Pergamon

Tetrahedron Letters, Vol. 36, No. 34, pp. 6067-6068, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4039/95 $9.50+0.00

0040-4039(95)01065-3

Ultrasound-Promoted Lipase-Catalyzed Reactions

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Abstract: Lipase from porcine pancreas is first demonstrated to catalyze reactions under ultrasonic condition. Reaction rates are significantly enhanced 7 to 83-fold and enantioselectivities are retained.

The recent development of enzyme catalysis in organic synthesis for kinetic resolutions of racemates has attracted the attention of organic chemists because of their synthetic utility.[1-5] The lipase-catalyzed acylations and transacylations have become popular methods in asymmetric synthesis.[3] A major drawback of applying lipase-catalyzed reactions in organic synthesis is the low reaction rate. We described a method based on azeotropic distillation to increase the lipase-catalyzed transacylation reaction rate by 4 to 70-fold.[6,7] We reported here another method based on ultrasonication to increase the lipase-catalyzed reaction rates in organic solvents.

Porcine pancreatic lipase (PPL) catalyzes the hydrolysis of *(R)*-1,2,3,4-tetrahydro-1-naphthylbutyrate (*R*-**1**) from its racemates (*rac*-**1**) to produce *(R)*-1,2,3,4-tetrahydro-1-naphthol (*R*-**2**) and recover *(S)*-1,2,3,4-tetrahydro-1-naphthylbutyrate (*S*-**1**) with high stereoselectivity. Under probe-ultrasonic conditions, the reaction rates and stereoselectivities decrease (Table 1). Low reaction rates may be from enzyme denaturation because of the increase of local temperature under probe-ultrasonic conditions. Low stereoselectivities are probably due to processes of chemical racemerization at locally high temperature. However, the reaction rate increases 7-fold and stereoselectivity remains under a bath-ultrasonic condition (Table 1).

Table 1. Porcine pancreatic lipase[a]-catalyzed hydrolysis of *rac*-**1** with water[b] in hexane

reaction type	temp./°C	first order rate constants[c]/h^{-1}	% ee (*S*-**1**)[d]	% ee (*R*-**2**)[d]	conv.[e]	E[f]
control	33	0.055±0.004	84	96	0.47	98
probe[g]/156W	n.d.[h]	0.0118±0.0005	34	94	0.27	33
probe/360W	n.d.	0.0079±0.0003	22	92	0.19	39
probe/600W	n.d.	$<10^{-4}$	-	-	-	-
bath[i]/375W	33	0.39±0.03	95	96	0.5	146

a. 5 mass equivalents of enzyme (Sigma L0382) was used. b. 100 mole equivalents were used c. First order rate constants were determined from the number of moles of the product formed (from HPLC) *vs* time plot by nonlinear curve fitting to first order kinetic equation. d. % ee was calculated by % ee = OP - (1- OP). Optical purity of reactive alcohol *R*-**2** was calculated by OP = $[\alpha]_{D\ exp.}$ / $[\alpha]_{D\ lit.}$. $[\alpha]_{D\ exp.}$ of *R*-**2** is measured from a polarimeter at 25°C, c2.5, $CHCl_3$. $[\alpha]_{D\ lit.}$ at 17°C of *R*-**2** is -32° which is obtained from Aldrich Catalog 1992-1993. *S*-**2** is obtained from basic hydrolysis (0.1 N KOH, EtOH, 25°C, 18h, 94%) of the unreactive ester *S*-**1**. e. the extent of conversion[8] f. the enantiomeric ratio[8] g. Vibra-cell high-intensity ultrasonic processor (VC600, up to 600 W, Sonics & Materials) equiped with a Suslick sonochemical reaction vessel h. not determined i. Bransonic ultrasonic cleaner (Branson 3200, 375 W)

For the PPL-catalyzed acylation of *rac*-**2** with vinyl acetate to produce *(R)*-1,2,3,4-tetrahydro-1-naphthyl-acetate (*R*-**3**) and recover *S*-**2**, the stereoselectivity remains but reaction rate increases 83-fold by applying the bath-ultrasonication (Table 2).

Table 2. PPL[a]-catalyzed acylation of *rac*-**2** with vinyl acetate[b] in benzene-ether (5/1, v/v)

reaction type	temp./°C	first order rate constants/h^{-1}	% ee[c] (*S*-**2**)	% ee (*R*-**3**)	conv.	E
control	33	0.003±0.001	92	95	0.49	152
probe[d]	n.d.	$<10^{-4}$	-	-	-	-
bath/375W	33	0.25±0.01	92	96	0.49	152

a. 1 mass equivalents of enzyme (Sigma L0382) was used. b. 100 mole equivalents were used c. similar to those described in Table 1. d. 156, 360, or 600 W

It is generally accepted that the rate enhancement caused by ultrasonication for most nonenzymatic reactions is due to the locally high pressures (up to 1000 atm) and temperature (up to 5000 K)[9,10] and the increase in usable surface area for catalysis.[11] For most enzyme catalyzed reactions, the reaction rates increase with temperature up to a certain limit. Above a certain temperature, enzyme activity decreases with temperature because of enzyme denaturation.[12] For PPL-catalyzed both hydrolysis of *rac*-**1** and acylation of *rac*-**2** in organic solvents, the reaction rates increase with temperature up to 37°C (activation energies are 74 and 81 kJ/mol, respectively). Above 37°C, enzyme activity decreases with temperature (deactivation energies are 83 and 90 kJ/mol, respectively). Therefore, the rate enhancements from ultrasonication of PPL-catalyzed reactions are not due to the locally high temperature but due to the locally high pressures or the increase in usable surface area for catalysis.

In summary, ultrasonication can be a simple, effective method to promote enzyme-catalyzed reactions in organic solvents.

Acknowledgement: We thank the National Science Council of Republic of China for financial support (NSF 84-2113-M005-001).

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(*Received in Japan* 9 *May* 1995; *revised* 7 *June* 1995; *accepted* 12 *June* 1995)