Enzymatic Syntheses of *N*-Lauroyl-β-Alanine Homologs in Organic Media

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ABSTRACT: Enyzmatic amidation of the primary amines β-alanine ethyl ester and 3-aminopropionitrile with methyl laurate by means of immobilized lipase (*Candida antarctica* lipase, CAL) resulted in the formation in good yield of *N*-lauroyl-β-alanine ethyl ester and 3-(*N*-lauroylamino)-propionitrile, respectively. When 3-amino-propionitrile was used as substrate, diisopropyl ether was a suitable solvent. Changing the reaction temperature (12–80°C) did not affect the yields, and room temperature was a suitable temperature for this reaction. In the investigation of reaction conditions, the use of equimolar amounts (5 mmol) of substrate and ester, along with 0.5 g of CAL, in diisopropyl ether gave the best yield (99.3%) after 24 h of incubation at 24°C. The enzyme activity in the amidation reaction did not decrease even after six uses. With β-alanine ethyl ester hydrochloride as substrate, diisopropyl ether was unsuited as a solvent owing to the low solubility of the substrate in this solvent.

In this reaction, the best yield (82.0%) was attained by using dioxane as solvent. CAL achieved higher extents of amide synthesis with long-chain than with short-chain ester substrates. The enzyme accepted only nonbulky primary amines as substrates.

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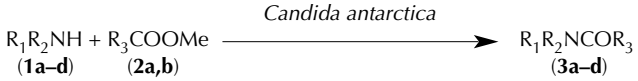
KEY WORDS: *Candida antarctica* lipase, enzymatic amidation, *N*-lauroyl-β-alanine, 3-(*N*-lauroylamino)propionitrile.

Oleochemical products have numerous advantages compared to their mineral counterparts. They are renewable and cause less damage to the environment than petroleum chemicals, the availability of which is finite. These aspects support the interest in surfactants produced from natural fats and oils. *N*-(long-chain acyl)-β-alanines are well known as useful and mild anionic surfactants. For example, it has been reported that *N*-lauroyl-β-alanine (LBA) has a low potential for inducing epidermal cell inflammation (1). Permeability experiments with hairless pigs indicate that this material permeates skin much less than soap, sodium dodecyl sulfate, sodium cocoyl isothionate, acylmethyl taurine, or monoalkyl phosphates (1). LBA is usually prepared by the reaction of β-alanine with lauroyl chloride (2). On the other hand, enzyme-mediated processes are becoming standard synthetic technologies to perform selective transformations in organic synthesis (3). Among the biocatalysts of synthetic interest, lipases are among the most useful enzymes and have been widely used in enantio- or regioselective transformations involving acylation, transesterification, hydrolysis, lactonization, hydrazinolysis, aminolysis, and transamidation, especially in organic media. Klibanov and coworkers (4) have reported that subtilisin can catalyze the enantioselective amidation of simple racemic amines by using 2,2,2-trifluoroethyl butyrate as acyl donor, and other authors have reported the enzymatic amidolysis of amines with lipases (5–8). Furthermore, Gotor and coworkers have demonstrated that *Candida antarctica* lipase catalyzes the aminolysis of dimethyl succinate (9), β-ketoesters (10), and α,β-unsaturated esters (11,12). In this report, we describe the enzymatic amidation of 3-aminopropionitrile and β-alanine ethyl ester with methyl caproate or methyl laurate by means of immobilized *C. antarctica* lipase (see Scheme 1).

EXPERIMENTAL PROCEDURES

All melting points are uncorrected. Infrared (IR) spectra were obtained with a Hitachi 260-10 IR spectrometer (Tokyo, Japan). ^{1}H Nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ in a Hitachi R-90H NMR spectrometer. Chemical shifts were measured in ppm downfield from internal tetramethylsilane ($\delta = 0$). The abbreviations *s*, *d, t*, and *m* denote singlet, doublet, triplet, and multiplet resonances, respectively.



1b: R_1 = H; R_2 = -CH_2CH_2CN,
1b: R_1 = H; R_2 = -CH_2CH_2COOEt,
1c: R_1 = H; R_2 = -$CH(COOEt)CH_2CH_2COOEt$,
1d: R_1 = Me; R_2 = -CH_2COOEt,
2a: R_3 = -$(CH_2)_4CH_3$,
2b: R_3 = -$(CH_2)_{10}CH_3$,
3a: R_1 = H; R_2 = -CH_2CH_2CN; R_3 = -$(CH_2)_4CH_3$,
3b: R_1 = H; R_2 = -CH_2CH_2CN; R_3 = -$(CH_2)_{10}CH_3$,
3c: R_1 = H; R_2 = -CH_2CH_2COOEt; R_3 = -$(CH_2)_{10}CH_3$,
3d: R_1 = H; R_2 = -CH_2CH_2COOH; R_3 = -$(CH_2)_{10}CH_3$.

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Mass spectra were obtained using a JEOL JMS-AX505HA spectrometer (JEOL Ltd., Akishima, Tokyo, Japan) operating in the electron impact mode with an ionization energy of 70 eV.

Immobilized lipase from *C. antarctica*, CAL (Novozym 435) was purchased from Novo Nordisk Bioindustry (Chiba, Japan). 3-Aminopropionitrile (**1a**), diethyl L-glutamate (**1c**), and *N*-methylglycine ethyl ester (sarcosine ethyl ester) (**1d**) were obtained from Sigma Chemical Co. (St. Louis, MO). β-Alanine ethyl ester hydrochloride (**1b**), methyl caproate (**2a**), and methyl laurate (**2b**) were gifts from Kawaken Fine Chemicals Co. (Kawagoe, Japan). Methyl caproate (**2a**) and methyl laurate (**2b**) were distilled before use. All solvents were of commercial special grade, were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan), and were stored over an adequate desiccant. Silica gel (Wakogel C-200) and molecular sieves 4A 1/16 were purchased from Wako Pure Chemical Ind. Ltd. (Osaka, Japan). Molecular sieves were activated by heating at 120°C for 5 h and pulverized before use.

*Reaction of the esters **2a** and **2b** with amine **1a** in diisopropyl ether catalyzed by CAL.* Prior to use of CAL, an equal weight of deionized water was sprayed on the enzyme, which was then gently tumbled for 30 min. In an Erlenmeyer flask, CAL (0.50 g) was added to the mixture of the amine (**1a**, 5 mmol) and the ester (**2a** and **2b**, 5 or 10 mmol) in the solvent (20 mL). The mixture was shaken, and the conversion was monitored by thin-layer chromatography (TLC) analysis (silica gel, benzene). White precipitate of product gradually separated out. When the starting amine had almost disappeared on TLC (24 or 48 h), 300 mL of chloroform was added to dissolve the precipitates. Then the enzyme was removed by filtration, and the filtrate was evaporated to dryness under reduced pressure. The product was purified by column chromatography on silica gel (Wakogel C-200) with chloroform, affording 3-(*N*-caproylamino)propionitrile (**3a**) or 3-(*N*-lauroylamino)propionitrile (**3b**). The results are summarized in Table 1. The spectroscopic and analytical data of products were as follows.

*3-(Caproylamino)propionitrile (**3a**).* This compound was obtained by enzymatic amidation with **2a**; colorless crystals, m.p. 62–64°C, IR (KBr): 3300 (-NH), 2240 (-CN), 1640, 1550 cm^{-1} (-NHCO-). ^1H NMR: δ = 0.89 (*t*, 3H, $-CH_3$), 1.28 (*m*, 6H, $-CH_2-$), 2.24 (*t*, 2H, $-CH_2CO-$), 2.60 (*t*, 2H, $-CH_2CN$), 3.58 (*d-d*, 2H, $-CH_2N-$), (*d-d*, 2H, $-CH_2N-$), 6.54 ppm (*br-s*, 1H, -NH). MS: *m/z* 168 (M^+). Elemental analyses: C, 64.16; H, 9.51; N, 16.52%; calculated for $C_9H_{16}N_2O$: C, 64.25; H, 9.59; N, 16.65%.

*3-(Lauroylamino)propionitrile (**3b**).* This compound was obtained by the enzymatic amidation of **1a** and **2b**; colorless crystals, m.p. 95–96°C [lit. (13), m.p. 96–97°C], IR (KBr): 3300 (-NH), 2240 (-CN), 1640, 1550 cm^{-1} (-NHCO-). ^1H NMR: δ = 0.88 (*t*, 3H, $-CH_3$), 1.27 (*m*, 18H, $-CH_2-$), 2.22 (*t*, 2H, $-CH_2CO-$), 2.62 (*t*, 2H, $-CH_2CN$), 3.55 (*d-d*, 2H, $-CH_2N-$), 6.51 ppm (*br-s*, 1H, -NH). MS: *m/z* 252 (M^+). Elemental analyses: C, 71.25; H, 11.04; N, 10.97%; calculated for $C_{15}H_{28}N_2O$: C, 71.38; H, 11.18; N, 11.10%.

TABLE 1
Enzymatic Amidation of 3-Aminopropionitrile (1a), β-Alanine Ethyl Ester (1b), L-Glutamic Acid Diethyl Ester (1c) and *N*-Methyl-Glycine Ethyl Ester (1d) with Methyl Hexanoate (2a) and Methyl Laurate (2b) by Immobilized *Candida antarctica* Lipase

Entry	Substrate (mmol)	Ester (mmol)	Solvent[a]	Reaction time (h)	Temp (°C)	Product[b] (% yield)[c]	
1	**1a** (5)	**2a** (10)	A	48	24	**3a**	(82.6)
2	**1a** (5)	**2b** (10)	A	24	24	**3b**	(84.9)
3[d]	**1a** (5)	**2b** (10)	A	24	24	**3b**	(93.9)
4	**1a** (5)	**2b** (5)	A	24	12	**3b**	(95.2)
5	**1a** (5)	**2b** (5)	A	24	24	**3b**	(99.3)
6	**1a** (5)	**2b** (5)	A	24	40	**3b**	(96.4)
7	**1a** (5)	**2b** (5)	A	24	80	**3b**	(98.7)
8[e]	**1a** (5)	**2b** (5)	A	24	24	**3b**	(97.0)
9[f]	**1a** (5)	**2b** (5)	A	24	24	**3b**	(96.7)
10[g]	**1a** (5)	**2b** (5)	A	24	24	**3b**	(95.8)
11[h]	**1a** (5)	**2b** (5)	A	24	24	**3b**	(95.2)
12[i]	**1a** (5)	**2b** (5)	A	24	24	**3b**	(94.3)
13	**1b** (5)	**2b** (5)	B	168	24	**3c**	(12.5)
14	**1b** (5)	**2b** (5)	C	168	24	**3c**	(45.5)
15	**1b** (5)	**2b** (5)	D	168	24	**3c**	(31.2)
16	**1b** (5)	**2b** (5)	E	168	24	**3c**	(82.0)
17	**1d** (5)	**2b** (5)	D	168	24	—	
18	**1d** (5)	**2b** (5)	F	168	24	—	

[a]A = diisopropyl ether; B = dimethylformamide; C = ethanol; D = tetrahydrofuran; E = dioxane; F = chloroform.
[b]**3a** = 3-(Caproylamino)propionitrile; **3b** = 3-(lauroylamino)propionitrile; **3c** = *N*-lauroyl-β-alanine ethyl ester.
[c]Isolated yield.
[d]The experiment was carried out in the presence of molecular sieves 4A powder (Wako Pure Chemical Ind. Ltd. Osaka, Japan).
[e]The recovered enzyme of entry 7 was reused.
[f]The recovered enzyme of entry 8 was reused.
[g]The recovered enzyme of entry 9 was reused.
[h]The recovered enzyme of entry 10 was reused.
[i]The recovered enzyme of entry 11 was reused.

Compound **3b** was hydrolyzed by refluxing for 6 h with 20% aqueous potassium hydroxide solution (5 mL) and ethylene glycol monomethyl ether (10 mL), and afforded *N*-lauroyl-β-alanine (**3d**), m.p. 109–110°C [lit. (14) m.p. 108–110°C] in 85% yield.

*Reaction of the ester **2b** with β-alanine ethyl ester **1b** catalyzed by CAL.* When β-alanine ethyl ester was used after converting to free amine, the yield was 30% down. Therefore, when β-alanine ethyl ester (**1b**) was used in the hydrochloride salt form, an equimolar sodium hydroxide aqueous solution was necessary to convert to free amine during enzymatic reaction. In an Erlenmeyer flask, ester **1b** (5 mmol) and ester **2b** (5 mmol) in the appropriate solvent (20 mL) were mixed with 1 mL of sodium hydroxide aqueous solution (5 M/L), and CAL (0.5 g) was added. The procedures for 3-aminopropionitrile **1a** as described above were followed.

N-*Lauroyl*-β-*alanine ethyl ester (**3c**)*. This compound was obtained by the enzymatic amidation of **1b** with **2b**; colorless crystals, m.p. 45–46°C IR (KBr): 3300 (-NH), 1730 (ester), 1630, 1545 cm^{-1} (-NHCO-). ^1H NMR: δ = 0.92 (*t*, 3H, -CH_3 of lauroyl group), 1.26 (*m*, 18H, -CH_2-), 1.34 (*t*, 3H, -CH_3 of ester group), 2.15 (*t*, 2H, -CH_2CON-), 2.51 (*t*, 2H, -CH_2COO-), 3.54 (*d-d*, 2H, -CH_2N-). 4.11 (*q*, 2H, -CH_2OOC-) 6.10 (*br-s*, 1H, -NH). MS: *m*/*z* 299 (M^+). Elemental analyses: C, 68.07; H, 11.03; N, 4.86%; 1 calculated for $C_{17}H_{33}NO_3$:1 C, 68.19; H, 11.11; N, 4.68%.

The hydrolysis of **3c** with 10% aqueous potassium hydroxide solution (5 mL) and ethylene glycol monomethyl ether (10 mL) after 3 h refluxing afforded **3d** in 80% yield.

RESULTS AND DISCUSSION

Candida antarctica lipase (CAL) has been widely used for the amidation of esters and amines (9–12). Our interest in developing improved methods for the preparation of *N*-lauroyl-β-alanine led us to examine the enzymatic amidation of **1a** and **1b** with the esters **2a** or **2b** by CAL. Our results for this enzymatic amidation by means of CAL are summarized in Table 1. The amine **1a** (1 equivalent) was incubated with CAL and the ester **2a** (2 equiv.) in diisopropyl ether at 24°C for 48 h, resulting in the formation of 3-(caproylamino)propionitrile (**3a**) in 82.6% yield (entry 1 in Table 1). Similarly, the reaction of **1a** (1 equiv.) with **2b** (2 equiv.) and CAL at 24°C for 24 h resulted in the formation of 3-(lauroylamino)propionitrile (**3b**) in 84.9% yield (entry 2). Hydrolysis of **3b** with aqueous potassium hydroxide solution afforded *N*-lauroyl-β-alanine (**3d**). In these studies, and in all work reported here, no product was formed if enzyme was not present in the reactions.

Previously, Wang and coworkers (15), in studies of the enzymatic transesterification of alcohols with vinyl esters as acylating agents by means of lipase, reported that long-chain esters were generally faster than short-chain esters. In the enzymatic amidation of **1a** with esters and CAL, we also found that the rate of amidation with the longer-chain ester **2b** is about two times faster than that with the shorter-chain ester **2a** (Table 1, entries 1 and 2). Furthermore, in the lipase-catalyzed transesterification of alcohols with vinyl esters, the addition of molecular sieves has a dramatic effect on the reaction rate and chemical yields (16,17). In the CAL-catalyzed amidation of **1a** in the presence of molecular sieves, the reaction rate was not significantly different from that in the absence of molecular sieves. However, the chemical yield was higher than that without molecular sieves (entry 3). The effect can be explained on the basis of molecular sieves adsorbing some unknown impurity, produced in a side reaction, which disturbs the amidation. The CAL-mediated reaction of **1a** (1 equiv.) with **2b** (1 equiv.) at 24°C for 24 h proceeded to afford **3b** with higher yield (entry 5), compared with that in the CAL-catalyzed reaction of **1a** (1 equiv.) with **2b** (2 equiv.) (entry 2). Moreover, yields of enzymatic reactions of equimolecular amounts of **1a** and **2b** were not affected by a change in the reaction temperature (entries 4–7). After 24 h of reaction in diisopropyl ether, the enzyme does not lose its catalytic activity and can be reused six times (entries 8–12). CAL can be reutilized without any subsequent treatment and with little los of activity.

The CAL-catalyzed reaction of equimolar amounts of **1b** and **2b** in various organic solvents (entries 13–16) resulted in the formation of *N*-lauroyl-β-alanine ethyl ester (**3c**). Among the organic solvents tested, dioxane was the best (entry 16). The reaction of **1b** with **2b** was less complete than that of **1a** with **2b** (entries 5 and 16). It is not clear if this is due to the difference in the substrates or the difference in the solvents used in the two reactions. Because **1b** did not dissolve in diisopropyl ether, it was not possible to compare the two reactions in that solvent.

The hydrolysis of **3c** with 10% aqueous potassium hydroxide solution and ethylene glycol monomethyl ether afforded **3d**.

Neither the enzymatic reaction of diethyl glutamate (**1c**) nor *N*-methylglycine ethyl ester (**1d**) with **2b** by means of CAL afforded amidation products (entries 17 and 18). These results show that the CAL enzyme catalyzes reactions only with nonbulky primary amines. This fact suggests that the presence of the bulky group in the α-position of the amine hinders adequate fitting of the substrates or product on the catalytic site of the enzyme.

Results similar to ours have been obtained by Goto and coworkers who have reported that CAL efficiently catalyzes the preparation of β-ketoamides from β-ketoesters with primary aliphatic amines and ammonia (10).

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