Lipase-catalyzed enantioselective transesterification of 3-hydroxy-3-(2-thienyl) propanenitrile in liquid carbon dioxide

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ABSTRACT
The transesterification of 3-hydroxy-3-(2-thienyl) propanenitrile catalyzed by \textit{Pseudomonas fluorescens} lipase (PFL) in liquid carbon dioxide (CO\textsubscript{2}) was reported. Compared with that in organic solvent (\textit{n}-hexane), the catalytic performance of PFL was dramatically enhanced in liquid CO\textsubscript{2}. Under the optimal reaction conditions, PFL exhibited an excellent enantioselectivity (\textit{E}-value: 92.9) with a high enzyme activity (82.5 \textmu mol/g/min). Besides, the remained (S)-3-hydroxy-3-(2-thienyl) propanenitrile with high enantiomeric purity (\textit{ee} > 99\%) was obtained in 4 h when the conversion was about 52%.

\begin{center}
\begin{tikzpicture}
\node[below=0.5cm]{\textbf{Lipase-catalyzed transesterification of 3-hydroxy-3-[2-thienyl] propanenitrile in liquid CO\textsubscript{2}}};
\end{tikzpicture}
\end{center}

Introduction
Duloxetine is a dual inhibitor of serotonin and norepinephrine reuptake and used as an important anti-depressant drug (1, 2). It is well known that racemic duloxetine has the therapeutic activity residing mainly in its (S)-enantiomer form (3–5), which can be synthesized from (S)-3-hydroxy-3-(2-thienyl) propanenitrile (6). Many chemical methods have been presented for the synthesis of this chiral building block and various expensive chiral catalysts have been used, which is inefficient and toxic (7–9). Comparatively, enzymatic method is more advantageous in terms of mild reaction conditions, environmental friendliness and excellent enantioselectivity. For instance, Ahmed Kamal et al. have used a commercial immobilized lipase (lipase PS-D) to prepare (S)-3-hydroxy-3-(2-thienyl) propanenitrile in diisopropyl ether at 14 h (10). A dynamic kinetic resolution of 3-hydroxy-3-(2-thienyl) propanenitrile using lipase and ruthenium catalyst has been reported by Bäckvall et al. to afford (S)-3-hydroxy-3-(2-thienyl) propanenitrile (11). Liu and his coworkers have reported an ultrasound assistant enantioselective transesterification of 3-hydroxy-3-(2-thienyl) propanenitrile catalyzed by lipase to increase the catalytic performance of lipase in \textit{n}-hexane (12). However, these reported enzymatic resolutions have encountered some drawbacks, such as low enzyme activity, high temperature and utilization of volatile organic solvents.

Liquid carbon dioxide (CO\textsubscript{2}) is often used as an environmentally benign reaction medium in chemical reaction for its nontoxicity, non-flammability, low cost, tunable density, high diffusivity and chemically inert (13, 14). Compared with supercritical CO\textsubscript{2}, liquid CO\textsubscript{2} is different in many physical properties, and the advantage of using liquid CO\textsubscript{2} is that it can be maintained under relatively modest pressure (e.g. 4.5 MPa at 10°C) that reduces the cost of specialized equipment for high-pressure reaction in supercritical CO\textsubscript{2} (over 7.4 Mpa at 31°C) (15). Furthermore, it can be employed at low temperature, which has the potential to enhance the
enatioselectivity of enzyme by the low-temperature strategy. Recently, some elegant works have been reported that liquid CO$_2$ could effectively enhance lipase activity and enantioselectivity in lipase-catalyzed transesterifications (16–18). These findings encouraged us to further investigate the application of liquid CO$_2$ in the lipase-catalyzed resolution of racemic pharmaceutical intermediates.

In this work, lipase-catalyzed transesterification of 3-hydroxy-3-(2-thienyl) propanenitrile in liquid CO$_2$ was presented (Scheme 1). To determine the optimal conditions for this lipase-catalyzed reaction, the effect of reaction parameters, such as the type of lipase, acyl donor, water content, substrate molar ratio and enzyme dosage, was studied.

**Results and discussion**

Generally, a low reaction temperature and pressure are beneficial to the catalytic performance of enzyme in liquid CO$_2$. Thus, we fixed the temperature and pressure of liquid CO$_2$ at 20°C and 6.5 Mpa in this work. Initially, various lipases have been selected as the catalysts using vinyl butyrate as the acyl donor in liquid CO$_2$ and results were shown in Table 1. Among these enzymes, *Pseudomonas fluorescens* lipase (PFL) exhibited the highest enantioselectivity with a high activity. It is known that liquid CO$_2$ has very similar properties with a hydrophobic solvent with very low polarizability, such as n-hexane (19, 20). Then, the catalytic performance of PFL in liquid CO$_2$ was compared with n-hexane. The activity of lipase in liquid CO$_2$ is much higher than that in n-hexane. It could be attributed to the important changes in the enzyme structure which are induced by pressurized CO$_2$. Liquid CO$_2$ could also avoid stripping off the essential water present on the surface of enzyme, and keep the active conformation of enzyme. Moreover, the excellent diffusivity and low viscosity and surface tension of liquid CO$_2$ are responsible for the reduction of interphase transport limitations, thus increasing the reaction rate in this transesterification. As for the enantioselectivity in liquid CO$_2$, PFL also exhibited a higher enantioselectivity than that in n-hexane, which could be explained by the fact that lipase obtained a desired change on the conformation in liquid CO$_2$. Therefore, PFL was selected as the optimal enzyme for this transesterification in liquid CO$_2$.

Source of acyl donor can obviously affect the enantioselective transesterification catalyzed by lipase (21). Thus, five different vinyl esters with various chain lengths were screened to investigate the effect of acyl donors in this study. As shown in Table 2, the enzyme activity was changed distinctly by the chain length of the acyl donor. The highest enzyme activity could be obtained when vinyl acetate was used as the acyl donor, and the activity decreased when the chain length of the acyl donor was increased. Acyl donors with longer chain lengths might access the active pocket of lipase with more difficulty due to steric strain, which may decrease the enzyme activity. As for the enantioselectivity, the highest enantioselectivity was obtained when vinyl acetate was used as the acyl donor. Therefore, the performance of PFL in liquid CO$_2$ was compared with n-hexane, which could be explained by the fact that lipase obtained a desired change on the conformation in liquid CO$_2$. Therefore, PFL was selected as the optimal enzyme for this transesterification in liquid CO$_2$.

**Table 1.** Lipase-catalyzed transesterification of 3-hydroxy-3-(2-thienyl) propanenitrile in liquid CO$_2$.

| Enzyme from | Enzyme activity (μmol/g/min) | Enantioselectivity E-value |
|-------------|----------------------------|---------------------------|
| Lipase from *Candida* sp. (CSL) | 11.2 | 26.2 |
| Pseudomonas sp. Lipase (PSL) | 52.6 | 78.2 |
| Porcine pancreas lipase (PPL) | 26.9 | 23.1 |
| *Candida antarctica* lipase (CalB) | 61.7 | 64.7 |
| *Pseudomonas fluorescens* lipase (PFL) | 82.5 | 92.9 |
| *Pseudomonas fluorescens* lipase (PFL)$^b$ | 26.2 | 57.3 |

$a$Reaction condition: 3-hydroxy-3-(2-thienyl) propanenitrile (1 mmol), vinyl butyrate (6 mmol), lipase (protein content: 40 mg), water content (0.04% v/v), 20°C, 6.5 Mpa, liquid CO$_2$ (25 mL) in a pressure-resistant vessel. $b$The reaction was performed in n-hexane (25 mL).

**Table 2.** Effect of acyl donor on the lipase-catalyzed transesterification in liquid CO$_2$.

| Acyl donor | Enzyme activity (μmol/g/min) | Enantioselectivity E-value |
|------------|-----------------------------|---------------------------|
| Vinyl acetate | 97.2 | 71.5 |
| Vinyl butyrate | 82.5 | 92.9 |
| Vinyl Hexanoate | 62.6 | 79.7 |
| Vinyl octanoate | 56.8 | 87.6 |
| Vinyl decanoate | 31.5 | 60.4 |

$a$Reaction condition: 3-hydroxy-3-(2-thienyl) propanenitrile (1 mmol), acyl donor (6 mmol), PFL (40 mg), water content (0.04% v/v), 20°C, 6.5 Mpa, liquid CO$_2$ (25 mL).
butyrate was used as acyl donor. Generally, the enantioselectivity of lipase, which is sensitive to the chain length of the acyl donor, mainly results from the disatereomeric interaction in the acyl-lipase-substrate complex (22). When different vinyl esters were used as acyl donors, the structures of the acyl-enzyme complex may differ from one another. Since vinyl butyrate could exhibit the highest enantioselectivity with a satisfactory enzyme activity (82.5 μmol/g/min), vinyl butyrate was adopted as the acyl donor in this study.

Water content is considered as a key parameter in the lipase-catalyzed transesterification in non-aqueous medium (23). In this study, a stainless steel pressure-resistant vessel (internal volume: 25 mL) was used as the reactor. Thus a certain amount of water was added into the reactor to control the water content. We investigated the effect of water content (0.01–0.06%v/v) on the lipase-catalyzed transesterification in liquid CO₂ (Figure 1). The activity of lipase presented a bell shaped curve with the water content increasing, and the highest activity was obtained when water content was 0.04%v/v. In general, the conformation of lipase was excessively rigid at low water content, which may hinder the "induced-fit" process of enzyme and reduce the activity of lipase. However, the excessively flexible conformation of lipase at high water content inhibits the transesterification activity. In addition, an excess of water can hinder the transesterification by promoting hydrolysis. Considering the enantioselectivity of lipase, it remained approximately constant. Considering the cost of the enzyme, 40 mg of PFL was proved to be the most efficient amount.

In an enzymatic transesterification, the enantiomeric excess (ee) of the residual substrate mainly depends on the reaction conversion (25). It was observed in Figure 4 that the ee of the remaining (S)-3-hydroxy-3-(2-thienyl) propanenitrile increased with prolonging the reaction time. (S)-3-hydroxy-3-(2-thienyl) propanenitrile with 99%ee and about 52.1% conversion was obtained when the reaction time was 4 h. We scaled up the reaction system in a pressure-resistant vessel with internal volume of 1 L (3-hydroxy-3-(2-thienyl) propanenitrile...
Porcine pancreatic lipase (PPL), and Candida antarctica lipase B (CalB) were obtained from Sigma-Aldrich Inc. Lipase from Pseudomonas sp. (PSL) and PFL were purchased from Amano Pharmaceutical Co., Ltd. These enzymes were used after lyophilization for enzymatic reaction. All the other reagents were purchased from J&K Scientific Ltd. Commercially available reagents and solvents were used without further purification. Solvents and acyl donors were dried over freshly activated molecular sieves (4 Å). Carbon dioxide with a purity of 99.99% was from Changchun Juyang Gas Co., Ltd. The pressure in the system was controlled by liquid CO2 pump (Spe-ed SFE, Applied Separations, Inc.). Chiral HPLC analyses were performed with a Shimadzu LC-10AD apparatus equipped with a SPD-M10A UV detector.

**Experimental materials**

Lipase from Candida sp. (CSL) was purchased from Beijing CTA New Century Biotechnology Co., Ltd. Lipase from Candida antarctica lipase B (CalB) were obtained from Sigma-Aldrich Inc. Lipase from Pseudomonas sp. (PSL) and PFL were purchased from Amano Pharmaceutical Co., Ltd. These enzymes were used after lyophilization for enzymatic reaction. All the other reagents were purchased from J&K Scientific Ltd. Commercially available reagents and solvents were used without further purification. Solvents and acyl donors were dried over freshly activated molecular sieves (4 Å). Carbon dioxide with a purity of 99.99% was from Changchun Juyang Gas Co., Ltd. The pressure in the system was controlled by liquid CO2 pump (Spe-ed SFE, Applied Separations, Inc.). Chiral HPLC analyses were performed with a Shimadzu LC-10AD apparatus equipped with a SPD-M10A UV detector.

**Lipase-catalyzed transesterification of 3-hydroxy-3-(2-thienyl) propanenitrile in liquid CO2**

Racemic 3-hydroxy-3-(2-thienyl) propanenitrile (1 mmol), acyl donor (6 mmol), deionized water (0.04%v/v) and enzyme (protein content: 40 mg) were mixed and sealed in a batch reactor (high-pressure-resistant stainless-steel vessel, 25 mL). The reaction was conducted at 6.5 MPa and 20°C. CO2 gas was sent in to the vessel by a CO2 pump. The vessel was stirred with a magnetic bar. After reaction to the desired time, depressurization and elution by n-hexane was conducted. To determine the conversion of 3-hydroxy-3-(2-thienyl) propanenitrile, the organic samples were withdrawn and analyzed by high performance liquid chromatography (HPLC). The enzyme activity (μmol/g/min) was defined as the amount (in micromoles) of the produced ester per minute per gram of protein content. The experiments were performed triplicate, and all data were obtained based on the average values.

**Determination of enantiomeric excess and enantioselectivity**

The withdrawn reaction mixture was analyzed by chiral HPLC (chiral OJ-H column, Diacel) employing hexane-isopropanol (85:15) as mobile phase at 0.75 mL/min and monitored by UV (254 nm). The degree of conversion (C) was calculated from the reduction of 3-hydroxy-3-(2-thienyl) propanenitrile. The enantiomeric excess (ee_S) was determined by calculating the peak areas of the two enantiomers and the enantiomeric ratio (E value) was determined from C and (ee_S) by using Equation (1)
(26):
\[
\text{enantiomeric excesses, } ee_{\text{S}}(\%) = \frac{[S - R]}{[S + R]} \times 100,
\]
\[
\text{enantioselectivity, } E = \frac{\ln [(1 - C)(1 - ee_{\text{S}})]}{\ln [(1 - C)(1 + ee_{\text{S}})]}.
\]

Conclusions

In this work, we demonstrated that liquid CO\(_2\) is feasible reaction medium for enantioselective transesterification of 3-hydroxy-3-(2-thienyl) propanenitrile catalyzed by lipase. Under the optimal conditions, PFL presented a much higher catalytic performance (enzyme activity: 82.5 \(\mu\)mol/g/min, \(E\)-value: 92.9) than that in n-hexane. When the conversion of racemic alcohol was 52.1\%, the enantiopure (S)-3-hydroxy-3-(2-thienyl) propanenitrile (>99%\text{ee}) could be achieved. Therefore, this mild and efficient method exhibits a high potential for practical preparation of (S)-3-hydroxy-3-(2-thienyl) propanenitrile. Moreover, this study expands the application of liquid CO\(_2\) in biocatalysis.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] Detke, M.J.; Lu, Y.; Goldstein, D.J.; Hayes, J.R.; Demitrack, M.A. Duloxetine, 60 mg Once Daily, for Major Depressive Disorder: A Randomized Double-Blind Placebo-Controlled Trial. J. Clin. Psychiat. 2002, 63, 308–315.

[2] Goldstein, D.J.; Mallinkrodt, C.; Lu, Y.; Demitrack, M.A. Duloxetine in the Treatment of Major Depressive Disorder: A Double-Blind Clinical Trial. J. Clin. Psychiat. 2002, 63, 225–231.

[3] Bymaster, F.P.; Beedle, E.E.; Findlay, J.; Gallagher, P.T.; Krushinski, J.H.; Mitchell, S.; Robertson, D.W.; Thompson, D.C.; Wallace, L.; Wong, D.T. Duloxetine (Cymbalta TM), a Dual Inhibitor of Serotonin and Norepinephrine Reuptake. Bioorg. Med. Chem. Lett. 2003, 13, 4477–4480.

[4] Fuller, R.W.; Hemrick-Luecke, S.K.; Snoddy, H.D. Effects of Duloxetine, an Antidepressant Drug Candidate, on Concentrations of Monoamines and Their Metabolites in Rats and Mice. J. Pharmacol. Exp. Ther. 1994, 269, 132–136.

[5] Perahia, D.G.S.; Wang, F.; Mallinkrodt, C.H.; Walker, D.J.; Detke, M.J. Duloxetine in the Treatment of Major Depressive Disorder: A Placebo-and Paroxetine-Controlled Trial. Eur. Psychiat. 2006, 21, 367–378.

[6] Kamal, A., Gollapalli, B. R. K., Rondla, R., Maddamsetty, V. R. (2006) Chemoenzymatic Process for Stereoselective Preparation of R and S Enantiomers of 2-hydroxy-3-(2-thienyl) Propanenitrile. US Patent 7,045,341.

[7] Venkatram, R.; Pai, V.K.; Nagaraj, S. Novel Enantioselective Synthesis and Dissolution Studies on Enteric Coated Pellets of (S)-Duloxetine Hydrochloride. Bulg. Chem. Commun. 2013, 45, 269–273.

[8] Majer, J.; Kwiatkowski, P.; Jurczak, J. Highly Enantioselective Friedel-Crafts Reaction of Thiophenes with Glyoxylates: Formal Synthesis of Duloxetine. Org. Lett. 2009, 11, 4636–4639.

[9] Kwak, S.H.; Seo, J.M.; Lee, K.I. A Facile Asymmetric Synthesis of (S)-Duloxetine. Archivoc 2010, 10, 55–61.

[10] Kamal, A.; Khanna, G.R.; Ramu, R.; Krishnaji, T. Chemoenzymatic Synthesis of Duloxetine and its Enantiomer: Lipase-Catalyzed Resolution of 3-Hydroxy-3-(2-Thienyl) Propanenitrile. Tetrahedron Lett. 2003, 44, 4783–4787.

[11] Träff, A.; Lihhammar, R.; Bäckvall, J.E. A Chemoenzymatic Dynamic Kinetic Resolution Approach to Enantiomerically Pure (R)- and (S)-Duloxetine. J. Org. Chem. 2011, 76, 3917–3921.

[12] Liu, S.; Xiong, G.; Gao, J.; Li, F.; Wei, S.; Wang, Z.; Wang, L. Ultrasound Promoted Enantioselective Transesterification of 3-Hydroxy-3-(2-Thienyl) Propanenitrile Catalyzed by Lipase. Green Chem. Lett. Rev. 2016, 9 (4), 190–195.

[13] Clark, M.R.; DeSimone, J.M. Cationic Polymerization of Vinyl and Cyclic Ethers in Supercritical and Liquid Carbon Dioxide. Macromolecules 1995, 28, 3002–3004.
[14] Hyatt, J.A. Liquid and Supercritical Carbon Dioxide as Organic Solvents. *J. Org. Chem.* **1984**, *49*, 5097–5101.

[15] King, M.B.; Mubarak, A.; Kim, J.D.; Bott, T.R. The Mutual Solubilities of Water with Supercritical and Liquid Carbon Dioxides. *J. Supercrit. Fluid.* **1992**, *5*, 296–302.

[16] Hoang, H.N.; Matsuda, T. Expanding Substrate Scope of Lipase-Catalyzed Transesterification by the Utilization of Liquid Carbon Dioxide. *Tetrahedron* **2016**, *72*, 7229–7234.

[17] Hoang, H.N.; Matsuda, T. Liquid Carbon Dioxide as an Effective Solvent for Immobilized *Candida Antarctica* Lipase B Catalyzed Transesterification. *Tetrahedron Lett.* **2015**, *56*, 639–641.

[18] Hoang, H.N.; Nagashima, Y.; Mori, S.; Kagechika, H.; Matsuda, T. CO2-Expanded Bio-Based Liquids as Novel Solvents for Enantioselective Biocatalysis. *Tetrahedron* **2017**, *73*, 2984–2989.

[19] Ohgaki, K.; Katayama, T. Isothermal Vapor-Liquid Equilibrium Data for Binary Systems Containing Carbon Dioxide at High Pressures: Methanol-Carbon Dioxide, n-Hexane-Carbon Dioxide, and Benzene-Carbon Dioxide Systems. *J. Chem. Eng. Data* **1976**, *21*, 53–55.

[20] Li, Y.H.; Dillard, K.H.; Robinson, R.L. Vapor-Liquid Phase Equilibrium for Carbon Dioxide-n-Hexane at 40, 80, and 120°C. *J. Chem. Eng. Data* **1981**, *26*, 53–55.

[21] Ottosson, J.; Hult, K. Influence of Acyl Chain Length on the Enantioselectivity of *Candida Antarctica* Lipase B and its Thermodynamic Components in Kinetic Resolution of Sec-Alcohols. *J. Mol. Catal. B-Enzym.* **2001**, *11*, 1025–1028.

[22] Chênevert, R.; Pelchat, N.; Morin, P. Lipase-Mediated Enantioselective Acylation of Alcohols with Functionalized Vinyl Esters: Acyl Donor Tolerance and Applications. *Tetrahedron: Asymmetr.* **2009**, *20*, 1191–1196.

[23] Iso, M.; Chen, B.; Eguchi, M.; Kudo, T.; Shrestha, S. Production of Biodiesel Fuel from Triglycerides and Alcohol Using Immobilized Lipase. *J. Mol. Catal. B-Enzym.* **2001**, *16*, 53–58.

[24] Xun, E.; Wang, J.; Zhang, H.; Chen, G.; Yue, H.; Zhao, J.; Wang, L.; Wang, Z. Resolution of N-Hydroxymethyl Vince Lactam Catalyzed by Lipase in Organic Solvent. *J. Chem. Technol. Biotechnol.* **2013**, *88*, 904–909.

[25] Hsu, C.H.; Tsai, S.W. Lipase-Catalysed Two-Step Desymmetrization of 2-Methylmalonic Dipyrazolide for Preparation of Optically Pure Enantiomer in Organic Solvents. *Biocatal. Biotransfor.* **2017**, *35*, 460–467.

[26] Chen, C.S.; Fujimoto, Y.; Girdaukas, G.; Sih, C.J. Quantitative Analyses of Biochemical Kinetic Resolutions of Enantiomers. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.