Journal of Molecular Catalysis B: Enzymatic 65 (2010) 49–51

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

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

Biocatalyzed irreversible esterification in the preparation of *S*-naproxen

R. Morrone*, N. D'Antona, D. Lambusta, G. Nicolosi

CNR – Istituto di Chimica Biomolecolare, Via Paolo Gaifami 18, 95126 Catania, Italy

ARTICLE INFO

Article history:
Available online 25 January 2010

Keywords:
Naproxen
NSAID
Profen
Lipase
Dimethyl carbonate
Irreversible esterification

ABSTRACT

Dimethyl carbonate was used as alcohol donor in the esterification of naproxen in the presence of immobilised lipase B from *Candida antarctica* (Novozym 435). The conjugation of hydrolysis of dimethyl carbonate and esterification of acid, created irreversible operative conditions so permitting the recovery of *S*-naproxen in high ee (>98%). The adopted procedure has a general use.

1. Introduction

Profen drugs are 2-phenylpropionic acid derivatives and constitute a structural class of NSAIDs widely used for the treatment of inflammatory diseases, such as rheumatoid arthritis and osteoarthritis. The anti-inflammatory property of profens resides primarily in the S enantiomer [1–3] and, in order to obtain the eutomer, numerous strategies have been developed, such as asymmetric synthesis [4,5], biocatalytic kinetic resolution [6,7], preferential crystallization [8] and chiral chromatography separations of racemates [9].

Among these procedures, the enantioselective catalysis by lipases has furnished good results [10–12]. In this way, the direct esterification in organic solvent of the specific profen of interest, in presence of a lipase with R stereopreference, is a valuable method because preliminary derivatizations are not required and the eutomer (S-enantioform) is recovered as unreacted substrate. However, this method needs to reach conversion values beyond 50% and therefore, the use of irreversible conditions is mandatory [13–15]. Otherwise, due to the water produced in the esterification reaction, at high conversion grade the favoured hydrolysis of the enantioform preferentially recognised by the lipase occurs, with consequent final damage of the enantiomeric excess of both ester (R-form) and unreacted acid (S-form).

Different approaches have been suggested to remove the water in a lipase catalyzed esterification, in order to shift the equilibrium towards the products, but they are not practicable for preparative purposes. The distillation of water [16] results impracticable when a low boiling point solvent or a volatile nucleophile (methanol) are used. The removal of water by the use of salt hydrates [17,18] has no real application since, due to the heterogenic operative conditions, the damage to the enzyme occurs during the shaking of the reaction mixture and moreover, the separation of water saturated salts is practically impossible. In the past we have envisaged, in an intrinsically chemical action, the possible procedure to overcome the problem, and we have indicated a valid alcohol generator system based on the reaction of water with alkyl orthoformates, although two molecules of unreacted alcohol are wasted for each ester molecule produced [13,19].

In this work, we report an improvement of the biocatalyzed irreversible esterification reaction using dimethyl carbonate as alcohol donor. This new procedure has successfully been applied to obtain *S*-naproxen.

2. Materials and methods

2.1. Materials

The chemicals and solvents were obtained from commercial sources and used without further purification. The (*S*)-(+)-naproxen was obtained from Sigma–Aldrich and racemic naproxen was obtained by a reported procedure [20]. Novozym 435® (lipase from *Candida antarctica*, CAL B), lipases from *Candida rugosa* and *Pseudomas cepacia* were purchased from Sigma; Lipozyme (lipase from *Mucor meihei*) was obtained from Fluka; *Rhizomucor miehei* lipase from Genzyme. The conversion values and enantiomeric excesses of the biocatalyzed reactions were determined by a Dionex HPLC apparatus equipped with LiChroCART® 250-4 (S,S)Whelk-O1 column and UV detector at 254 nm. The analyses were performed using a mixture of hexane, 2-propanol and acetic acid in the ratio

* Corresponding author. Fax: +39 095 7338310.
E-mail address: raffaele.morrone@icb.cnr.it (R. Morrone).

1381-1177/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2010.01.014

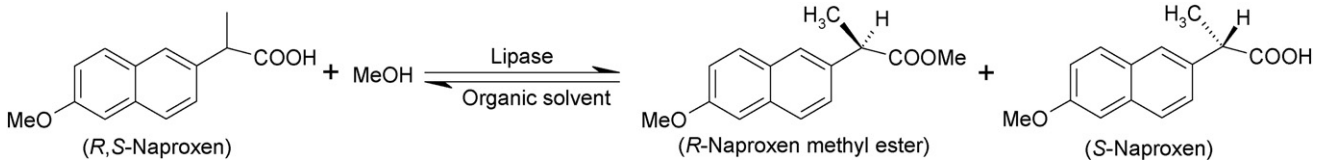

Fig. 1. Biocatalyzed esterification of (±)-naproxen.

of 80:20:0.5 as eluent. The enantiomeric ratio (*E*) was calculated from the extent of esterification and the enantiomeric excess of the unreacted acid using the equation reported in literature [21].

2.2. General procedure for esterification of (±)-naproxen by lipase

Rac-naproxen (10 mg, 0.043 mmol) was dissolved in 1 ml of solvent of choice and 40 mg of enzyme, 5.2 μl (0.129 mmol) of methanol were added to the solution and kept in a shaker at 45 °C and 300 rpm. At the opportune conversion value, the reaction was stopped by filtering off the lipase. The progress of the reaction was monitored by chiral HPLC on aliquots of 10 μl of the reaction mixture taken periodically.

2.3. (S)-(+)-Naproxen

To a solution of rac-naproxen (500 mg; 2.17 mmol) and dimethyl carbonate (585.9 mg; 6.51 mmol) in acetonitrile (50 ml) was added Novozym (2 g) and the resulting mixture was stirred at 45 °C and 300 rpm for 9 days. The reaction was stopped by filtering off the enzyme and the filtrate was partitioned with saturated bicarbonate solution. The aqueous phase was acidified to pH 2.0 with 2N H_2SO_4 and extracted with *tert*-butyl methyl ether to give 130 mg of unreacted (*S*)-(+)-naproxen (yield 52%; ee > 98%).

3. Results and discussion

3.1. Screening of lipases

In order to identify a suitable lipase possessing R stereopreference, (±)-naproxen was subjected to direct esterification in presence of immobilised [*Candida antarctica* (Novozym 435), *Mucor meihei* (Lipozyme)] and not immobilised (*Candida rugosa*, *Pseudomas cepacia*, *Rhizomucor meihei*) lipases (Fig. 1) in di-isopropyl ether as solvent and methanol as nucleophile.

All the investigated lipases catalyzed the esterification of naproxen but only Novozym 435 showed the desired R stereopreference, as reported in Table 1. All the next reactions were carried out in presence of Novozym 435 as catalyst.

3.2. Selection of solvent

With the intention of improving the enantioselectivity of Novozym 435 in the esterification of naproxen, in order to consider it for an enantiomeric preparative resolution, the reaction was repeated in different solvents as reported in Table 2.

Table 1
Esterification of (±)-naproxen with different lipases.

Lipase from	Time (d)	Conv. %	Est. ee	Ac. ee	Stereopref.
Candida antarctica (Novozym 435)	1	26	28	10	R
Candida rugosa	5	21	75	20	S
Pseudomonas cepacia	1	12	15	22	S
Mucor meihei (Lipozyme)	2	26	3	3	S
Rhizomucor meihei	2	14	12	2	S

Reaction conditions: 10 mg (0.043 mmol) (±)-naproxen, 5.2 μl (0.129 mmol) methanol, 40 mg lipase, 1 ml di-isopropyl ether, 45 °C, 300 rpm.

Table 2
Esterification of (±)-naproxen in different solvents.

Solvent	Time (d)	Conv. %	ee_{Ac}	ee_{Est}	*E*
Tetrahydrofuran	2	0	–	–	–
Diethyl ether	2	24	10	32	2
Di-isopropyl ether	1	26	10	28	2
tert-Butyl methyl ether	1	42	25	34	2
Toluene	1	34	15	29	2
1,2-Dichloropropane	1	21	15	56	4
1,4-Dioxan	4	12	8	59	4
Dichloromethane	4	28	23	59	5
tert-Amyl alcohol	7	15	11	62	5
Acetonitrile	3	29	29	71	8
Chloroform	2	21	20	73	8

Reaction conditions: 10 mg (0.043 mmol) (±)-naproxen, 5.2 μl (0.129 mmol) methanol, 40 mg Novozym 435, 1 ml solvent, 45 °C, 300 rpm.

All the considered solvents, except tetrahydrofuran, allowed to perform the esterification. Reactions carried out in acetonitrile and chloroform gave the best results in terms of enantioselectivity (*E* = 8). Although in chloroform the reaction rate is higher than in acetonitrile, this latter was chosen as solvent for further experiments in view of the lesser toxicity.

3.3. Reversible esterification

A resolution of (±)-naproxen by esterification catalyzed by Novozym 435 was attempted in acetonitrile in presence of methanol as nucleophile. In the adopted condition the equilibrium was reached in 7 days with a conversion value of substrate approaching 55%. Any lengthening of the reaction time led to the slow decline in the ee values, as indicated in Fig. 2.

3.4. Irreversible esterification procedure

The irreversible devised procedure contemplates the use of a dialkylcarbonate as “chemical utilizer” of the water produced during the biocatalyzed esterification reaction. In particular, the

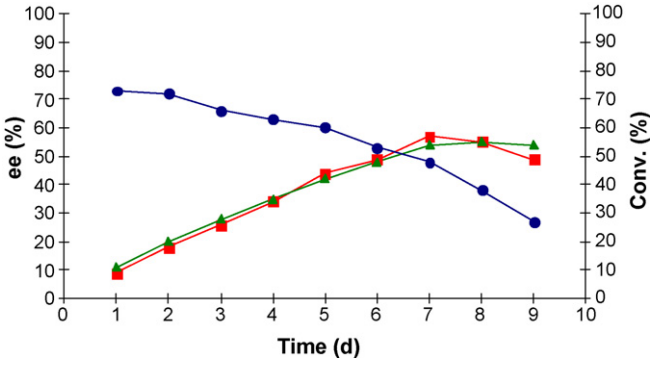

Fig. 2. Trend of biocatalyzed (±)-naproxen esterification under reversible conditions. (■) Enantiomeric excess of unreacted *S*-naproxen; (●) enantiomeric excess of *R*-naproxen methyl ester; (▲) conversion value.

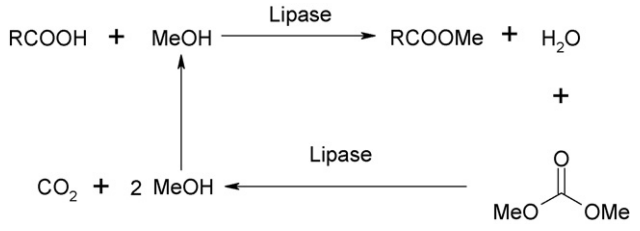

Fig. 3. Representation of biocatalyzed irreversible esterification process.

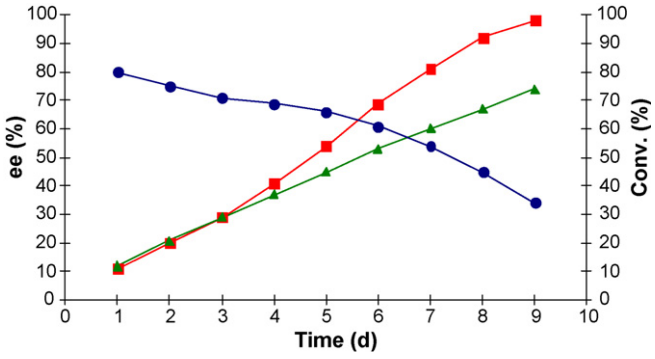

Fig. 4. Trend of irreversible biocatalyzed esterification of (±)-naproxen. (■) Enantiomeric excess of unreacted *S*-naproxen; (●) enantiomeric excess of *R*-naproxen methyl ester; (▲) conversion value.

coupling of two lipase catalyzed reactions working in synergy takes place, as reported in Fig. 3.

In an initial step, lipase catalyses the reversible esterification of the acid, generating water. At the same time, the produced water hydrolyses the dialkylcarbonate in presence of the lipase and as a consequence CO_2 and two molecules of methanol are generated allowing the progression of the esterification process in irreversible manner. At the final stage of the reaction, the presence of methanol in excess permits the dynamic transformation (transalcoholysis) of the produced ester but without any effect on the conversion and ee values. With this intention dimethyl carbonate [22] was added to a solution of naproxen in acetonitrile and the esterification reaction started using 0.001% of pure methanol. The progress of esterification was monitored by chiral HPLC (Fig. 4). After 9 days, when the conversion value was 74%, the reaction was stopped and *S*-naproxen was present in the mixture of reaction with a ee >98% (yield 52%) while the naproxen (*R*)-methyl ester was recovered with low enantiomeric purity (ee 34%). In the adopted reaction conditions, neither naproxen ester formation nor hydrolysis of dimethyl carbonate was observed in absence of Novozym 435.

4. Conclusion

We have applied a new method to achieve the esterification of a chiral acid in irreversible manner by the use of dimethyl carbonate as alcohol donor. The procedure preserves the enantiorecognition of the substrate during the entire esterification reaction, thus permitting the recovery of unreacted substrate in enantiopure grade. The procedure applied to the resolution of naproxen furnished *S*-naproxen with ee > 98% and yield 52%. The adopted method has general use, and work is in progress in our laboratory using this approach in the esterification/resolution of valuable organic acids.

References

[1] S.S. Adams, P. Bresloff, C.G. Mason, J. Pharm. Pharmacol. 28 (1976) 256–257.
[2] A.J. Hutt, J. Caldwell, J. Pharm. Pharmacol. 35 (1983) 693–704.
[3] J. Caldwell, A.J. Hutt, S. Fournel-Gigleux, Biochem. Pharmacol. 37 (1988) 105–114.
[4] C.R. Smith, T.V. RajanBabu, J. Org. Chem. 74 (2009) 3066–3072.
[5] H.R. Sonawane, N.S. Bellur, J.R. Ahuja, D.G. Kulkarni, Tetrahedron Asymmetr. 3 (1992) 163–192.
[6] H.Y. Lin, S.W. Tsai, J. Mol. Catal. B: Enzym. 24–25 (2003) 111–120.
[7] S. Panke, M.G. Wubbolts, Curr. Opin. Biotechnol. 13 (2002) 111–116.
[8] J.C. Wu, Y. Chow, R. Li, K. Carpenter, Sep. Purif. Technol. 53 (2007) 144–147.
[9] J. Bojarskil, H.Y. Aboul-Enein, A. Ghanem, Curr. Anal. Chem. 1 (2005) 59–77.
[10] R. Morrone, G. Nicolosi, M. Piattelli, Chirality 10 (1998) 321–324.
[11] R. Morrone, G. Nicolosi, A. Patti, M. Piattelli, Tetrahedron Asymmetr. 2 (1995) 1773–1778.
[12] P. Mertoli, G. Nicolosi, A. Patti, M. Piattelli, Chirality 8 (1996) 377–380.
[13] R. Morrone, M. Piattelli, G. Nicolosi, Eur. J. Org. Chem. 4 (2001) 1441–1442.
[14] R. Morrone, N. D'Antona, G. Nicolosi, Rasayan J. Chem. 1 (2008) 732–737.
[15] G.E. Jeromin, A. Zoor, Biotechnol. Lett. 30 (2008) 925–928.
[16] F. Roure, A. Ducret, M. Trani, R. Lortie, J. Chem. Technol. Biotechnol. 69 (1997) 266–270.
[17] P. Kubl, P.J. Hailing, Biochim. Biophys. Acta 1078 (1991) 326–328.
[18] M.G. Kim, S.B. Lee, J. Ferm. Bioeng. 81 (1996) 269–271.
[19] R. Morrone, G. Nicolosi, M. Piattelli, PCT/EP00/07102.
[20] S.W. Tsai, H.J. Wei, Enzym. Microb. Technol. 16 (1994) 328–333.
[21] C.-S. Chen, Y. Fujimoto, G. Girdaukas, C.J. Sih, J. Am. Chem. Soc. 104 (1982) 7294–7299.
[22] S. Memoli, M. Selva, P. Tundo, Chemosphere 43 (2001) 115–121.