Journal of Molecular Catalysis B: Enzymatic 60 (2009) 64–68

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Controllable enzymatic Markovnikov addition and acylation of thiols to vinyl esters

Feng-Wen Lou, Bo-Kai Liu, Jun-Liang Wang, Qian Pan, Xian-Fu Lin*

Department of Chemistry, Zhejiang University, Hangzhou 310027, People's Republic of China

ARTICLE INFO

Article history:
Received 7 January 2009
Received in revised form 18 March 2009
Accepted 20 March 2009
Available online 31 March 2009

Keywords:
Enzyme
Controllable
Markovnikov addition
Acylation
Vinyl esters
Thiols

ABSTRACT

The chemical selectivity between Markovnikov addition and acylation in the reaction of thiols and vinyl esters were developed. The addition reaction was efficiently catalyzed by CAL-B at 50 °C in isopropyl ether, affording the Markovnikov adducts with good yields. Acylation was carried out under the catalysis of D-amino acylase in DMF at 50 °C. A series of thioethers and thioesters were synthesized via the controllable enzymatic methods.

1. Introduction

Enzymes can be used as efficient catalysts with high selectivity in organic synthesis [1]. The control of the selectivity could be achieved by many methods. Riva reported the remote control of lipase PS site- and regioselectivity in the enzymatic acetylation of stevioside and steviolbioside by substrate modification [2]. Site-directed mutation was used to control the substrate selectivity of deacetoxycephalosporin/deacetylcephalosporin C synthase [3]. Media engineering was a simple one among the methods. The selectively enzymatic *N*- and *O*-acylation were achieved by organic solvents [4]. Furthermore, we also controlled the selectivity between anti-Markovnikov addition and Markovnikov addition using different organic media [5].

Recently, more and more studies have clearly demonstrated that enzymes have catalytic promiscuity which brings about an exponential increase in interest in the field of organic synthesis [6–12]. Among them, hydrolases particularly show great potential. Subtilisin not only catalyzed hydrolysis of a sulfonamide S–N bond in an *N*-acyl sulfonamide, but also showed a remarkable activity in Michael addition of *N*-nucleophiles to acrylates [13–18]. Acylase was proved to be able to promote aza-Markovnikov reaction [19–21]. In the further studies, it also displayed catalytic ability for Michael addition [22,23]. The recent progress in catalytic promiscuity of CAL-B has greatly extended its application. For example, CAL-B has the capability to promote the C-N Michael addition between acrylonitrile and a variety of secondary amine [24]. The wild-type and the Ser105Ala mutant of CAL-B could catalyze C–C bond formation through Michael addition of nucleophiles to a range of α, β-unsaturated carbonyl compounds, or the aldol reaction [25–28]. Thus, exploration of enzymes with new activities has continued to increase in popularity. Moreover, the more reactions could be promoted by enzyme, the more significant is it to control the selectivity among these reactions.

Herein, we report the unprecedented observation of C–S bond formation based on the promiscuous catalysis of enzyme. The selectivity of reaction type between Markovnikov addition and acylation was controlled by mediating enzymes in organic media. The Markovnikov addition was efficiently catalyzed by the CAL-B in isopropyl ether at 50 °C, affording the thiolethers in good yields with high regioselectivity. A series of thioesters were obtained under the catalysis of D-amino acylase in DMF at 50 °C (Scheme 1).

2. Experimental

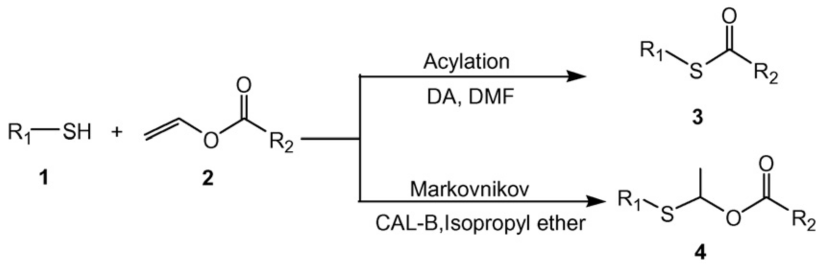
2.1. Materials

An immobilized lipase from *Candida antarctica* on macroporous acrylic resin (CAL-B) were purchased from Sigma. D-Amino acylase from *Escherichia coli* (DA) and Acylase "Amano" from *Aspergillus oryzae* (AA) were purchased from Amano Enzyme Inc. (Japan).

---

* Corresponding author. Fax: +86 571 87952618.
E-mail address: llc123@zju.edu.cn (X.-F. Lin).

1381-1177/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2009.03.013



Scheme 1. Reaction of thiols to vinyl esters.

Lipase from *Candida cylindracea* (CCL) was purchased from Fluka (Switzerland). Lipase Type VII from *Candida rugosa* (CRL) was purchased from Sigma (Steinheim, Germany). Immobilized Penicillin G Acylase from *Escherichia coli* (IPA) was obtained from Hunan Enzyme Co. Ltd. (China). All solvents were analytical grade and were dried by storing over activated 3 Å molecular sieves before use. All other reagents were used as received.

2.2. Analytical methods

The process of reactions was monitored by TLC on silica with Petroleum Ether/EtOAc (10/1 to 30/1, v/v). The ^1H and ^{13}C NMR spectra were recorded with TMS as internal standard using a Bruker AMX-500 MHz spectrometer. Chemical shifts were expressed in ppm and coupling constants (*J*) in Hz. Mass spectrometry data were obtained on Bruker Esquire-LC for electrospray (ESI-MS) measurements. Analytical GC was performed using Agilent 6890 series with SE-54 capillary column and FID detector. IR spectra were measured with a Nicolet Nexus FTIR 670 spectrophotometer.

2.3. Synthesis of S-benzyl thioacetate (3a) [29]

A suspension of benzyl thiol (1.0 mmol) and 20 mg DA in 1 mL DMF was incubated at 50 °C and 200 rpm (orbitally shaken) for 5 min. Then, 1.2 equiv. of vinyl ester was added to initiate the reaction. The mixture was shaken for 48 h. The GC yield was 96%. The enzyme was filtered off to terminate the reaction and washed with ether (3–5 mL). Solvent was evaporated under vacuum to dryness. The crude product was purified by chromatography on silica gel by eluting with petroleum/ethyl acetate (10:1, by vol.). The product was light yellow oil. IR (neat): 3063, 3029, 2964, 2932, 2874, 1689, 1602, 1495, 1454, 1411, 1112, 1006, 701 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz, δ, ppm): 7.29–7.25 (m, 5H, C_6H_5-), 4.12 (s, 2H, $C_6H_5-CH_2-S-C{=}O$), 2.34 (s, 3H, $O{=}C-CH_3$). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm): 195.3 ($-C{=}O$), 137.7 (C_6H_5-), 129.0 (C_6H_5-), 128.8 (C_6H_5-), 127.5 (C_6H_5-), 33.7 ($C_6H_5-CH_2-S-$), 30.5 ($O{=}C-CH_3$). ESI-MS *m*/*z* calcd. for $[M+Na]^+$ $C_9H_{10}OSNa$ 189.0, found 189.1.

2.4. Synthesis of S-butyl thioacetate (3b) [29]

S-Butyl thioacetate was synthesized using the same method as for **3a**. The yield of 48 h was 95%. The product was light yellow oil. IR (neat): 2960, 2932, 2874, 1693, 1466, 1427, 1354, 1136, 1109, 956 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz, δ, ppm): 2.86 (s, 2H, $O{=}C-S-CH_2-CH_2-$), 2.31 (s, 3H, $O{=}C-CH_3$), 1.53 (m, 2H, $O{=}C-S-CH_2-CH_2-$), 1.36 (m, 2H, $-CH_2-CH_3$), 0.90 (t, 3H, $-CH_2-CH_3$). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm): 196.0 ($-C{=}O$), 31.5 ($-S-CH_2-CH_2-CH_2-CH_3$), 30.5 ($O{=}C-CH_3$), 28.7 ($-S-CH_2-CH_2-CH_2-CH_3$), 21.8 ($-S-CH_2-CH_2-CH_2-CH_3$), 13.5 ($-S-CH_2-CH_2-CH_2-CH_3$). ESI-MS *m*/*z* calcd. for $[M+Na]^+$ $C_6H_{12}OSNa$ 155.0, found 154.9.

2.5. Synthesis of S-dodecyl thioacetate (3c) [30]

S-Dodecyl thioacetate was synthesized using the same method as for **3a**. The yield of product was 91% after 48 h. The product was light yellow oil. IR (neat): 2963, 2924, 2853, 1695, 1466, 1377, 1353, 1135, 954, 722, 627 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz, δ, ppm): 2.86 (t, 2H, $-S-CH_2-CH_2-$), 2.30 (s, 3H, $O{=}C-CH_3$), 1.56 (m, 2H, $-S-CH_2-CH_2-$), 1.36–1.26 (br, 18H, 9 $-CH_2-$), 0.88 (t, 3H, $-CH_2-CH_3$). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm): 195.9 ($-C{=}O$), 31.8, 30.5, 29.6, 29.5, 29.4, 29.4, 29.2, 29.0, 29.0, 28.9, 28.7, 22.6 ($-CH_2-CH_3$), 14.0 ($-CH_2-CH_3$). ESI-MS *m*/*z* calcd. for $[M+Na]^+$ $C_{14}H_{28}OSNa$ 267.0, found 267.0.

2.6. Synthesis of S-benzyl thiobutyrate (3d) [31]

S-Benzyl thiobutyrate was synthesized using the same synthesis method as for **3a**. The yield of product was 93% after 48 h. The product was light yellow oil. IR (neat): 3065, 3029, 2965, 2932, 2874, 1687, 1601, 1495, 1454, 1006, 705 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz, δ, ppm): 7.29–7.28 (m, 5H, C_6H_5-), 4.12 (s, 2H, $C_6H_5-CH_2-S-C{=}O$), 2.55 (m, 2H, $-CH_2-CH_2-CH_3$), 1.71 (m, 2H, $-CH_2-CH_2-CH_3$), 0.95 (t, 3H, $-CH_2-CH_2-CH_3$). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm): 198.9 ($-S(O{=}C)CH_2-CH_2-CH_3$), 137.7 (C_6H_5-), 129.0 (C_6H_5-), 128.8 (C_6H_5-), 127.5 (C_6H_5-), 45.9 ($-CH_2-CH_2-CH_3$), 33.3 ($C_6H_5-CH_2-S-$), 19.3 ($-CH_2-CH_2-CH_3$), 13.7 ($-CH_2-CH_2-CH_3$). ESI-MS *m*/*z* calcd. for $[M+Na]^+$ $C_{11}H_{14}OSNa$ 194.1, found 193.9.

2.7. Synthesis of 1-(benzylthio) ethyl acetate (4a) [5]

A suspension of benzyl thiol (1.0 mmol) and 20 mg CAL-B in 4 mL isopropyl ether was incubated at 50 °C and 200 rpm (orbitally shaken) for 5 min. Then, 4.0 equiv. of vinyl ester was added to initiate the reaction. The mixture was shaken for 48 h. The GC yield was 81%. The enzyme was filtered off to terminate the reaction and washed with ether (3–5 mL). Solvent was evaporated under vacuum to dryness. The crude product was purified by chromatography on silica gel by eluting with petroleum/ethyl acetate (20:1, by vol.). The product was light yellow oil. IR (neat): 3062, 3029, 2983, 2932, 1739, 1602, 1495, 1453, 1370, 1229, 934, 702 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz, δ, ppm): 7.32–7.23 (m, 5H, C_6H_5-), 6.00 (q, 1H, $J = 6.4$ Hz, $CH-CH_3$), 3.85 (q, 2H, $C_6H_5-CH_2-S-$), 1.92 (d, 3H, $O{=}C-CH_3$), 1.50 (d, 3H, $J = 6.4$ Hz, $-CH-CH_3$). ^{13}C NMR ($CDCl_3$, 100 MHz, δ, ppm): 170.2 ($-C{=}O$), 137.8 (C_6H_5-), 128.6 (C_6H_5-), 128.4 (C_6H_5-), 126.9 (C_6H_5-), 75.2 ($-CH-CH_3$), 35.3 ($C_6H_5-CH_2-S-$), 21.2 ($-CH-CH_3$), 20.9 ($O{=}C-CH_3$). ESI-MS *m*/*z* calcd. for $[M+Na]^+$ $C_{11}H_{14}O_2S$ Na 233.0, found 233.1.

2.8. Synthesis of 1-(benzylthio) ethyl butyrate (4b) [5]

1-(Benzylthio) ethyl butyrate was synthesized using the same method as for **4a**. The yield was 72% after 48 h. The product was light yellow oil. IR (neat): 3062, 3029, 2983, 2932, 1739, 1602, 1495, 1453, 1228, 935, 702 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): 7.32–7.22 (m, 5H, C_6H_5-), 6.02 (q, 1H, J = 6.8 Hz, $CH-CH_3$), 3.84 (q, 2H, $C_6H_5-CH_2-S-$), 2.21 (t, 2H, $O{=}C-CH_2-CH_2-CH_3$), 1.64 (m, 2H, $O{=}C-CH_2-CH_2-CH_3$), 1.51 (d, 3H, J = 6.8 Hz, $-CH-CH_3$), 0.95 (t, 3H, $O{=}C-CH_2-CH_2-CH_3$). ^{13}C NMR ($CDCl_3$, 100 MHz, δ, ppm): 172.9 ($-C{=}O$), 137.8 (C_6H_5-), 128.6 (C_6H_5-), 128.3 (C_6H_5-), 126.9 (C_6H_5-), 74.9 ($-CH-CH_3$), 36.0 ($O{=}C-CH_2-CH_2-CH_3$), 35.2 ($C_6H_5-CH_2-S-$), 21.2 ($-CH-CH_3$), 18.1 ($O{=}C-CH_2-CH_2-CH_3$), 13.4 ($O{=}C-CH_2-CH_2-CH_3$). ESI-MS m/z calcd. for $[M+Na]^+$ $C_{13}H_{18}O_2S$ Na 261.0, found 260.9.

2.9. Synthesis of 1-(benzylthio) ethyl caproate (**4c**) [5]

1-(Benzylthio) ethyl caproate was synthesized using the same method as for **4a**. The yield was 62% after 48 h. The product was light yellow oil. IR (neat): 3063, 3030, 2957, 2931, 2871, 1736, 1602, 1495, 1454, 1375, 1240, 1165, 1059, 1003, 701 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): 7.31–7.24 (m, 5H, C_6H_5-), 6.03 (q, 1H, J = 6.8 Hz, $CH-CH_3$), 3.86 (q, 2H, $C_6H_5-CH_2-S-$), 2.24 (s, 2H, $O{=}C-CH_2-$), 1.61 (t, 2H, $-CH_2-CH_2-CH_2-CH_3$), 1.52 (d, 3H, J = 6.8 Hz, $-CH-CH_3$), 1.31 (m, 4H, $-CH_2-CH_2-CH_2-CH_3$), 0.92 (t, 3H, $-CH_2-CH_2-CH_2-CH_3$). ^{13}C NMR ($CDCl_3$, 100 MHz, δ, ppm): 173.1 ($-C{=}O$), 137.8 (C_6H_5-), 128.7 (C_6H_5-), 128.4 (C_6H_5-), 126.9 (C_6H_5-), 74.9 ($-CH-CH_3$), 35.2, 34.2, 31.1, 24.3, 22.1, 21.2 ($-CH_2-CH_3$), 13.8 ($-CH_2-CH_3$). ESI-MS m/z calcd. for $[M+Na]^+$ $C_{15}H_{22}O_2S$ Na 289.1, found 289.0.

2.10. Synthesis of 1-(butylthio)ethyl acetate (**4d**) [5]

1-(Butylthio)ethyl acetate was synthesized using the same synthesis method as for **4a**. The yield of product was 67% after 48 h. The product was light yellow oil. IR (neat): 2959, 2930, 2873, 1742, 1457, 1370, 1231, 1053, 1018, 933, 838 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): 6.06 (d, 1H, J = 6.4 Hz, $-CH-CH_3$), 2.63 (t, 2H, $-S-CH_2-CH_2-$), 2.07 (s, 3H, $O{=}C-CH_3$), 1.56 (m, 5H, J = 6.4 Hz, $-CH-CH_3$, $-S-CH_2-CH_2-$), 1.38 (m, 2H, $-CH_2-CH_3$), 0.91 (m, 3H, $-CH_2-CH_3$). ^{13}C NMR ($CDCl_3$, 100 MHz, δ, ppm): 170.4 ($-C{=}O$), 75.4 ($-CH-CH_3$), 31.8, 30.3, 21.9, 21.4, 21.3, 13.6 ($-CH_2-CH_3$). ESI-MS m/z calcd. for $[M+Na]^+$ $C_8H_{16}O_2S$ Na 199.0, found 198.8.

2.11. Synthesis of 1-(butylthio)ethyl butyrate (**4e**) [5]

1-(Butylthio)ethyl butyrate was synthesized using the same synthesis method as for **4a**. The yield of product was 52% after 48 h. The product was light yellow oil. IR (neat): 2962, 2933, 2875, 1737, 1459, 1376, 1173, 1055, 1001, 929 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): 6.06 (d, 1H, J = 6.4 Hz, $-CH-CH_3$), 2.60 (t, 2H, $-S-CH_2-$), 2.27 (t, 2H, $O{=}C-CH_2-$), 1.50–1.38 (m, 7H, J = 6.4 Hz, $-CH-CH_3$, $-S-CH_2-CH_2-CH_2-CH_3$, $O{=}C-CH_2-CH_2-CH_3$), 1.33 (m, 2H, $-S-CH_2-CH_2-CH_2-CH_3$), 0.91 (m, 6H, $-CH_3$, $-CH_3$). ^{13}C NMR ($CDCl_3$, 100 MHz, δ, ppm): 172.8 ($-C{=}O$), 74.9 ($-CH-CH_3$), 36.2, 31.7, 30.1, 21.7, 21.3, 18.2, 13.4, 13.4 ($-CH_2-CH_3$). ESI-MS m/z calcd. for $[M+Na]^+$ $C_8H_{16}O_2S$ Na 199.0, found 198.8.

2.12. Synthesis of 1-(dodecylthio)ethyl acetate (**4f**) [5]

2-(Dodecylthio)ethanol acetate was synthesized by the same synthesis method as for **4a**. The yield of product was 38% after 48 h. The product was light yellow oil. IR (neat): 2961, 2925, 2874, 1743, 1466, 1370, 1228, 1058, 1018, 933, 838 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): 6.06 (d, 1H, $-CH-CH_3$), 2.63 (t, 2H, $-S-CH_2-CH_2-$), 2.07 (t, 2H, $O{=}C-CH_2-$), 1.62–1.53 (m, 5H, $-S-CH_2-CH_2-$, $-CH-CH_3$), 1.36–1.25 (br, 18H, 9 $-CH_2-$), 0.87 (t, 3H, CH_2-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz, δ, ppm): 170.4 ($-O-C{=}O$), 75.4, 31.9, 30.6, 29.8, 29.6, 29.6, 29.5, 29.5, 29.3, 29.1, 28.8, 22.6, 21.4, 21.3, 14.1. ESI-MS m/z calcd. for $[M+Na]^+$ $C_{16}H_{32}O_2S$ Na 311.2, found 311.2.

3. Results and discussion

3.1. Screening for acylation

Firstly, the reaction of benzyl thiol with 1.2 equiv of vinyl acetate **2a** was carried out in DMF at 50 °C under the catalysis of DA. The formation of a single product **3a** with 96.1% yield was obtained. The structure of this compound was characterized by ^{1}H NMR, ^{13}C NMR and ESI-MS. Results showed that an acylation reaction was occurred to give thiol ester products. It was surprising that DA, a zinc binding metalloenzyme which naturally catalyzes the hydrolysis of N-acetyl-D-amino acids, presented the promiscuity for C–S acylation activity. Other acylases, including AA and IPA (entries 1, 3 and 5, Table 1), also promoted the C–S acylation leading to the thioesters **3a** in good yield. Three widely used hydrolases, CCL, CRL and CAL-B, cannot promote the acylation reaction efficiently in DMF (entries 2 and 4, Table 1). In the control experiment, the reaction between benzyl thiol and vinyl acetate resulted in a very low yield (2.3%) after 48 h in the absence of catalyst, which prove that DA was in charge of the acylation. All these results suggested that the tertiary structure and the specific spatial conformation of acylase were necessary for the acylation reaction of benzyl thiol to vinyl acetate. In DMF, DA displayed the highest catalytic activity for the desired transformation. Thus, DA was chosen as catalyst in the following experiments.

3.2. Screening for Markovnikov addition

From Table 2, CAL-B showed the high selectivity for Markovnikov addition. In order to improve the activity of enzyme, some conventional organic solvents with different log P values were screened for the enzymatic reaction and the results were shown in Table 2. CAL-B showed high Markovnikov addition activity in isopropyl ether and led to 83.9% GC yield after 48 h (entry 6, Table 2). In other polar solvents such as acetonitrile, tetrahydrofuran, t-butanol, DMF and DMSO, the yields are far from satisfactory (entries 1–5, Table 2). In those solvents such as methyl cyclohexane, isopropyl ether, toluene, or n-hexane, the Markovnikov addition products were generated as major products. Furthermore, the isopropyl ether could not promote the reaction, because only 1.3% yield was observed after 48 h in the absence of enzyme. Thus, isopropyl ether was chosen as solvents in the following experiments. It has been reported that organic solvents would cause a significant change in enzyme conformation. Polar solvents such as DMSO could affect the unfolding mecha-

Table 1
Reaction between benzyl thiol (**1a**) and vinyl acetate (**2a**) in the presence of different catalysts[a].

Entry	Enzyme	Yield (%)[b]	
		3a	**4a**
1	AA	55.8	3.6
2	CRL	1.6	–
3	DA	96.1	0.2
4	CCL	1.4	–
5	IPA	74.3	0.8
6	CAL-B	0.6	2.5
7	–	2.3	2.6

Abbreviation: CAL-B, lipase from *Candida antarctica* on macroporous acrylic resin; DA, D-amino acylase from *Escherichia coli*; AA, Acylase "Amano" from *Aspergillus oryzae*; CCL, Lipase from *Candida cylindracea*; CRL, Lipase Type VII from *Candida rugosa*; IPA, Immobilized Penicillin G Acylase from *Escherichia coli*.

[a] Conditions: benzyl thiol (1.0 mmol), vinyl acetate (1.2 mmol), enzyme (20 mg), DMF (1 mL), 50 °C for 48 h.

[b] Product distribution was calculated from the GC results.

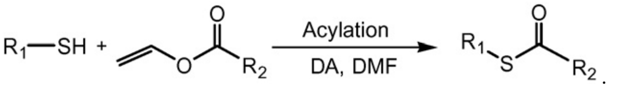
Table 2
CAL-B catalyzed reaction of benzyl thiol (**1a**) to vinyl acetate (**2a**) in different solvent (log *P*)[a].

Entry	Solvent	Log *P*	CAL-B (mg)	Yield (%)[b]	
				3a	**4a**
1	DMSO	−1.3	20	4.3	2.1
2	DMF	−1.0	20	0.6	2.5
3	Acetonitrile	−0.33	20	7.3	3.4
4	Tetrahydrofuran	0.49	20	7.1	4.8
5	t-Butanol	0.80	20	3.4	15.3
6	Isopropyl ether	1.9	20	7.6	83.0
7	Toluene	2.6	20	6.3	56.0
8	Methylcyclohexane	3.7	10	5.7	58.7.
9	n-Hexane	3.9	20	8.8	64.1
10	Isopropyl ether	1.9	–	0.6	1.3

[a] Reactions were carried out with 1.0 mmol of benzyl thiol, 4.0 mmol of vinyl acetate, and 20 mg of CAL-B in 1.0 mL of solvent at 50 °C for 48 h.

[b] Product distribution was calculated from the GC results.

Table 3
Acylation reaction between thiols and vinyl acetates[a].



Entry	Substrate R_1	Vinyl ester R_2	DA (mg)	Solvent	Product	**3** Yield (%)[b]
1	$C_6H_5CH_2$	CH_3	20	DMF	**3a**	96
2	n-C_4H_9	CH_3	20	DMF	**3b**	95
3	n-$C_{12}H_{25}$	CH_3	20	DMF	**3c**	91
4	$C_6H_5CH_2$	$(CH_2)_2CH_3$	20	DMF	**3d**	93
5	C_6H_5	CH_3	20	DMF	**3e**	–

[a] Reactions were carried out on 1.0 mmol scale of thiols with 1.2 equiv. of vinyl acetate in 1.0 mL of DMF at 50 °C for 48 h.

[b] Was calculated from the GC results.

nism and increase the conformational flexibility of enzyme [32–35]. In non-polar solvents, the hydrophilic surface of CALB decreased by 10% in comparison to water, while the hydrophobic surface is slightly increased by 1%. Based on these, we speculated that the selectivity in isopropyl ether may be attributed to the conformational changes of enzyme.

3.3. The selective synthesis of thiol esters via enzymatic acylation

The enzymatic acylation of a variety of structurally diverse thiols and vinyl esters compounds were carried out. The results were summarized in Table 3. All the thiols underwent the transesterification with vinyl acetate smoothly to give the corresponding products with excellent yields, except thiophenol (entries 1–5, Table 3). The reason that thiophenol displayed low activity could be ascribed to the existence of a strong acidic proton, which can inactivate enzyme. The reactivity decreased by the following order: benzyl thiol, butyl thiol, and n-dodecanethiol, which was in accordance with the nucleophilicity of thiols. As compared with butyl thiol, n-dodecanethiol had long chain and high steric hindrance, and underwent esterification in slightly decreasing yield.

3.4. The selective synthesis of thiol ethers via enzymatic Markovnikov addition

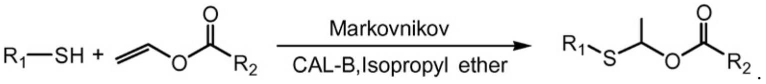
Different thiols and vinyl esters were employed as substrates for the synthesis of thiol ethers. The results were summarized in Table 4. In isopropyl ether all the addition reactions afforded the Markovnikov adduct exclusively or in high selectivities. The reaction of vinyl acetates in the catalysis of CAL-B ($20\,mg\,mL^{-1}$) in dipropyl ether at 50 °C gave the corresponding Markovnikov adducts in moderate to good yields. Small amount of thioesters were obtained as the by-product. Addition of benzyl thiol to vinyl acetate afforded Markovnikov adducts **4a** in 81% isolated yield and 87% of addition reaction selectivity, and the ratio of **4a** to **3a** was 87:13 (entry 1, Table 4). However, the reactivity of the acceptor decreased as the chain length increased. Lower yields were obtained from the vinyl esters with longer chain (entries 1–3, Table 4). Besides the acceptors, the donors also had important influence on the reaction. Vinyl butyrate and vinyl hexanoate reacted with benzyl thiol to give the corresponding adducts in moderate yields and high selectivity as the same as vinyl acetate, and the ratio of Markovnikov adducts to acylations was about 83:17 (entries 2 and 3, Table 4). Butyl mercaptan and dodecyl mercaptan could easily afford the desired products with good selectivity (entries 4–6, Table 4). Comparatively, when the activities of mercaptan became weaker gradually, the yield of the corresponding products also decreased (entries 1, 4, 6 and entries 2, 5; Table 4). However, n-dodecanethiol showed rather lower activity because of the strong steric hindrance (entry 6, Table 4).

The enantioselectivity of catalyst was also tested. To our disappointment, none or little optical activity was observed for any tested Markovnikov adducts. These revealed that the active sites might perform the promiscuous activity in some specific way. The study on the enantioselectivity will be further investigated.

4. Conclusion

In conclusion, we have developed a facile biotransformation path to perform Markovnikov additions and acylations of thiols and vinyl esters under the catalysis of CAL-B and DA, respectively. These two enzymatic promiscuous reactions could be controlled in organic media and were successfully applied for the synthesis of thiolesters and thiolethers. The works on enzyme engineering to investigate the reaction mechanism are being further considered in our lab.

Table 4
Markovnikov addition between thiols and vinyl acetates in the presence of different catalysts[a].



Entry	Nu-H	Substrate	**1** Conversion (%)[b]	**4** Yield (%)[c]	Selectivity[b] **4/3**
1	$C_6H_5CH_2$	CH_3	99.6	81	86.7/13.2
2	$C_6H_5CH_2$	$(CH_2)_2CH_3$	91.3	72	82.6/17.1
3	$C_6H_5CH_2$	$(CH_2)_4CH_3$	80.5	62	84.6/14.4
4	n-C_4H_9	CH_3	92.1	67	79.4/20.4
5	n-C_4H_9	$(CH_2)_2CH_3$	75.8	52	85.2/10.4
6	n-$C_{12}H_{25}$	CH_3	51.9	38	80.3/17.5

[a] Reactions were carried out on 1.0 mmol scale of thiol with 4 equiv. of vinyl acetates in 4.0 mL of isopropyl ether at 50 °C for 48 h.

[b] Conversion was calculated from the GC results.

[c] Was calculated from the GC results.

Acknowledgements

This investigation has enjoyed financial support from the Natural Science Foundation of China (no. 20572099, no. 20872130), Ph.D. Programs Foundation of Ministry of Education of China (20060335031) and The Zhejiang Provincial Natural Science Foundation (Project no. 2008-Y407086).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcatb.2009.03.013.

References

[1] V. Gotor-Fernandez, E. Busto, V. Gtor, Adv. Synth. Catal. 348 (2006) 797–812.
[2] G. Colombo, S. Riva, B. Danieli, Tetrahedron 60 (2004) 741–746.
[3] M.D. Lloyd, S.J. Lipscomb, K.S. Hewitson, C.M.H. Hensgens, J.E. Baldwin, C.J. Schofield, J. Biol. Chem. 279 (2004) 15420–15426.
[4] J. Quan, N. Wang, X.Q. Cai, Q. Wu, X.F. Lin, J. Mol. Catal. B: Enzym. 44 (2007) 1–7.
[5] F.W. Lou, B.K. Liu, Q. Wu, D.S. Lv, X.F. Lin, Adv. Synth. Catal. 350 (2008) 1959–1962.
[6] A. Zaks, A.M. Klibanov, Science 224 (1984) 1249–1253.
[7] K. Tao, A.M. Klibanov, J. Am. Chem. Soc. 121 (1999) 3334–3348.
[8] S. Kobayashi, H. Uyama, S. Kimura, Chem. Rev. 101 (2001) 3793–3818.
[9] R.A. Gross, A. Kumar, B. Kalra, Chem. Rev. 101 (2001) 2097–2124.
[10] L. Ferreiraa, M.H. Gila, J.S. Dordick, Biomaterials 23 (2002) 3957–3967.
[11] J.H. Xu, J. Mol. Catal. B: Enzym. 18 (2002) 199–203.
[12] E. Garcia-Urdiales, I. Alfonso, V. Gotor, Chem. Rev. 105 (2005) 313–354.
[13] P.F. Mugford, V.P. Magloire, R.J. Kazlauskas, J. Am. Chem. Soc. 127 (2005) 6536–6537.
[14] Y. Cai, X.F. Sun, N. Wang, X.F. Lin, Synthesis 5 (2004) 671–674.
[15] Y. Cai, S.P. Yao, Q. Wu, X.F. Lin, Biotechnol. Lett. 26 (2004) 525–528.
[16] Y. Cai, Q. Wu, Y.M. Xiao, D.S. Lv, X.F. Lin, J. Biotechnol. 121 (2006) 330–337.
[17] S.P. Yao, D.S. Lv, Q. Wu, Y. Cai, S.H. Xu, X.F. Lin, Chem. Commun. 17 (2004) 2006–2007.
[18] D.R.J. Palmer, J.B. Garrett, V. Sharma, R. Meganathan, P.C. Babbitt, J.A. Gerlt, Biochemistry 38 (1999) 4252–4258.
[19] W.B. Wu, N. Wang, J.M. Xu, Q. Wu, X.F. Lin, Chem. Commun. 18 (2005) 2348–2350.
[20] W.B. Wu, J.M. Xu, Q. Wu, D.S. Lv, X.F. Lin, Synlett 16 (2005) 2433–2436.
[21] W.B. Wu, J.M.Q. Xu, D.S. Lv, X.F. Lin, Adv. Synth. Catal. 348 (2006) 487–492.
[22] C. Qian, J.M. Xu, Q. Wu, D.S. Lv, X.F. Lin, Tetrahedron Lett. 48 (2007) 6100–6104.
[23] J.M. Xu, F. Zhang, B.K. Liu, Q. Wu, X.F. Lin, Chem. Commun. 20 (2007) 2078–2080.
[24] O. Torre, I. Alfonso, V. Gotor, Chem. Commun. 15 (2004) 1724–1725.
[25] C. Branneby, P. Carlqvist, K. Hult, T. Brinck, P. Berglund, J. Mol. Catal. B: Enzym. 31 (2004) 123–128.
[26] O. Torre, V. Gotor-Fernandez, I. Alfonso, L.F. Garcia-Alles, V. Gotor, Adv. Synth. Catal. 347 (2005) 1007–1014.
[27] A.O. Magnusson, M. Takwa, A. Hamberg, K. Hult, Angew. Chem. Int. Ed. 44 (2005) 4582–4585.
[28] M. Svendendahl, K. Hult, P. Berglund, J. Am. Chem. Soc. 127 (2005) 17988–17989.
[29] Y.Q. Cao, Y.F. Du, B.H. Chen, Z. Dai, J. Chem. Res. 11 (2004) 762–763.
[30] P. Ferraboschi, A. Fiecchi, P. Grisenti, E. Santaniello, S. Trave, Synth. Commun. 17 (1987) 1569–1575.
[31] S. Ahmad, J. Iqbal, Tetrahedron Lett. 27 (1986) 3791–3794.
[32] E. Castillo, F. Pezzotti, A. Navarro, A. López-Munguía, J. Biotechnol. 102 (2003) 251–259.
[33] H. Cheng, I. Nikolic-Hughes, J.H. Wang, H. Deng, P.J. O'Brien, L. Wu, Z.Y. Zhang, D. Herschlag, R. Callender, J. Am. Chem. Soc. 124 (2002) 11295–11306.
[34] K. Watanabe, S. Ueji, Biotechnol. Lett. 22 (2000) 599–603.
[35] Z.W. Yang, S.W. Tendian, W.M. Carson, W.J. Brouillette, L.J. Delucas, C.G. Brouillette, Protein Sci. 13 (2004) 830–841.