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Journal of Molecular Catalysis B: Enzymatic 2 (1996) 19–25

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

A new enzymatic one-pot procedure for the synthesis of carboxylic amides from carboxylic acids

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Received 6 October 1995; accepted 29 January 1996

Abstract

An efficient enzymatic procedure is described for the one-pot conversion of carboxylic acids to the corresponding amides via in situ formation of the ester and subsequent ammoniolysis. The procedure was optimized for the formation of octanamide from octanoic acid via butyl octanoate. It is best performed with lipase SP525 from *Candida antarctica* B immobilized on Accurel EP100 as catalyst with two equivalents of n-butanol in *t*-amyl alcohol as solvent. The resulting octanamide was isolated in 93% yield. The one-pot procedure was also applied to oleic acid, which resulted in the isolation of oleamide in 90% yield.

Keywords: Lipase; Esterification; Ammoniolysis; One-pot procedure; Inhibition; Alcohols

1. Introduction

During the last decade it has become clear that lipases efficiently catalyze transformations with non-natural acyl acceptors such as hydrogen peroxide and amines. This feature has become a dominant part of the synthetic repertoire [1]. We [2] and others [3] recently reported that ammonia also acts as unnatural acyl acceptor. Carboxylic esters are converted to the corresponding carboxylic amides in essentially quantitative yield in an exothermic (see Appendix) and, hence, irreversible reaction. Moreover, ammoniolysis of chiral esters proved to be up to an order more enantioselective than hydrolysis or transesterification [2].

It would often be convenient to prepare carboxylic amides directly from the acids, but a straightforward reaction of carboxylic acid and ammonia is not feasible because the unreactive ammonium carboxylate results. We reasoned that it should be possible, however, to develop a one-pot procedure by combining lipase-catalyzed esterification and subsequent ammoniolysis in the same reaction medium. Accordingly, we have investigated the conversion of octanoic acid via butyl octanoate into octanamide as a simple model reaction. The results which we obtained with this procedure, as well as the inhibition and support effects which profoundly influenced the final outcome, are reported in the present paper.

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PII S1381-1177(96)00009-4

2. Experimental

2.1. Analysis and materials

^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ with TMS as internal standard using a Varian VXR-400S spectrometer. Analytical HPLC was performed using a Waters M590 pump, an 8 × 100 mm Waters Novapak C_{18} column at ambient temperature and an Erma ERC-7510 RI detector. Mobile phase: 80/20 methanol/water, pH 4.3, 1.0 ml/min. Analytical GC was performed with a Varian Star 3400 equipped with a CP Sil 5 CB, 50 m × 0.53 mm, df = 100 μm column. For TLC, Merck (Darmstadt, Germany) plastic roll silica gel 60 F_{254}, layer thickness 0.2 mm was used.

Melting points are uncorrected and were measured on a Büchi 510 melting-point apparatus.

All solvents and reagents were of reagent grade and were dried on molecular sieves before use. Oleic acid was 83% pure according to GC. The contaminants are probably saturated and unsaturated fatty acids, such as linoleic acid. Novozym 435 (*Candida antarctica* B lipase on a proprietary carrier, activity 7550 PLU/g), SP525 (a lyophilized lipase preparation from *Candida antarctica* B), SP398 (a liquid lipase preparation from *Humicola*), SP523 (a lyophilized lipase preparation from *Humicola*) and Lipozym IM20 (*Rhizomucor miehei* lipase on anionic resin, activity 29 BIU/g) were kindly donated by Novo-Nordisk A/S (Bagsværd, Denmark). Lipomax, a lyophilized lipase preparation from *Pseudomonas alcaligenes*, was received from Gist-brocades B.V. (Delft, The Netherlands) as a gift. Accurel EP100 was kindly donated by Akzo Nobel (Obernburg, Germany).

2.1.1. Immobilization and activity test

Novozym 435 and Lipozym IM20 were used as received; immobilization of the enzymes on Accurel EP100 was performed according to a published procedure [4].

The activity of the immobilized enzyme preparations was measured in the hydrolysis of tributyrin at pH 7.2 with a pH-stat. The activity of the lipase preparations is given in kilo-lipase units per gram (KLU/g). One unit will hydrolyze 1 microequivalent of butanoic acid from tributyrin in 1 min. For results see Table 1.

2.2. Esterification with ethanol followed by ammoniolysis in t-butyl alcohol

Lipase Novozym 435 (100 mg) was added to a solution of octanoic acid (0.5 ml, 3.1 mmol) and diethylene glycol dibutyl ether (100 μl, internal standard) in ethanol (5 ml). The reaction mixture was shaken at 40°C for 24 h. Then, the enzyme was filtered off and the ethanol evaporated in vacuo. *t*-Butyl alcohol (5 ml) and fresh lipase Novozym 435 (100 mg) were added and the reaction mixture was saturated with ammonia. The reaction mixture was shaken at 40°C for 24 h. HPLC yields: ethyl octanoate: 95% (24 h), octanamide 80% (24 h).

2.3. Esterification of octanoic acid with n-butyl alcohol

n-Butyl alcohol (5 ml), octanoic acid (1 ml), diethylene glycol dibutyl ether (200 μl, internal standard) and 50 mg of enzyme preparation were shaken at 40°C for 24 h. The conversion was determined by HPLC (Table 2). To establish the effect of water, additional experiments were performed with lipase SP398 in the presence of 2% (v/v) of water. Further conditions were as described above.

Table 1
Activity of immobilized lipase preparations

Lipase preparation	Activity (KLU/g)
C. antarctica B (SP525) on Accurel EP100	12.4
Pseudomonas alcaligenes on Accurel EP100	7.5
Humicola (SP523) on Accurel EP100	14.3
Humicola (SP398) on Accurel EP100	8.9

Table 2
Esterification of octanoic acid with butanol [a]

	Esterification	Esterification	Transesterification
% water added	0	2	2
Lipase preparation			
C. antarctica B on acrylic resin (Novozym 435)	98	n.d.	n.d.
C. antarctica B (SP525) on Accurel EP100	94	n.d.	n.d.
Humicola (SP523) on Accurel EP100	79	n.d.	90
Humicola (SP398) on Accurel EP100	0	9	54

[a] % Reaction in 24 h as determined by HPLC.

2.4. Transesterification of ethyl octanoate with n-butyl alcohol

n-Butyl alcohol (5 ml), ethyl octanoate (1 ml), diethylene glycol dibutyl ether (200 μl, internal standard) and 50 mg of enzyme preparation were shaken at 40°C. After 24 h the conversion was determined by HPLC (Table 2). To establish the effect of water, additional experiments were performed with lipase SP525 and SP398 in the presence of 2% (v/v) of water. Further conditions were as described above.

2.5. Ammoniolysis of butyl octanoate

2.5.1. Ammoniolysis of butyl octanoate in t-butyl alcohol–n-butyl alcohol mixtures

Lipase (50 mg) was added to a solution of butyl octanoate (0.5 ml) and diethylene glycol dibutyl ether (50 μl, internal standard) in ammonia-saturated *t*-butyl alcohol/n-butyl alcohol mixtures (5 ml of various composition: 5/0, 2.5/2.5 and 0/5). The reaction mixture was shaken at 40°C and the course of the reaction was monitored by HPLC. The lipases which have been used and the conversions are given in Table 3.

2.5.2. Ammoniolysis in i-butyl alcohol and s-butyl alcohol

Lipase SP525 immobilized on Accurel EP100 was added to a solution of n-butyl octanoate (1 ml) or *s*-butyl octanoate (1 ml) and 100 μl of diethylene glycol dibutyl ether (internal standard) in ammonia-saturated *i*-butyl alcohol/*t*-butyl alcohol mixtures (total volume 5 ml) or *s*-butyl alcohol (5 ml). The reaction mixture was shaken at 40°C, and analyzed with HPLC. For results see Table 4.

2.6. One-pot procedure

2.6.1. Novozym 435

Lipase Novozym 435 (250 mg) was added to a solution of octanoic acid (5 ml, 32 mmol) in

Table 3
Inhibition of ammoniolysis by n-butyl alcohol

Lipase preparation	Conversion (% in 24 h)		
	n-Butyl alcohol/*t*-butyl alcohol (% v/v)		
	0	50	100
C. antarctica B on acrylic resin (Novozym 435)	77	9	5
C. antarctica B (SP525) on EP100	99	42	13
Humicola (SP523) on EP100	41	27	8
P. alcaligenes (Lipomax) on EP100	80	23	11
Rh. miehei on anionic resin (Lipozym IM20) [a]	15	0	0

[a] 1 equiv. of NH_3 was used.

t-amyl alcohol (50 ml) and n-butyl alcohol (3 ml, 32 mmol), which were contained in a 100 ml round bottomed flask equipped with a reflux condenser and a Soxhlet extractor containing 15 g of activated zeolite CaA. The reaction mixture was stirred for 24 h under reflux at 50°C in vacuo. Subsequently the reflux condenser and Soxhlet extractor were removed; the reaction mixture was saturated with ammonia and stirred for 48 h at 40°C. The course of the reaction was monitored by HPLC. After 48 h, the enzyme was filtered off and the solvent was evaporated in vacuo. The product was crystallized from methanol/petroleum ether 40–60. HPLC yields: butyl octanoate: 98% in 24 h (70% in 2 h), octanamide: 85% in 48 h. Isolated yield of octanamide: 3.43 g (24 mmol, 76%).

2.6.2. SP525 on Accurel EP100

The esterification step was carried out using the equipment described above, starting with lipase SP525 immobilized on Accurel EP100 (250 mg), octanoic acid (5 g, 35 mmol) and n-butyl alcohol (6 g, 81 mmol) in *t*-amyl alcohol (50 ml). The reaction mixture was stirred for 24 h under reflux at 50°C in vacuo. The reaction mixture was subsequently saturated with ammonia and stirred for 168 h at 40°C. The course of the reaction was monitored by HPLC. After 168 h, the enzyme was filtered off and the solvent was evaporated in vacuo. HPLC yields: butyl octanoate: 98% in 24 h, octanamide: 89% in 6 h, 97% in 168 h. Octanamide (isolated yield): 4.62 g (32.3 mmol, 93%). m.p.: 104°C, lit. 106–110°C, 99% pure on HPLC.

2.7. One-pot synthesis of oleamide from oleic acid

Lipase SP525 immobilized on Accurel EP100 (940 mg, 6.28 kLU/g) was added to a solution of oleic acid (5 g, 17.7 mmol, 83% pure according to GC) and n-butyl alcohol (3.0 g, 40.7 mmol) in *t*-amyl alcohol (45 ml) which were contained in a 100 ml round bottomed flask equipped with a reflux condenser and a Soxhlet extractor containing 15 g of activated zeolite CaA. The reaction mixture was stirred for 24 h under reflux at 60°C in vacuo; the progress of the reaction was monitored by TLC (hexane/ethyl acetate: 10/1). When the esterification was complete the reflux condenser and Soxhlet extractor were removed, the reaction mixture was saturated with ammonia and stirred for 96 h at 60°C. The course of the reaction was followed with TLC (hexane/ethyl acetate: 10/1). After 96 h, the enzyme was filtered off and the solvent evaporated in vacuo, which yielded 6.2 g of raw oleamide. The product was crystallised from hexane, which yielded 3.0 g oleamide (m.p. 73–74°C). A second crop of 1.5 g oleamide (m.p. 71–72°C) was obtained from the filtrate. Oleamide (total isolated yield): 4.5 g, 15.9 mmol, 90%, (84% pure according to GC).

3. Results and discussion

3.1. Preliminary experiment

We first tested the feasibility of a one-pot procedure by performing the two reaction steps consecutively. Esterification of octanoic acid catalyzed by Novozym 435 (lipase from *Candida antarctica* B immobilized on a macroporous acrylic resin) at 40°C using ethanol as reactant and solvent gave 95% of ethyl octanoate in 24 h. The enzyme was filtered off, the ethanol was evaporated in vacuo and *t*-butyl alcohol and fresh Novozym 435 were added. The reaction mixture was saturated with ammonia and after 24 h 80% of octanamide and 10% of octanoic acid were formed; unreacted ethyl octanoate accounted for the remainder. From these results we concluded, that it should, in principle, be possible to develop a real 'one-pot synthesis' of carboxylic amides from carboxylic acids via carboxylic esters. To establish the optimal reaction conditions for the one-pot procedure we studied the two consecutive reaction steps separately.

3.2. First step: esterification of octanoic acid

Octanoic acid was esterified with n-butyl alcohol at 40°C with several lipase preparations as catalyst. An almost quantitative conversion in 24 h was achieved with Novozym 435 and with SP525 (a lyophilized *C. antarctica* lipase preparation) immobilized on Accurel EP100 (see Table 2). *Humicola* lipase SP523 (a lyophilized lipase preparation) on Accurel EP100 was less active, *Humicola* lipase SP398, (a liquid lipase preparation) on Accurel EP100 showed no esterification activity at all. A small amount of water (2% v/v) proved to be indispensable for the activity of these *Humicola* lipases, both in esterification and transesterification reactions (see Table 2). Because water causes undesired hydrolysis, the *C. antarctica* lipases were an obvious choice for the one-pot procedure.

3.3. Second step: ammoniolysis in n-butyl alcohol

We first attempted the ammoniolysis of butyl octanoate in n-butyl alcohol with Novozym 435 as catalyst. Although n-butyl alcohol competes with ammonia as nucleophile in the ammoniolysis, complete conversion to octanamide can be reached, in principle, because the amide is the thermodynamically favoured product (see Appendix). Only traces of octanamide were formed, however. Comparison with ammoniolysis reactions in *t*-butyl alcohol and a 1:1 mixture of *t*- and n-butyl alcohol made it clear that n-butyl alcohol strongly inhibited Novozym 435 in the ammoniolysis reaction, because the conversion of butyl octanoate dramatically decreased in the presence of n-butyl alcohol (Table 3). From literature data it is known that the reaction rates of Novozym 435-catalyzed transformations are affected by alcohols [5], which may be the same phenomenon as we observe here.

To study whether inhibition by n-butyl alcohol was specific for Novozym 435 or an intrinsic feature of all lipases, similar experiments were performed with a number of other lipase preparations. In all cases a decrease in rate with increasing amounts of n-butyl alcohol was observed (see Table 3). In 50% n-butyl alcohol very strong inhibition of Novozym 435 was observed (9% conversion in 24 h) whereas SP525 (42% conversion in 24 h) was the least affected lipase. Since both are *C. antarctica* B lipases which differ only in the carrier material, this result clearly demonstrates the crucial role of the support in enzymatic reactions.

Although it would be possible to perform the one-pot procedure in n-butyl alcohol with lipase SP525 on Accurel EP100 as catalyst, the reaction rate is too low to be of practical value.

3.4. Second step: the use of other alcohols

Because it would be convenient to use the acyl acceptor of the esterification step as reaction medium in both steps, we subsequently investigated the use of *sec*-butyl and *i*-butyl alcohol in this role. We assumed that the ammoniolysis reaction would again be the bottle-neck and therefore we carried out the SP525-catalyzed ammoniolysis of n-butyl octanoate (as a model for the ammoniolysis of *i*-butyl octanoate) in *i*-butyl alcohol and the ammoniolysis of *sec*-butyl octanoate in *sec*-butyl alcohol. The results are given in Table 4.

From the data in Table 4 it can be seen that the SP525-catalyzed ammoniolyses in *i*-butyl alcohol and *sec*-butyl alcohol give slightly better results than the reaction in n-butyl alcohol (conversion 13% in 24 h, Table 2). However, the rate of ammoniolysis is too low to give a

Table 4
Inhibition of ammoniolysis by *i*-butyl alcohol and *sec*-butyl alcohol

Lipase preparation	*i*-Butyl alcohol/ *t*-butyl alcohol (% v/v)	Conversion (% in 24 h)
C. antarctica (SP525) on EP100	0	99
	50	48
	100	23
	sec-Butyl alcohol/ *t*-butyl alcohol (% v/v)	
C. antarctica (SP525) on EP100	100	20

sufficient yield of octanamide within a reasonable reaction time.

3.5. A practical one-pot procedure

To avoid the inhibition of Novozym 435 by n-butyl alcohol in the ammoniolysis step, the esterification was performed with one equivalent of n-butyl alcohol in *t*-amyl alcohol as solvent. The reaction was performed at diminished pressure and under reflux over molecular sieves to absorb the water side product. Butyl octanoate was readily formed (98% in 20 h). Subsequently, ammoniolysis was performed. After 45 h of reaction the conversion of butyl octanoate was 98% (determined by HPLC), and octanamide and octanoic acid were formed in 85% or 10% yield. After standard work-up procedures 76% of octanamide (based on the amount of octanoic acid started with) was isolated. The formation of octanoic acid during the ammoniolysis is attributed to traces of water which were not removed by reflux over molecular sieves. We assumed that the enzyme preparation absorbs a small amount of strongly bound water in the course of the esterification step, which is freed by ammonia upon saturation of the reaction mixture with ammonia, thus causing the hydrolysis side-reaction.

Accordingly, we carried out a preparative scale reaction with lipase SP525 (*Candida antarctica* B) immobilized on Accurel EP100 as catalyst, because this very apolar support (polypropene) would not absorb water. The reaction was carried out in the same way as described above: the use of two equivalents of n-butanol together with water removal in the esterification step resulted in 98% butyl octanoate in 24 h. Subsequent ammoniolysis yielded 97% of octanamide, and only traces of octanoic acid could be detected. After normal work-up procedures octanamide was isolated in 93% yield.

3.6. One-pot synthesis of oleamide

Long chain fatty amides [6] are industrially produced in thousands of tons a year from the fatty acids by reaction with anhydrous ammonia at approximately 200°C at a pressure of 345–690 kPa. They are primarily used for their lubricating and surfactant properties. Recently, *cis*-9,10-octadecenoamide was reported [7] to induce physiological sleep in rats, thus being a promising new sedative. The commercial importance of the long fatty amides and the harsh reaction conditions which are required for their synthesis, prompted us to investigate their synthesis from the carboxylic acid via the mild, enzymatic one-pot procedure.

Oleic acid was chosen as a model compound. The esterification was performed with 2.3 equiv. of n-butanol in *t*-amyl alcohol as solvent and with lipase SP525 on EP100 as catalyst. The reaction was performed at 60°C at diminished pressure and under reflux over molecular sieves. Complete conversion to butyl oleate was obtained after 20 h of reaction. Subsequent ammoniolysis yielded complete conversion to the oleamide in 96 h of reaction. After normal work-up procedures oleamide was isolated in 90% yield.

4. Conclusions

A new and efficient one-pot procedure for the synthesis of carboxylic amides from the corresponding carboxylic acids via in situ formation of carboxylic esters is developed. Because primary and secondary alcohols tend to retard the reaction rate of the ammoniolysis reaction, the one-pot procedure is best performed in a tertiary alcohol as solvent with one or two equivalents of the primary or secondary alcohol as esterifying agent. The one-pot procedure yields a simple and mild alternative method for the synthesis of (long chain) amides. We expect that this procedure can be advantageously applied to the synthesis of chiral amides from a racemic mixture of carboxylic acid, because this one-pot procedure offers two consecutive resolution steps, yielding chiral amides with high enantioselectivity. Work is in progress

Table 5

Component	ΔH_f(g) (kJ/mol)	ΔH_f(l) (kJ/mol)	$\Delta G_{(sol)}$ (kJ/mol)
butyl octanoate	−592.8 [8]		−1.007
ammonia	−45.9 [9]		−5.16
octanamide	−362.7 [8]		−1.66
butanol		−327.3 [9]	

to demonstrate the synthetic utility of this approach.

Acknowledgements

Generous gifts of enzymes by Novo-Nordisk A/S (Bagsvård, Denmark) and Gist-brocades B.V. (Delft, The Netherlands) are gratefully acknowledged. The authors thank Akzo Nobel (Obernburg, Germany) for donations of Accurel EP100.

Appendix A. Estimated heat of reaction of ammoniolysis

$\Delta H_{reaction}$ was estimated from ΔH_f and the heat of solvation ΔG_{sol}.

For the reaction components ΔH_f(g) was used, except for butanol for which ΔH_f(l) was used because butanol is the continuous phase (see Table 5).

ΔG_{sol} was calculated from the formula:

$$\Delta G = -14.39325214 \frac{\mu^2}{r^3} \frac{(\epsilon - 1)}{(2\epsilon + 1)} \text{ kcal/mol}$$

From the data in Table 5 $\Delta H_{reaction}$ was calculated to be −46.79 kJ/mol.

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