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LIPASE CATALYZED REACTIONS AND STRATEGY FOR ALCOHOL RESOLUTION

Georges Langrand, Jacques Baratti[+], Gérard Buono and Christian Triantaphylides*

Ecole Supérieure de Chimie de Marseille, Centre de Saint-Jérôme
Rue Henri Poincaré, 13397 Marseille Cédex 13, FRANCE
Laboratoire de Chimie Bactérienne du CNRS[+]
BP 71, 13277 Marseille Cédex 9, FRANCE

Abstract : *Asymmetric hydrolysis, ester interchange and ester formation were carried out for menthol resolution with Candida cylindracea lipase. The reactions were realized under emulsified conditions with water soluble enzyme and in organic medium with an insoluble enzyme preparation. In the latter case, an enhancement of the enantioselectivity was observed. The potentialities of ester interchange and ester formation for preparative alcohol resolutions are discussed.*

Lipases (triacylglycerol hydrolases E.C. 3.1.1.3.) catalyze two reversible reactions : ester hydrolysis-synthesis (1) and ester interchange (2) which is less classical. These reactions are well known in the fats and oil chemistry (3), and have been recently applied to organic asymmetric synthesis since these enzymes exhibit high enantioselectivity towards alcohols (4-5-6) and acids (7). We report in this paper several strategies for alcohol resolution by using lipase catalyzed reactions. In this context, a similar approach although less exhaustive was recently described (7). Racemic menthol has been chosen as a model compound and the resolution carried out as previously described with *Candida cylindracea* lipase (Trade Name, "Lipase My", Meito Sangyo Co.) (6).

The lipases catalyze acyl transfer reactions (8), and the mode of action of these enzymes is related to the presence of an interface (9). As far as the racemic alcohol is concerned, the ester hydrolysis-synthesis and ester interchange reactions can be highly enantioselective. Thus it is possible to conceive four reactions for alcohol resolution :

Hydrolysis

1. (±) menthyl ester + water ————> (-) menthol + (+) menthyl ester + acid

Ester interchange

2. (±) menthyl ester + alcohol ————> (-) menthol + (+) menthyl ester + ester
3. (±) menthol + ester ————> (+) menthol + (-) menthyl ester + alcohol

Ester formation

4. (±) menthol + acid ————> (+) menthol + (-) menthyl ester + water

Table 1 : Reactions under emulsified conditions ; enzyme in aqueous phase

Reactions	Experimental conditions with purified *Candida cylindracea* lipase (11) (250000 eu/g)(10)			Results	
				Initial rate (Relative rate to tributyrin hydrolysis)(10)	Products (% ee) (13) % Conversion based on the racemic substrate (time)
Hydrolysis	t = 37°C				(−) menthol
1. (±) menthyl laurate	(±) menthyl laurate	0.36 g		3400	(70 % ee)
	lipase	10 mg		(μmole/mn.g enz.)	
	borate buffer	1 mM	25.6 ml	(1/74)	38 % (60 mn)
	pH monitored at	7.5			
Ester interchange	t = 37°C				(−) menthol
2. (±) menthyl laurate	(±) menthyl laurate	0.25 M	10 ml	1.7	(80 % ee)
	1-pentanol	1 M		(μmole/mn.g enz.)	
	heptane				
	lipase	30 mg	2 ml	(1/150000)	48 % (15 days, equilibrium)
	borate buffer 1 mM, pH7.3				
Ester formation	t = 45°C				(−) menthyl laurate
4. (±) menthol with lauric acid	(±) menthol	4 g		130-250	
	lauric acid	5.2 g		(μmole/mn.g enz.)	(86 % ee)
	lipase	80 mg			
	phosphate buffer	0.1 M,	2 ml	(1/1900 - 1/1000)	41 % (10 hours)
	pH 7.0				

These reactions have been studied in the two ways :

- under emulsified conditions, with water soluble enzyme and immiscible substrates (Table 1)
- in an organic solvent, with insoluble enzymatic preparation and soluble substrates (Table 2).

The results obtained are discussed in terms of initial rate, degree of conversion, and enantioselectivity.

Relative rates to tributyrin hydrolysis have been given for the two lipase preparations which have been used in order to facilitate comparisons. (see Table 1 and 2). The highest initial rate has been obtained with the hydrolytic reaction. However it is difficult to reach the required conversion ratio, owing to product inhibition, as will be reported later. Ester interchange and ester formation reactions occurred at similar rates with trilaurin or lauric acid, while ester interchange starting from (±) menthyl laurate as acyl donor compound proceeded slowly.

The enantiomeric excess observed for ca. 40 % conversion ratio is very high (> 95 %) when the reaction is carried out in an organic solvent. Optical purities are lower (70-85 %) under emulsified conditions. This difference results probably from an enhancement of the enzymatic conformation rigidity in the former case. Therefore, reactions carried out in organic solvent with the powdered "Lipase My" or with the enzyme supported on glass beads (12) should be preferred.

Table 2 : Reactions in organic solvent, with insoluble enzyme

Reactions	Experimental conditions with "lipase My" (14000 eu/g) (10)			Results	
				Initial rate (Relative rate to tributyrin hydrolysis)(10)	Products (% ee)(13) % Conversion based on the racemic substrate(time)
Ester interchange	t = 40°C			0.35	(-) menthol
2. (±)menthyl laurate with isobutanol	(±) menthyl laurate Isobutanol heptane	0.25 M 1 M	} 10 ml	(µmole/mn.g enz.) (1/40000)	(94 % ee) 45 % (15 days, equilibrium)
	lipase My	0.5 g			
Ester interchange	t = 40°C				(-) menthyl laurate
3. (±) menthol with trilaurin	(±) menthol trilaurin heptane	0.5 M 0.5 M	} 10 ml	5.0 (µmole/mn.g enz.)	(95 % ee)
	lipase My	0.5 g		(1/2800)	33 % (15 days, equilibrium)
Ester formation	t = 40°C				(-) menthyl laurate
4. (±) menthol with lauric acid	(±) menthol lauric acid heptane	0.25 M 0.25 M	} 10 ml	7.0 (µmole/mn.g enz.)	(95 % ee)
	lipase My	0.5 g		(1/2000)	45 % (10 hours)

Under the conditions described in Table 2, ester formation is a complete reaction and the water which is produced is adsorbed by the biocatalyst. Ester interchanges are subjected to equilibrium conditions. For preparative purposes, it is of prime importance to choose conveniently the substrates and their concentrations in order to obtain a good degree of conversion and to separate easily the reaction products.

Menthol resolution with *Candida cylindracea's* lipase has been achieved starting either from the racemic alcohol or an ester of this alcohol. Thus, the combination of two enzymatic steps, ester synthesis and ester solvolysis, should open a way for the preparation of various optically pure alcohols.

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