reaction was stopped in about 4 h, the reaction mixture was filtered by 0.22 μm PDVF filters to remove the enzyme. The solvent was evaporated and the crude product was purified by chromatography on a silica-gel column using heptane/ethyl acetate (2:1, v/v) as eluent. The isolated yields of 4′-O-acetyl-resveratrol and 3, 4′-di-O-acetyl-resveratrol were about 75% and 6%, respectively.

**Conclusions**

In this study, a novel commercially available biocatalyst (CSL, Candida sp. 99–125 lipase) has been selected for the regioselective acylation of resveratrol in one step. It was found that CSL was highly specific at 4′-OH of resveratrol. Furthermore, microwave irradiation has been successfully applied to improve the catalytic activity of CSL and accelerate the regioselective acylation dramatically, which extends the application of microwave in the study of enzymatic reaction.

**Disclosure statement**

No potential conflict of interest was reported by the authors.
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