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Candida antarctica Lipase B (CAL-B)-Catalyzed Carbon-Sulfur Bond Addition and Controllable Selectivity in Organic Media

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Abstract: A novel enzymatic, promiscuous protocol for *Candida antarctica* lipase B (CAL-B)-catalyzed carbon-sulfur bond addition is described. Some control experiments have been designed to demonstrate the catalytic specificity of CAL-B. Selectivity between anti-Markovnikov addition and Markovnikov addition was achieved in different organic media. A series of thioether-containing ester functional groups was synthesized under the catalysis of CAL-B at 50 °C. All the products were characterized by spectroscopic methods (IR, NMR, ESI-MS).

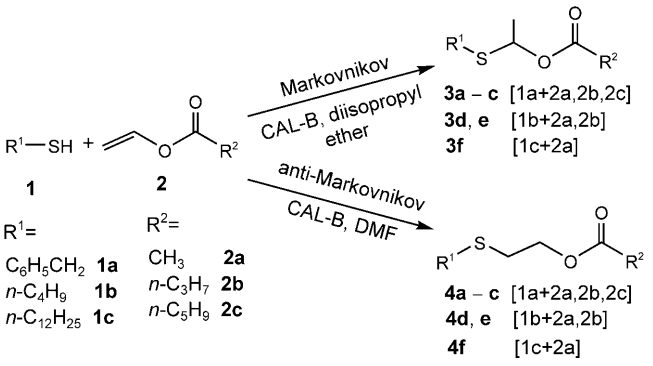
Keywords: anti-Markovnikov addition; catalytic promiscuity; enzyme catalysis; regioselectivity; thiols

Enzymatic synthesis in organic media has attracted an exponentially increasing interest in the field of organic synthesis.[1] The recent progress in catalytic promiscuity of enzymes has greatly extended the application of biocatalysis in organic chemistry. Subtilisin not only catalyzed the hydrolysis of a sulfonamide S–N bond, but also showed a remarkable activity in the Michael addition of N-nucleophiles to acrylates.[2] Acylase proved to be able to promote C–N bond formation *via* the aza-Markovnikov reaction.[3] Subsequent studies revealed that it also displayed catalytic ability for the Michael addition between aromatic N-heterocycles and α,β-unsaturated compounds.[4] Some lipases showed their great potential application as carboxylic acid esterases, thioesterases, peptidases, dehalogenases, epoxide hydrolases and halo peroxidases, etc.[5] Thus, the exploration of enzymes with new activities becomes particularly fascinating and remains a great challenge.

Among all the enzymes, *Candida antarctica* lipase B (CAL-B) shows the advantages of commercial availability and easy use and recycling. It is a very effective catalyst for the synthesis and resolution of a wide range of compounds for pharmaceuticals and industry. In recent years, CAL-B showed promiscuity in addition reactions. For example, Gotor used CAL-B to catalyze the Michael addition of a variety of secondary amines with acrylonitrile and obtained the corresponding adducts.[6] Berglund reported that the Ser105Ala mutant of CAL-B showed catalytic ability for the aldol reaction and the Michael addition of nucleophiles to α,β-unsaturated carbonyl compounds to form C–C bonds.[7]

In the present work, CAL-B was first found to be able to catalyze C–S bond addition. Controllable selectivity in the Markovnikov and anti-Markovnikov addition between thiols and vinyl esters was achieved in different organic solvents. A series of thioethers containing ester functional groups were prepared *via* controllable C–S bond addition under the catalysis of CAL-B.

We first examined the addition reaction of benzyl thiol (**1a**) and vinyl acetate (**2a**) (Scheme 1) in DMF. When 0.25 mmol **1a** was added to a solution containing 10 mg *Candida antarctica* lipase B (CAL-B) and 0.3 mmol **2a** in DMF at 50 °C, a single product was



Scheme 1. CAL-B-catalyzed reaction of thiols with vinyl esters.

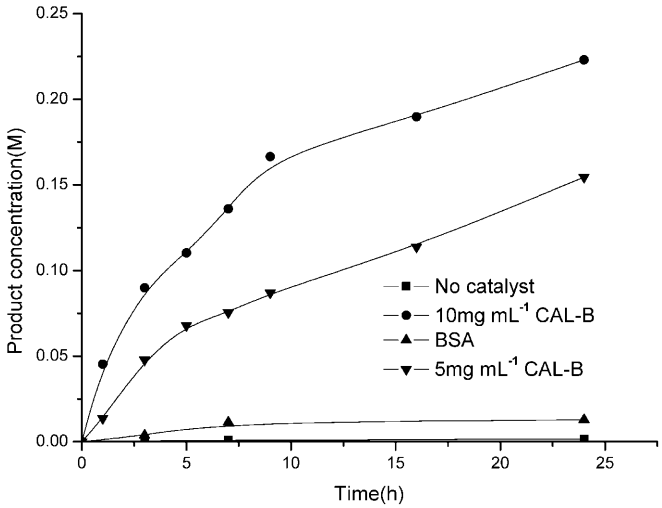


Figure 1. Progress curves of the anti-Markovnikov addition of benzyl thiol (**1a**) to vinyl acetate (**2a**).

prepared in 86% isolated yield after 24 h. The structure of this compound assigned as **4a** was confirmed by IR, ^{1}H NMR, ^{13}C NMR. It showed that the C–S bond addition *via* an anti-Markovnikov addition reaction in DMF was promoted by CAL-B. The progress curves are shown in Figure 1 and the initial reaction rates were calculated accordingly. The reaction proceeded efficiently under the catalysis of CAL-B and the initial reaction rate was up to 550.13 μM min^{-1}. 91.6% yield was obtained in 24 h as detected by GC. As seen from Figure 1, the initial reaction rate is practically proportional to the enzyme amount, suggesting the catalytic effect of the enzyme.

Taking into account these results, some control experiments were performed focusing on the specific catalytic effect of the anti-Markovnikov reaction (Table 1). The reaction of benzyl thiol with vinyl acetate in the absence of enzyme led to the anti-Markovnikov adduct in very low yield (Entry 1, Table 1) after 24 h. In contrast, the reactions in the presence of CAL-B were up to 100-fold faster (entry 2, Table 1). When the reactions were incubated with denatured CAL-B or bovine serum albumin (BSA), both the initial rates were almost equal to the background reaction (entries 4 and 8, Table 1), ruling out the possibility that the similar amino acid distribution on the protein surface has promoted the process. Another five lipases, including AYL, AL, PS-C , CCL and CRL, cannot catalyze the reaction efficiently in DMF (entries 5–7, 9, 10, Table 1). All these results suggest that the tertiary structure and the specific spatial conformation of CAL-B are responsible for the anti-Markovnikov addition reaction of benzyl thiol to vinyl acetate. Examining the influence of temperature showed that the anti-Markovnikov addition activity of CAL-B increased from 15 °C to 50 °C and reached the highest activity at 50 °C. When the temperature exceeded 50 °C, the activity decreased sharply, and the yield at 65 °C was only 35% (Figure 2). This illustrates that the existence of organic solvents increased the thermal stability of enzymes.

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

Entry	Catalyst	Amount [mg]	Yield [%][b]	V_0 [μM min^{-1}]	V_r[c]
1	No catalyst	-	0.6	3.63	1.0
2	CAL-B	10	91.6	550.13	151.6
3	CAL-B	5	61.8	246.51	67.9
4	CAL-B[d]	10	2.9	17.57	4.8
5	AYL[e]	10	28.0	111.7	30.8
6	AL[e]	10	13.6	82.3	22.7
7	PS-C[e]	10	33.3	132.8	36.6
8	BSA[e]	10	1.5	9.1	2.5
9	CCL[e]	10	3.4	20.6	5.7
10	CRL[e]	10	0.9	5.4	1.5

[a] *Reaction conditions:* benzyl thiol (0.25 mmol), vinyl acetate (0.30 mmol), enzyme, DMF (1 mL), 50 °C for 24 h.
[b] Conversion was calculated from the GC results.
[c] Relative initial reaction rate to the reaction in absence of enzyme.
[d] CAL-B predenatured with urea at 100 °C for 10 h.
[e] AYL: lipase AY 30; AL: Amano lipase M from *Mucor javanicus*; CCL: lipase from *Candida cylindracea*; CRL: lipase from *Candida rugosa*; PS-C: immobilized lipase from *Pseudomonas cepacia*.

In order to improve the activity of the enzyme, some conventional organic solvents with different log P values were screened for the enzymatic reaction and the results are shown in Table 2. Besides the anti-Markovnikov addition product **4a**, Markovnikov addi-

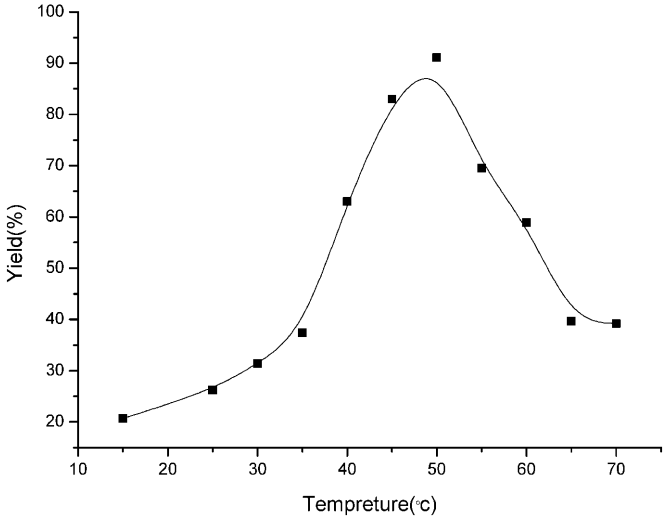


Figure 2. Effect of temperature on the CAL-B catalyzed anti-Markovnikov addition.

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

Entry	Solvent	Log P	CAL-B [mg]	Product [%][b]	
				3a	**4a**
1	DMSO	−1.3	10	2.1	50.7
2	DMF	−1.0	10	2.3	93.1
3	Acetonitrile	−0.33	10	3.4	21.3
4	Tetrahydrofuran	0.49	10	4.8	30.3
5	*tert*-Butyl alcohol	0.80	10	15.3	25.8
6	Diisopropyl ether	1.9	10	83.9	0.3
7	Toluene	2.6	10	56.0	2.6
8	Methylcyclohexane	3.7	10	58.7.	2.7
9	*n*-Hexane	3.9	10	64.1	0.9
10	Diisopropyl ether	1.9	-	1.3	0.1

[a] Reactions were carried out with 0.25 mmol of benzyl thiol, 0.3 mmol of vinyl acetate, and CAL-B in 1.0 mL of solvent at 50 °C for 48 h.

[b] Conversion was determined by gas chromatographic analysis, using dodecane as an internal standard.

tion product **3a** was detected by GC-MS in the reaction system. CAL-B showed higher anti-Markovnikov addition activity in DMF and led to a 93.1% GC yield after 48 h (entry 2, Table 2). In other polar solvents such as acetonitrile, tetrahydrofuran, *tert*-butyl alcohol and DMSO, the yields are far from satisfactory (entries 3–5 and entry 1, Table 1). In solvents such as methyl cyclohexane, diisopropyl ether, toluene, or *n*-hexane, the anti-Markovnikov additions proceeded very slowly and the yields were less than 3% (entries 6–9, Table 2). However, the Markovnikov addition product was generated as a major product. The Markovnikov addition in diisopropyl ether led to a 83.9% GC yield after 48 h. The diisopropyl ether could not promote the reaction, because only 1.3% yield was observed after 48 h in the absence of enzyme. Thus, DMF and diisopropyl ether were chosen as solvents in the following experiments. It has been reported that organic solvents could cause a significant change in enzyme conformation. Polar solvents such as DMSO could affect the unfolding mechanism and increase the conformational flexibility of enzyme.[8] In non-polar solvents, the hydrophilic surface of CALB decreased by 10% in comparison to that in water, while the hydrophobic surface is slightly increased by 1%.[9] Based on these findings, we speculated that the selectivites in DMF and diisopropyl ether may be attributed to the conformational changes of enzyme.

The scope of this method was investigated with different thiols and vinyl esters. The results are summarized in Table 3. In diisopropyl ether, all the addition reactions afforded exclusively the Markovnikov adduct in high selectivity. Addition of benzyl thiol **1a** to **2a** afforded Markovnikov adducts **3a** in 81% isolated yield with 99.6% conversion, and the ratio of **3a** to **4a** was 99.6:0.4 (Table 3, entry 2). Benzyl thiol reacted with **2b** and **2c** to give adducts in moderate yields with high selectivity, and the ratio of Markovnikov adducts **3a** to anti-Markovnikov adducts **4a** exceeded 98:2 (entries 4 and 6). However, the reactivity of the acceptor decreased as the chain length increased. Lower yields were obtained from the vinyl esters with longer chain (entries 2, 4 and 6). Besides the acceptors, the influence of donors on the reaction was also investigated. Butyl mercaptan and dodecyl mercaptan also facilitated the Markovnikov addition for the synthesis of the corresponding adducts with good selectivities (entries 8 and 12). However, *n*-dodecanethiol showed rather lower activity because of the strong steric hindrance. As the activities of the mercaptan became gradually weaker, the yields of the Markovni-

Table 3. Addition between thiols **1** and vinyl esters **2** in the presence of different catalysts.[a]

Entry	**1**	**2**	Solvent	**1** Conversion [%][b]	**3** Yield [%][c]	**4** Yield [%][c]	Selectivity[b] **3/4**
1	**1a**	**2a**	DMF	95.4	2.3	93.1	2.5/97.6
2			Diisopropyl ether	99.6	83.9	0.3	99.6/0.4
3	**1a**	**2b**	DMF	87.1	3.6	83.5	4.1/95.9
4			Diisopropyl ether	91.8	75.4	0.5	99.3/0.7
5	**1a**	**2c**	DMF	70.7	5.5	65.2	8.4/92.2
6			Diisopropyl ether	80.2	68.1	0.8	98.8/1.2
7	**1b**	**2a**	DMF	87.7	0.8	86.9	0.9/99.1
8			Diisopropyl ether	92.3	73.1	0.2	99.7/0.3
9	**1b**	**2b**	DMF	82.9	1.9	81.0	2.3/97.7
10			Diisopropyl ether	75.8	64.6	3.3	95.1/4.9
11	**1c**	**2a**	DMF	48.5	0.7	47.8	1.4/98.6
12			Diisopropyl ether	53.9	41.7	1.1	97.4/2.6

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

[b] Conversion was determined by gas chromatographic analysis, using dodecane as an internal standard.

[c] Yields refer to those of pure isolated addition products characterized by IR, ^{1}H and ^{13}C NMR.

kov products decreased (entries 2, 8 and 12). A similar observation was found when the reactions were carried out in DMF. The anti-Markovnikov products were obtained, leaving a trace of the Markovnikov products.

In conclusion, we herein report an unprecedented CAL-B-catalyzed C–S bond addition. The catalytic effect of the enzyme is demonstrated by the combination of different experiments. This new addition activity of CAL-B provides a clear example of catalytic promiscuity. It is worthy of note that the selectivity of CAL-B can be controlled by solvents. Anti-Markovnikov and Markovnikov addition were achieved in DMF and diisopropyl ether, respectively. This novel protocol provides a potential synthetic route for β-(substituted thio)-ethanol acetate compounds.

Experimental Section

Materials and General Methods

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-500 MHz spectrometer in $CDCl_3$, respectively. Chemical shifts are reported in ppm (δ), relative to the internal standard of tetramethylsilane (TMS). HR-MS were obtained on a Bruker 7-Tesla FT-ICR MS equipped with an electrospray source (Billelica, MA, USA). All chemicals were obtained from commercial suppliers and used without further purification. For all reactions dry (molecular sieve) analytical grade solvents were used. Solvents for column chromatography were distilled before use. The progress curves of the enzymatic addition were analyzed by GC. Samples were analyzed using a GC (SE-54 capillary column, FID detection; oven temperature: from 80 to 240 °C, rate of heating 20 °C min^{-1}) and using dodecane as an internal standard. All the compounds were characterized (IR, ^{1}H, ^{13}C NMR and MS) and analytically compared (GC) with authentic samples prepared by conventional methods. The enzymes used included CAL-B (lipase B acrylic resin from *Candida antarctica*, E.C. 3.1.1.3, 10,000 U/g), AYL (lipase AY 30, E.C. 3.1.1.3), AL (Amano lipase M from *Mucor javanicus*, E.C. 3.1.1.3, 10 U/mg), CCL (lipase from *Candida cylindracea*, E.C. 3.1.1.3, 2.08 U/mg), CRL (lipase from *Candida rugosa*, E.C. 3.1.1.3, 706 U/mg), PS-C (an immobilized lipase from *Pseudomonas cepacia,* E.C. 3.1.1.3, 730 U/g).

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