A mild and efficient Dakin reaction mediated by lipase

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ABSTRACT

We have developed a novel lipase-mediated method to realize the Dakin reaction. A wide range of hydroxylated benzaldehydes could be oxidized with high yields (from 90% to 97%) under mild reaction conditions. Moreover, this lipase-mediated reaction could be scaled up easily and Novozym 435 could be reused more than 10 runs without an obvious decrease in enzyme activity. This protocol expands the application of lipase in organic synthesis and offers a complementary route for the Dakin reaction.

ARTICLE HISTORY

Received 30 May 2017
Accepted 30 August 2017

KEYWORDS

Lipase; promiscuity; Dakin reaction; hydroxylated benzaldehyde

Introduction

Enzyme catalytic promiscuity is the hidden ability of an enzyme to catalyze more than one type of chemical transformation (1, 2). Lipase is one of the most popular biocatalysts in this research area due to its excellent catalytic performance in many kinds of reactions (Aldol reaction, Morita-Baylis–Hillman reaction, Mannich reaction, Henry reaction, Markovnikov addition, Michael addition, Hantzsch reaction, etc.) (3–9). Over the past few years, many reports have demonstrated that lipase could mediate the in situ generation of peracids through a perhydrolysis of carboxylic acids or esters (10–12). And the in situ formed peracids have been successfully utilized in the epoxidation of alkenes, Baeyer–Villiger reactions, the oxidation of N-heteroaromatic compounds and sulfanyl compounds (13–18).

The Dakin reaction replaces an aldehyde group with a hydroxyl group in an aromatic aldehyde or ketone that has a hydroxyl group in the ortho- or para- position (19). The conventional procedure for the Dakin reaction utilizes excess hydrogen peroxide and sodium hydroxide at elevated temperatures. Up until now, numerous stoichiometric alkylperoxides and catalytic transition metal complexes have been developed for Dakin oxidations (20–25). However, most of these reported methods suffered from unstable oxidants, the use of transition metals, expensive reagents or extreme reaction conditions. To the best of our knowledge, the enzymatic process for the Dakin reaction has not been reported yet. Herein we report a mild and efficient method for the Dakin reaction mediated by lipase (Scheme 1), which can provide a new case of enzyme catalytic promiscuity.
Results and discussion

As a starting point, we selected salicylaldehyde as the substrate in this lipase-mediated oxidation (Table 1). In this study, ethyl acetate (EA) was used as the peracid source (in situ generated by lipase-catalyzed perhydrolysis) and the reaction medium, meanwhile urea hydrogen peroxide (UHP) was used to release the oxidant controlably. We screened some lipases as the catalysts for the enzymatic Dakin reaction. As shown in Table 1, the yields of catechol were dramatically influenced by the type and origin of the enzyme. Only ANL, CalB and Novozym 435 (a commercial immobilized CalB) could mediate the Dakin reaction and the highest yield (96%) could be obtained when Novozym 435 was selected as the catalyst. The results of Entry 7 showed that UHP only exhibited a poor catalytic activity in this reaction. And the denatured Novozym 435 could not improve the Dakin reaction (Entry 6), which indicated that the active conformation of enzyme was necessary for this reaction. Moreover, the oxidation mediated by lipase obtained a much higher yield than that catalyzed by mCPBA (meta-chloroperoxybenzoic acid, a commercial organic peracid), which suggested that the lipase-mediated Dakin reaction was worthy of further study.

The effect of enzyme dosage on the Dakin reaction has been studied and the results were shown in Figure 1. The concentrations of salicylaldehyde and UHP were kept constant, while enzyme dosage was changed from 0 to 400 U. The yield of catechol improved when the amount of Novozym 435 was increased. However, the yield could not be increased further when the amount of enzyme was beyond 300 U. By increasing the amount of enzyme, the number of active sites that take part in the generation of peracid would increase. Thus, more in situ-produced peracids would oxidize salicylaldehyde into catechol. Considering the cost of the enzyme, 300 U of Novozym 435 was selected as the catalyst for this reaction.

Table 1. The effect of enzyme source on the lipase-mediated Dakin reaction.

| Entry | Enzyme                     | Yield (%) |
|-------|----------------------------|-----------|
| 1     | ANL (Lipase from Aspergillus niger) | 76        |
| 2     | CalB (Candida antarctica lipase B) | 84        |
| 3     | PSL (Lipase from Pseudomonas sp.) | 7         |
| 4     | PPL (Porcine pancreatic lipase) | 6         |
| 5     | Novozym 435\(^{b}\) | 96        |
| 6     | denatured Novozym 435\(^{c}\) | 5         |
| 7     | No enzyme | 5         |
| 8     | mCPBA | 53        |

\(^{a}\)Reaction condition: salicylaldehyde (1 mmol), EA (1 mL), UHP (1.2 mmol), enzyme (300 U), room temperature, 0.5 h.

\(^{b}\)Commercial immobilized CalB.

\(^{c}\)Novozym 435 was denatured by heating it to 100°C for 12 h in water.

Figure 1. The effect of enzyme dosage on the lipase-mediated Dakin reaction.

Note: Reaction condition: salicylaldehyde (1 mmol), EA (1 mL), Novozym 435 (300 U), room temperature, 0.5 h.

Figure 2. The effect of UHP on the lipase-mediated Dakin reaction.

Note: Reaction condition: salicylaldehyde (1 mmol), EA (1 mL), Novozym 435 (300 U), room temperature, 0.5 h.

Figure 3. The time course of the lipase-mediated Dakin reaction.

Note: Reaction condition: salicylaldehyde (50 mmol), EA (50 mL), UHP (60 mmol), Novozym 435 (15,000 U), room temperature.
adopted as the optimal enzyme dosage for further investigation.

Generally, the amount of oxidant might affect the yield of Dakin reaction. In this study, the effect of the amount of UHP was investigated (Figure 2). The yield of Dakin reaction increased when the amount of UHP was increased from 0.4 to 1.2 equiv.; after that, the yield did not improve markedly. Therefore, 1.2 equiv. of UHP was selected for this lipase-mediated Dakin reaction.

Under the optimized reaction conditions, we scaled up this lipase-mediated process to 50-fold (salicylaldehyde (50 mmol), EA (50 mL), Novozym 435 (15,000 U) and UHP (60 mmol)) and the time course was illustrated in Figure 3. The yield of catechol was up to 97% after 0.5 h, which demonstrated that this green and efficient method had high potential for industrial application.

It is known that the immobilized enzyme is easily recovered and reused, which can drive down the product cost and make the enzymatic process economically viable (26–28). Thus, we have studied the reusability of Novozym 435 for this lipase-mediated reaction. Novozym 435 can be easily recovered after each run by filtration. The recovered Novozym 435 was then reused directly for the oxidation of salicylaldehyde under the same conditions. As shown in Figure 4, Novozym 435 exhibited an excellent reusability, and up to 90% oxidation yield could be obtained even after 10 reaction cycles. The slight decrease of enzyme activity might be attributed to the deactivation of enzyme by the oxidants or the leakage of enzyme from the support.

To evaluate the scope and limitation of this method, a wide variety of hydroxylated benzaldehydes were used for this lipase-mediated Dakin reaction. As depicted in Table 2, all the listed hydroxylated benzaldehydes have been oxidized efficiently to provide the corresponding products. The results also indicated that the electronic nature of substituents of the hydroxylated benzaldehyde has no distinct effect on the lipase-mediated Dakin reaction.

| Entry | Hydroxylated benzaldehyde | Yield (%) |
|-------|---------------------------|-----------|
| 1     | ![](image1)               | 96        |
| 2     | ![](image2)               | 91        |
| 3     | ![](image3)               | 96        |
| 4     | ![](image4)               | 94        |
| 5     | ![](image5)               | 93        |
| 6     | ![](image6)               | 90        |
| 7     | ![](image7)               | 97        |
| 8     | ![](image8)               | 97        |
| 9     | ![](image9)               | 95        |
| 10    | ![](image10)              | 93        |

Note: Reaction condition: hydroxylated benzaldehyde (1 mmol), EA (5 mL), UHP (1.5 mmol), Novozym 435 (300 U), room temperature, 0.5 h.
reaction. Furthermore, some other aromatic aldehydes (such as benzaldehyde, 2-chlorobenzaldehyde, 4-chlorobenzaldehyde, 4-nitrobenzaldehyde and 4-methoxybenzaldehyde) have also been investigated as the substrates at the same reaction conditions. However, only the corresponding aromatic acids were obtained instead of the corresponding phenols. Therefore, the valid substrates for this lipase-mediated Dakin reaction should be the benzaldehydes with ortho- or para- hydroxyl group.

Experimental

Materials

Lipase from Aspergillus niger (ANL), Candida antarctica lipase B (CaB) and Porcine pancreatic lipase (PPL) were purchased from Sigma (Beijing, China). Lipase from Pseudomonas sp. (PSL) was purchased from Amano Pharmaceutical Co., Ltd (Japan). Hydroxylated benzaldehydes and UHP were purchased from J&K Scientific (Beijing, China). All the other chemical reagents were purchased from Shanghai Chemical Reagent Company (Shanghai, China). All the commercially available reagents and solvents were used without further purification. NMR spectra were recorded on an Inova 500 (500 MHz) spectrometer.

General procedure of the lipase-mediated Dakin reaction

A mixture of hydroxylated benzaldehyde (1 mmol), UHP (1.5 mmol), Novozym 435 (300 U) in EA (5 mL) was stirred at room temperature in a round-bottom flask for 0.5 h. The reaction was monitored by thin-layer chromatography. Then, the mixture was filtered and the residue was washed with EA. The combined organic phases were concentrated under vacuum, and the resulting residue was purified by column chromatography on silica gel with EA/hexane (1/4) to afford the desired phenol. All the isolated products were well characterized by their 1H-NMR spectral analysis. Each experiment was performed triplicate, and all the data were obtained based on the average values.

Conclusion

In this study, a mild and efficient method for the Dakin reaction mediated by lipase was reported. After thorough optimization of the reaction conditions, all the products could be obtained in high yields (from 90% to 97%). This lipase-mediated method could be easily scaled up and the catalyst (Novozym 435) presented an excellent reusability. The notable features of this new synthetic route are atom economy, environmental friendliness and simple operational process. More importantly, this work provides a new case of enzyme catalytic promiscuity which can contribute to the development of novel synthetic approach and green technology.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The authors gratefully acknowledge the Foundation of Changchun BC&HC Pharmaceutical Technology Co., Ltd (grant no. 3R116V401465) and the Fund of Basic Scientific Research from Jilin University (for Constructing the Scientific and Technological Platform, 2017).

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