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Solvent engineering: an effective tool to direct chemoselectivity in a lipase-catalyzed Michael addition

Jaime Priego [a], Claudia Ortíz-Nava [b], Manuel Carrillo-Morales [b], Agustín López-Munguía [a], Jaime Escalante [b,*], Edmundo Castillo [a,*]

[a] Instituto de Biotecnología, Universidad Nacional Autónoma de México, Apartado Postal 510-3, C.P. 62271 Cuernavaca, Morelos, Mexico
[b] Centro de Investigaciones Químicas, Universidad Autónoma del Estado de Morelos, Av. Universidad No. 1001, C.P. 62209 Cuernavaca, Morelos, Mexico

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ABSTRACT

A solvent engineering strategy was implemented in order to control the chemoselectivity in a lipase-catalyzed Michael addition reaction. This strategy was revealed as a high-effective tool for the selective synthesis of Michael adduct **3** or aminolysis product **4** from benzylamine **1** and methyl crotonate **2**. Chemoselectivity of the enzymatic process was elucidated in terms of polarity of the medium, hence, adduct **3** was preferentially accumulated in hydrophobic medium, whereas in polar solvents the amide **4** was preferentially formed.

1. Introduction

In the last years, enzymes in organic media have become a useful alternative in organic synthesis due to their chemo-, regio-, and enantioselective properties.[1] Particular attention has been paid to lipase B from *Candida antarctica* (CaLB), because of its high stability and activity suitable for a broad spectrum of reactions, substrates, and media. This biocatalyst represents an effective tool to overcome significant limitations found in traditional organic synthesis.[2]

Recently, novel and valuable applications of CaLB were reported: Michael addition type reactions involving addition of secondary amines to acrylonitrile[3] or addition of imidazoles to acrylic monomers in organic medium[4] among other reactions. These additions take place when α,β-unsaturated systems are used as the electrophile moiety and amines as the nucleophile substrate. Some evidences of the mechanism of this promiscuous activity of CaLB point out that the oxyanion hole (Thr_{40} and Gln_{106}) of the active site stabilizes the negative charge of the transition state while the His_{224}-Asp_{187} pair facilitates proton transfer during the catalysis. Consequently, it is proposed that solvents of low polarity may induce interaction between the oxyanion hole and the carbonyl oxygen in the catalytic intermediate complex, allowing the ability of CaLB to carry out this reaction (Fig. 1).[5]

Although the mechanism described above may help to explain the effect of reaction conditions on the selectivity of the enzymatic reaction, alternating between a Michael addition and an aminolysis product, it is important to point out the essential effect that solvents can also have on the reaction thermodynamics.

2. Results and discussions

We have previously reported the use of solvent engineering strategies in lipase-catalyzed reactions in order to manipulate the

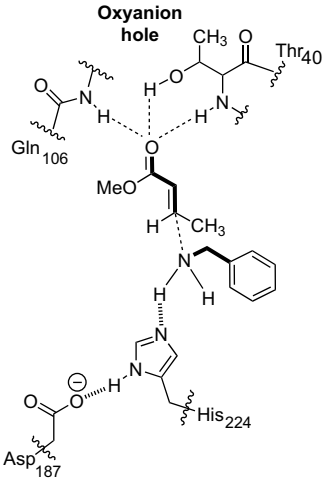

Figure 1. Hypothetical catalytic addition of benzylamine to methyl crotonate in the active site of CaLB. The carbonyl oxygen of the α,β-unsaturated is bound to the oxyanion hole and the nucleophile is activated by His.[5]

* Corresponding authors. Tel.: +52 55 56 22 76 09; fax: +52 77 73 11 49 03.
E-mail addresses: jaime@ciq.uaem.mx (J. Escalante), edmundo@ibt.unam.mx (E. Castillo).

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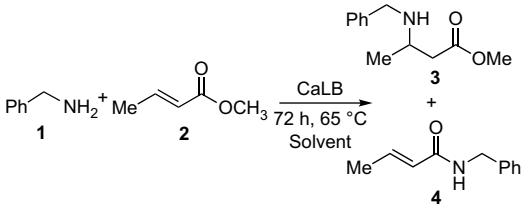
thermodynamic activity of the reactants, allowing the accumulation of a specific product in the reaction medium at equilibrium.[6] We have also demonstrated that in inverse hydrolysis reactions, polar products such as amides or polar esters are preferentially accumulated in polar solvents while in hydrophobic solvents their synthesis is quite disfavored.[7] In fact, the higher the solubility of a product in a given solvent the lower its thermodynamic activity coefficient favoring the accumulation of the product at equilibrium and vice versa.[6]

Based on these thermodynamic considerations, in this work we make use of a solvent engineering strategy as an effective tool to control chemoselectivity between a Michael addition and the aminolysis product when benzylamine **1** and methyl crotonate **2** are exposed to CaLB as catalyst (Scheme 1).



Scheme 1. Addition of benzylamine to methyl crotonate catalyzed by CaLB in organic medium to yield Michael addition **3** or aminolysis product **4**.

The hypothesis of this work is that rational changes in the polarity of the reaction medium can control the selective formation of the Michael adduct **3** or the aminolysis product **4**.

In the first step, the polarity of the reaction medium was selected according to the empirical polarity parameter $E_T(30)$ described by Reichardt[8] for pure solvents and adapted by Castillo et al. for solvent mixtures.[6c] Contrary to dielectric constants, which rather reflect bulk polarities, $E_T(30)$ take into account solvent–solute interactions at the molecular level.[9]

Seven media ranging from $E_T(30)$=30.9 (non-polar) to $E_T(30)$=41.0 (polar) were screened in enzymatic reactions at 65 °C with the amount of Michael adduct **3** and aminolysis product **4** quantified by HPLC. While neither product was formed in the absence of CaLB, a large range of ratio between **3** and **4** was obtained under enzyme-catalyzed condition which, as expected, was strongly driven by the polarity of the reaction medium.

From the results in Table 1, one can observe that in hydrophobic medium such as *n*-hexane or toluene Michael adduct is the predominant product while the aminonolysis product is the preferred one in more polar solvents. Accordingly, a variety of ratios of **3** and **4** can be reliably obtained through controlling of the polarity of solvent mixtures.

Table 1
Influence of polarity of the enzymatic reaction medium on the chemoselectivity between Michael addition and aminolysis

Entry	Solvent	$E_T(30)$	Ratio (%)		**3** (mmol/mL)	**4** (mmol/mL)
			3	**4**		
1	*n*-Hexane	30.9	95	5	0.40	0.02
2	Toluene	33.9	71	29	0.25	0.10
3	Diisopropyl ether	34.0	40	60	0.11	0.17
4	THF	37.4	30	70	0.09	0.20
5	*n*-Hexane/2M2B, 1:1	39.2	35	65	0.14	0.25
6	*n*-Hexane/2M2B, 1:3	40.1	26	74	0.09	0.26
7	2M2B	41.0	20	80	0.08	0.30

Reaction conditions: 0.5 M benzylamine **1**, 0.75 M of methyl crotonate **2**, and 10 mg/mL of CaLB at 65 °C for 72 h. **3** Michael addition and **4** aminolysis products.

In particular, for *n*-hexane, a non-polar solvent with an $E_T(30)$=30.9 (entry 1), 95% of converted **1** corresponds to the Michael adduct product **3** while only 5% is transformed to **4**. On the other hand, in 2M2B, a polar solvent with an $E_T(30)$=41.0 (entry 7), the proportion of converted **1** to **3** and **4** was 20 and 80%, respectively. Actually, we have reported earlier[7,10] that a lower thermodynamic activity of amides in 2M2B leads to its preferential formation while in non-polar solvent a higher thermodynamic activity results in lower yield. It is noteworthy that the product **1** obtained in *n*-hexane did not show significant specific optical rotation [$[\alpha]_D^{20}$ 0.45 (*c* 1.0, $CHCl_3$)], on the contrary, product **1** obtained in 2M2B showed a specific optical rotation of 11.0 [$[\alpha]_D^{20}$ 11.0 (*c* 1.0, $CHCl_3$)]. In fact, when comparing this optical rotation to the specific optical rotation observed for enantiomerically pure compound the ee of **1** corresponds to an only ee <60%.

Although, from a thermodynamical point of view these observations are in agreement with our previous reports concerning the preferred formation of the amides in lipase-catalyzed reactions carried out in polar medium,[6,7] the highly selective accumulation of the Michael adduct as a function of the nature of reaction media, arises as a new and original application of solvent engineering.

A closer look at these results, in Figure 2, shows a clear correlation between the Michael addition reaction selectivity and the reaction medium polarity [as measured by the empirical parameter $E_T(30)$]. This confirmed that the rational selection of the solvent (or solvent mixtures) directs the chemoselective process toward the

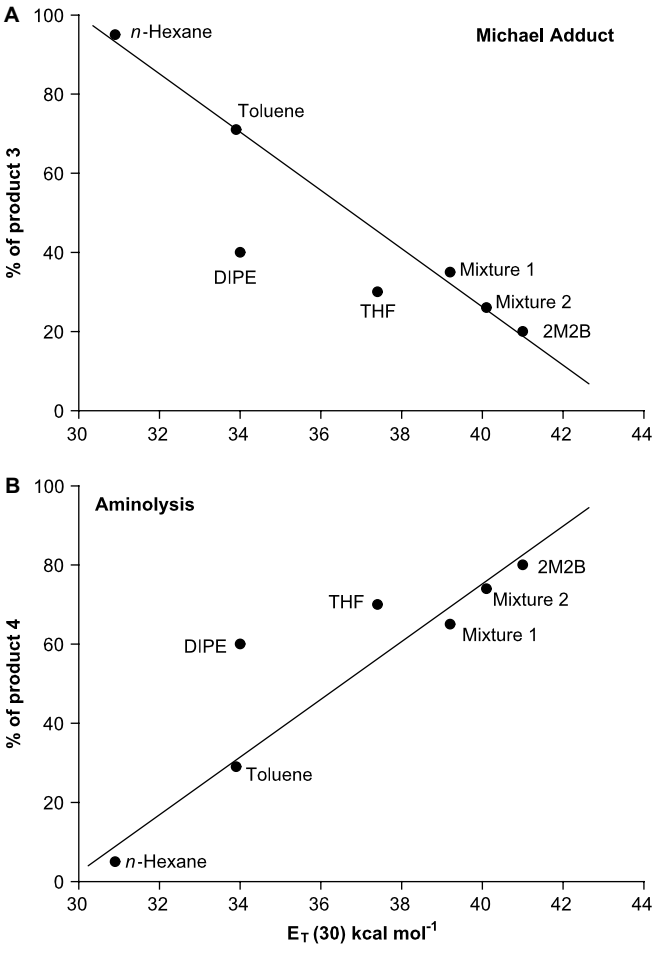


Figure 2. Plots of % of product **3** and **4** as a function of the solvent polarity parameter $E_T(30)$.

Table 2
Influence of the initial benzylamine **1** concentration in *n*-hexane on the ratio of Michael adduct **3** to aminolysis products **4**

Entry[a]	**1** (mmol/mL)	Ratio (%)		Yield **1**[b] (%)	**3** (mmol/mL)	**4** (mmol/mL)
		3	**4**			
1	0.50	>95	<5	85	0.400	0.025
2	0.25	75	25	68	0.130	0.042
3	0.10	8	92	81	0.003	0.036
4	0.05	—	100	79	—	0.039

[a] In all entries, a ratio of 1.5 mmol of methyl crotonate **2** by each mmol of benzylamine **1** were used.
[b] Determined by HPLC and referred to the initial concentration of benzylamine **1**. Ratios also were determined by HPLC after 72 h of reaction.

Michael addition or aminolysis and shows that solvent engineering may be used as an effective tool to design or predict the lipase-catalyzed chemoselectivity.

In an apparent contradiction to these results, two previous reports of reactions between amines and α,β-unsaturated carboxyl groups in hydrophobic solvents at low concentrations of amine (0.13 M), and high ratio of enzyme/amine (50 mg of CaLB/mmol of benzylamine) resulted in the preferential formation of the amide over the Michael addition product.[11a,b] As the main difference with our results is the low substrate concentration used by the authors, a series of experiments were performed in *n*-hexane, varying the initial concentration of amine **1** [molar ratio 1:1.5 with respect to **2**] with 10 mg of enzyme/mL in all experiments.

Interestingly, after 72 h of reaction, a maximal concentration of the amide **4** was obtained in all cases (~0.035 M). However, in reactions with low initial substrate concentration of amine **1** a strong decrease in the Michael adduct product **3** occurs. It is clear that under these circumstances, chemoselectivity of the process at initial amine concentration lower than 0.25 M is favored toward the synthesis of **4** (Table 2). In fact, in agreement to Puertas et al. (1993),[11a,c] at amine concentrations lower than 0.1 M the reaction is practically selective for the amide **4** synthesis.

From a kinetic point of view, it has been reported that aminolysis reactions are faster than the Michael adduct formation with CaLB.[6c] However, the rate is influenced by the nature of the substrates and their initial concentration. In experiments carried out in *n*-hexane at a high initial substrate concentration (0.5 M), the initial rate for the Michael adduct reaction was higher than that of aminolysis. On the other hand, at a low initial amine concentration (0.1 and 0.05 M) the aminolysis reaction was faster while the Michael

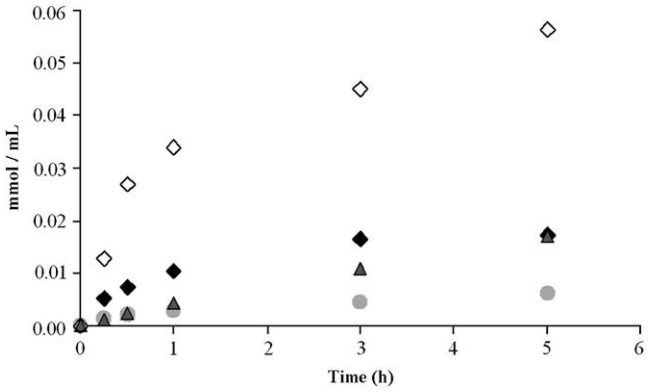


Figure 3. Initial reaction experiments for the lipase-catalyzed synthesis of Michael adduct and aminolysis products. Reaction at 0.5 M initial substrate concentration: ◇ Michael adduct **3** and ◆ aminolysis product **4**. Aminolysis product at ▲=0.1 M and ●=0.05 M.

Table 3
Influence of the presence of **4** in the course of the reaction of benzylamine **1** and methyl crotonate **2** using 10 mg/mL of CaLB at 65 °C for 72 h

S_0 (mmol/mL)		Products (mmol/mL)		Ratio (%)		Yield (%)
1 and **2**	**4**	**3**	**4**	**3**	**4**	**1**
0.5	0.020	0.420	0.033	93	7	90.6

S_0=initial concentration of substrates.

adduct formation became extremely slow. Actually, product **3** is only detected after 6 h of reaction (Fig. 3). Surprisingly, in all experiments after 72 h of reaction, the concentration of **4** never exceeded 0.04 M.

Although these initial rate experiments describe a kinetic control on chemoselectivity, the effect is observed only at very low substrate concentrations.

In order to further explore the thermodynamic effect of initial substrates concentration on chemoselectivity, we decided to vary the substrates concentration after it has reached its maximal value, with the purpose to switch from aminolysis to Michael adduct products. The experiment was started with low substrate concentration (0.1 M of both substrates in *n*-hexane) and the products measured after 72 h of reaction resulting in 0.039 M of **4** and 0.03 M of **3**. At this point, the concentrations of both substrates were raised to 0.4 M and again after 72 h of reaction the products yield was measured. As expected, in this second step only product **3** was formed once **4** reached its maximal concentration (0.035 M).

A second experiment was also carried out, this time a reaction medium with the purified product **4** at its maximal solubility in *n*-hexane (0.02 M) and 0.5 M initial concentration of **1** and **2** was prepared. Again, and as predicted from our previous results, after 72 h of reaction, 0.42 M of **3** was observed, while **4** increased only to 0.033 M (Table 3).

From these results, it is clear that at high initial substrate concentration, the maximal concentration of amide is independent of the reaction rate and is only a function of thermodynamic conditions. We have shown previously[6,7] that maximum solubility of polar products in hydrophobic solvents such as hexane, limit the yield of product. Therefore, even if the amide synthesis is faster at low substrate concentrations, once the maximum concentration is reached, its formation ceases, while the formation of adduct **3** continues until the global thermodynamic equilibrium is reached.

In short, substrate concentration can also be considered as a factor affecting chemoselectivity, but only at very low concentrations and in a limited range of chemoselectivity (Table 2). Therefore, if high productivities of either aminolysis or Michael adduct products are required, then the optimum conditions for the reaction is best controlled via the solvent polarity.

3. Conclusions

We have demonstrated that solvent engineering is an effective tool to control chemoselectivity in the reaction between methyl crotonate **2** and benzylamine **1** catalyzed by CaLB, the results show that the accumulation of the Michael adduct **3** is favored in more hydrophobic media while the amide product **4** in more polar solvents, as long as high substrates concentration are used in the reaction.

In addition, an appropriate solvent or solvent mixture may be designed according to the empirical polarity parameter $E_T(30)$ and used as an effective tool to predict chemoselectivity. In addition, these results open a wide spectrum of possibilities to rationally modify reaction media in biocatalytic reactions controlled by equilibrium.

4. Experimental

4.1. Materials and methods

candida antarctica lipase B, CaLB Novozyme 435, was obtained from Novozyme-México A/C. Benzylamine and methyl crotonate were obtained from Aldrich. All the solvents were distilled over an appropriate desiccant under nitrogen. Silica gel of 70–230 mesh of Merck was used for purification by flash chromatography.

4.2. Analytical methods

Spectra data of ^{1}H and ^{13}C NMR of Michael adduct **3** and aminolysis products **4** as well as the proportions of each product in the reaction mixture were recorded on a Varian Gemini 200 Spectrometer using $CDCl_3$ as solvent and TMS as an internal standard. The conversions were determined on an HPLC Waters 510 pump, using a diodes array detector at 210 nm equipped with a C18 reversed phase Novapack column (3.9×75 mm). Substrates and products were eluted with a mixture 80:20 of phosphate buffer 50 mM pH 2.6/MeOH at a flow rate of 1.0 mL min^{-1}, retention times: **1**=0.86, **3**=1.49, **2**=3.70, and **4**=6.83 min.

4.3. Lipase-catalyzed Michael addition and aminolysis

The typical procedure consisted in the preparation of a solution containing 0.39 g of benzylamine (3.67 mmol), 0.55 g (1.5 equiv, 5.51 mmol) of methyl crotonate, and 0.07 g of CaLB in 7.5 mL of a pure dry solvent or a solvent mixture. The reaction medium was heated to 65 °C for 72 h in a 10 mL sealed vial. Afterward, the reaction mixture was filtered and the enzyme washed with ethyl acetate (3×3 mL). The filtrated solvents were evaporated under reduced pressure to give crude yellow oil, which was analyzed by HPLC to determine the composition of **3** and **4**. Both products were purified by silica gel flash chromatography with an *n*-hexane/ethyl acetate gradient from 80:20 to 60:40 and their structures were confirmed by ^{1}H and ^{13}C NMR.

*4.3.1. (±)-Methyl 3-(benzylamine)butanoate **3***

Yellow oil, yield 70%, 0.53 g, $[\alpha]_D^{20}$ 0.45 (*c* 1.0, $CHCl_3$) for product obtained in *n*-hexane, $[\alpha]_D^{20}$ 11.0 (*c* 1.0, $CHCl_3$) for product obtained in 2M2B. Spectral data of ^{1}H and ^{13}C NMR are consistent with the literature reports.[12]

*4.3.2. N-Benzylcrotonamide **4***

White solid, mp 114–116 °C (lit.[11a] 113–115 °C), yield 76%, 0.488 g. Spectral data of ^{1}H and ^{13}C NMR are consistent with the literature reports.

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References and notes

1. (a) Bornscheuer, U. T.; Kazlauskas, R. J. *Hydrolases in Organic Synthesis: Regio- and Stereoselective Biotransformations*; Wiley-VCH: Weinheim, 1999; (b) Carrea, G.; Riva, S. *Angew. Chem., Int. Ed.* **2000**, *39*, 2226–2254; (c) Lutz, S. *Tetrahedron: Asymmetry* **2004**, *15*, 2743–2748; (d) Flores-Sánchez, P.; Escalante, J.; Castillo, E. *Tetrahedron: Asymmetry* **2005**, *16*, 629–634.
2. (a) Bertau, M. *Curr. Org. Chem.* **2002**, *6*, 987–1014; (b) Bornscheuer, U. T.; Bessler, C.; Srinivas, R.; Krishna, S. H. *Trends Biotechnol.* **2002**, *20*, 433–437; (c) Shaw, N. M.; Robins, K. T.; Kiener, A. *Adv. Synth. Catal.* **2003**, *345*, 425–435.
3. Torre, O.; Alfonso, I.; Gotor, V. *Chem. Commun.* **2004**, 1724–1725.
4. (a) Cai, Y.; Yao, S. P.; Wu, Q.; Lin, X. F. *Biotechnol. Lett.* **2004**, *26*, 525–528; (b) Cai, Y.; Wu, Q.; Xiao, Y. M.; Lv, D. S.; Lin, X. F. *J. Biotechnol.* **2006**, *121*, 330–337.
5. (a) Branneby, C.; Carlqvist, P.; Magnusson, A.; Hult, K.; Brinck, T.; Berglund, P. *J. Am. Chem. Soc.* **2003**, *125*, 874–875; (b) Branneby, C.; Carlqvist, P.; Hult, K.; Brinck, T.; Berglund, P. *J. Mol. Catal. B: Enzym.* **2004**, *31*, 123–128; (c) Carlqvist, P.; Svedendahl, M.; Branneby, C.; Hult, K.; Brinck, T.; Berglund, P. *ChemBioChem* **2005**, *6*, 331–336; (d) Svedendahl, M.; Hult, K.; Berglund, P. *J. Am. Chem. Soc.* **2005**, *127*, 17988–17989.
6. (a) Bellot, J. C.; Choisnard, L.; Castillo, E.; Marty, A. *Enzyme Microb. Technol.* **2001**, *28*, 362–369; (b) Rendón, X.; López-Munguía, A.; Castillo, E. *J. Am. Oil Chem. Soc.* **2001**, *78*, 1061–1066; (c) Castillo, E.; Pezzotti, F.; Navarro, A.; López-Munguía, A. *J. Biotechnol.* **2003**, *102*, 251–259.
7. (a) Reyes-Duarte, D.; Castillo, E.; Martínez, R.; López-Munguía, A. *Biotechnol. Lett.* **2002**, *24*, 2057–2061; (b) Castillo, E.; López-González, I.; De Regil-Hernández, R.; Reyes-Duarte, D.; Sánchez-Herrera, D.; López-Munguía, A.; Darszon, A. *Biochem. Biophys. Res. Commun.* **2007**, *356*, 424–430.
8. (a) Reichardt, C. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 98–110; (b) Reichardt, C. *Chem. Rev.* **1994**, *94*, 2319–2358.
9. Sinkeldam, R. W.; Tor, Y. *Org. Biomol. Chem.* **2007**, *5*, 2523–2528.
10. Torres-Gavilán, A.; Castillo, E.; López-Munguía, A. *J. Mol. Catal. B: Enzym.* **2006**, *41*, 136–140.
11. (a) Puertas, S.; Brieva, R.; Rebolledo, F.; Gotor, V. *Tetrahedron* **1993**, *49*, 4007–4014; (b) Sánchez, V.; Rebolledo, F.; Gotor, V. *Synlett* **1994**, 529–530; (c) Torre, O.; Gotor-Fernández, V.; Alfonso, I.; García-Alles, L. F.; Gotor, V. *Adv. Synth. Catal.* **2005**, *347*, 1007–1014.
12. Asao, N.; Uyehara, T.; Yamamoto, Y. *Tetrahedron* **1988**, *44*, 4173–4180.