Microwave-Promoted Lipase-Catalyzed Reactions. Resolution of (±)-1-Phenylethanol

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Microwave irradiation increased enzymatic affinity and selectivity of supported lipases in esterification and transesterification reactions carried out in dry media and at temperature near 100 °C.

Introduction

In recent years, chemoenzymatic methodology has become a standard technique for the preparation of a wide variety of enantiomerically pure molecules.[2] Among them, lipase-catalyzed acylations and transacylations represent an important class of enzymatic transformations in organic chemistry. The use of biological catalysts for technological purposes requires that enzymes be stable and functional in nonphysiological environments. Reactions in organic media, under pressure or at elevated temperatures, are increasingly used.[3a]

Until now no general correlation of reaction enantioselectivity with physicochemical constants of solvents could be established.[4] Temperature has long been known to be inversely correlated with enzyme enantiospecificity (*E*). However, since 1992, numerous examples in the opposite way have been accumulated.[3] To date, temperature dependent variations in *E* values are employed as a practical means to achieve reaction stereoselectivity even near 100 °C.[3a]

The devising of enzymatic systems for use in organic media is in constant progress. New thermostable enzymes now available through recombinant DNA technology (Novozym SP 435) or isolation from thermophilic microorganisms allow one to work at relatively high temperatures.[19]

One of the major limitations of enzymatic synthesis is the reversible nature of the reactions which result in low rates and low selectivities.[2] A technique to displace the equilibrium toward the desired direction, which has industrial applications, is to continuously remove molecules such as water or ethanol formed in the process by azeotropic distillation[5] or evaporation under reduced pressure.[6]

The purpose of this paper is to show it is possible to take advantage of microwave exposure to induce equilibrium shifting by evaporation of light polar molecules which are strongly interacting with the electromagnetic field. Electromagnetic field of high frequency (2450 MHz) induces molecular rotation which is accompanied by intermolecular friction of polar species and subsequent dissipation of energy by heating in the core. Toward this aim, microwave activation has to be coupled with solvent-free methods to facilitate and to keep the procedure safe.[7] The use of focused microwaves allows one to combine the advantages of an homogenous field with very high energetic yields[8] (therefore without local heating effects) and with close control of the reaction temperature.[9] Results are then compared with those obtained under classical heating strictly under the same conditions.

Results and Discussion

To work under dry media conditions, enzymes were immobilized on solid supports[10] of adequate pH; thus only weak interactions with microwaves would occur, avoiding high-temperature enhancement. Four types of solid supports were *a priori* considered: three of them are mineral (Florisil, Celite 545, and Hyflo Super Cel = HSC[11]) and the last one is organic (a polypropylene resin = Accurel). Their behavior was tested under microwaves for 30 min at a power level of 90 W (Figure 1 and Table 1).

On the basis of experimental results, Florisil was eliminated on the ground that it absorbs microwaves too strongly. We subsequently found HSC and Accurel to be the best supports for lipase-catalyzed transesterification, and only results related to these two supports will be presented in this report.

The lipases selected for our study were the *Pseudomonas cepacia* lipase (LP) and *Candida antarctica* lipase (SP 435).

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(11) Hyflo Super Cel composition according to Fluka informations: silica 89.3%; Al_2O_3 4.2%; Na_2O,K_2O 3.5%; Fe_2O_3 1.4%. Silica structures are a mixture of α-cristobalite and α-tridymite.

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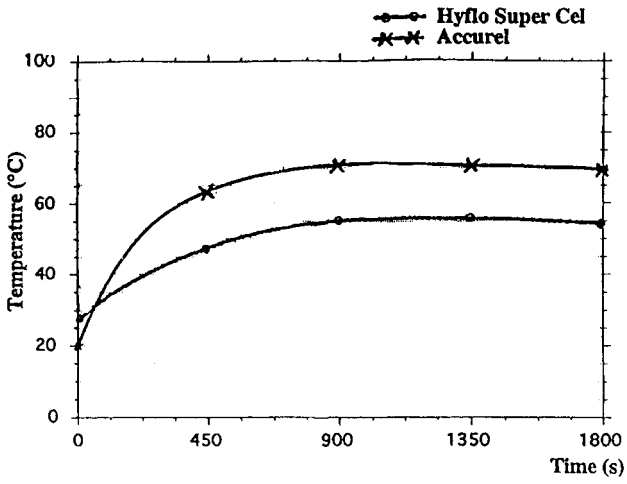

Figure 1. Thermal behavior of supports (1 g) under microwave exposure (power = 90 W)

Table 1. Support Behavior and Property

		final temperature (°C) after MW exposure	
Support	pH	30 W	90 W
Florisil	8.5−9	120	
Celite 545	7.5	50	55
HSC	8.5−9	50	55
Accurel			65−70

[a] MW = microwave.

Specification of the enzyme purchased indicates that (i) LP presents good stability in the dry state during at least 10 h at 100 °C (Figure 2a)[13] and (ii) Novozym 435 is thermostable with a maximum activity in the range of 70−90 °C (Figure 2b).[12]

We first studied the stability of these enzymes under our reaction conditions. *P. cepacia* lipase was therefore dispersed on HSC (10−30% w/w) whereas *C. antarctica* was impregnated on Accurel from pH 7 buffered solution (commercial product Novozym SP 435[12]).

The supported enzymes were placed during 30 min under microwave irradiation at the temperature range of 70−100 °C and subsequently tested in the transesterification reactions of racemic 1-phenylethanol. Within experimental errors, their activities were found to be the same as those of freshly prepared, nonheated, supported enzymes.

The resolution of racemic 1-phenylethanol through esterification or transesterification, a reaction quite often described in the literature about enzymatic reaction,[14] was then studied under microwave irradiation. Transesterification was performed both with nonactivated (ethyl valerate) and with activated esters (isopropenyl acetate).

The substrates were solubilized in organic solvents and then impregnated on the enzyme-loaded supports by subsequent evaporation of these solutions (Scheme 1).

(12) Novozym 435, product sheet from Enzyme Process Division, Novo-Nordisk, Bagsvaerd, Denmark.

(13) Lipase LP, a new lipolytic enzyme preparation, product sheet from Amano Pharmaceutical Co. Ltd, Nagoya, Japan.

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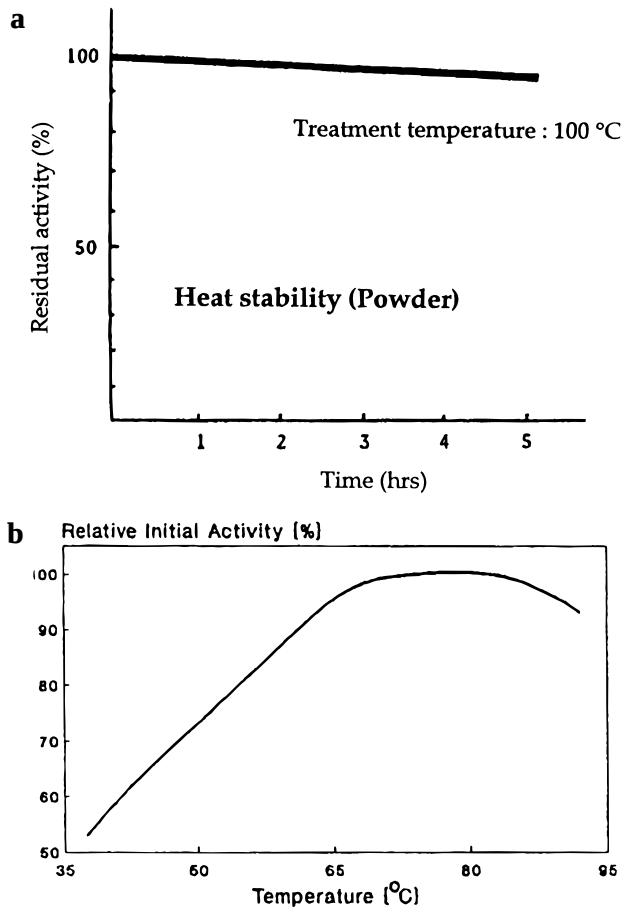


Figure 2. (a) Heat stability of LP (powder). (b) Activity of Novozym versus temperature.

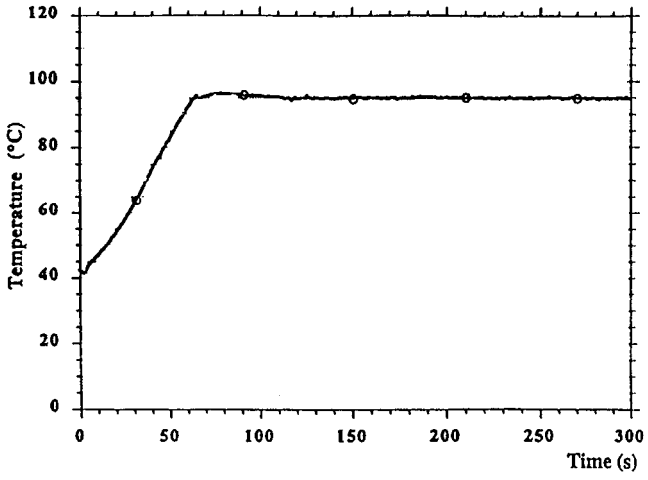


Figure 3. Thermal evolution of SP 435 catalyzed phenylethanol transesterification.

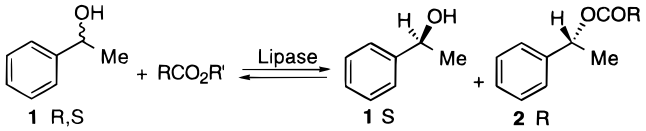
Reactions were then performed in dry media inside a monomode microwave reactor with focused waves, since the energy distribution is much more homogeneous and consequently much more efficient than with domestic ovens. The temperature was maintained at a fixed value through control of incident power[9] (Figure 3). For the sake of comparison, and to check specific microwave (purely nonthermal) effects, reactions were also performed in a thermostatted oil bath at the same temperature for the same time with as close as possible heating profiles.

Table 2. Acylation of (*RS*)-1-Phenylethanol under Microwave Irradiation or Classical Heating

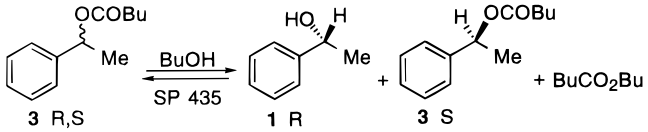
lipase	R	R′	run no.	mode of activtn	*t* (mn)	*T* (°C)	conv (%)	ee alcohol *S* (%)	*E*
LP/HSC	C_4H_9	C_2H_5	1	Δ[a]	180	77	46	32	3
			2	MW[b] 90 W	180	77	50	47	8
	CH_3	$H_2C{=}CCH_3$	3	Δ	15	85	38	50	16
			4	MW 240 W	15	85	47	79	42
SP 435	C_7H_{15}	C_2H_5	5	Δ	60	70	34	46	28
			6	MW 60 W	60	70	36	51	36
			7	Δ	10	90	40	62	**50**
			8	MW 90 W	10	90	43	75	>**100**
			9	MW 90 W	15	90	47	88	>100
		H	10	MW 30 W	10	67	45	66	18
			11	Δ	10	78	48	62	10
			12	MW 60 W + 20 W	10 (5 + 5)	78	52	93	44
			13	MW 300 W + 80 W	5 (1 + 4)	95	47	86	>**100**

[a] Δ = classical heating. [b] MW = microwave exposure.

Scheme 1



Scheme 2



We found that the supported enzymes could be reused three more times in the reactions under study without loss of activity, thus showing the good stability and the possibility of recycling of such systems.

These results (average values of three experiments) in terms of reaction time (*t*), temperature (*T*), substrate conversion (conv), enantiomeric excess (ee), and enantiomeric ratio of the reaction (*E*) calculated according to the Sih equation[15] are reported in Table 2.

In both cases (classical heating and microwave irradiation) all reactions were very fast when compared, for instance, to the results obtained under reduced pressure in solution of acylating agent where 24 h were needed.[6b] At optimal temperature (90 °C for water or 85 °C for ethanol elimination), the initial rates and enantiomeric ratios *E* are significantly enhanced under microwave irradiation. When compared to ethyl ester (runs 1 and 2), the increase in selectivity is more pronounced for isopropenyl acetate (runs 3 and 4) and octanoic acid (runs 11 and 12), a result which may be connected with the enhanced polarities of these species and leading to their more pronounced interactions with microwaves.[16]

Another advantage of microwaves in terms of reactivity lies in the fact that the associated rate enhancements may allow the reaction to go to completion, in cases where it is barely attainable under classical heating. To illustrate this point, we have studied the resolution of (±)-1-phenylethyl valerate (**3**) by transesterification with butanol (Scheme 2, Table 3).

Quantitative yield could be obtained (50%) under microwave exposure whereas it is limited to 47% (which was constant even after prolongation of the reaction time) by conventional heating, thus necessitating further separation of enantiomers.

Table 3. Resolution of (*RS*)-1-Phenylethyl Valerate (3)

mode of activtn[a]	*t* (mn)	*T* (°C)	conv (%)	ee alcohol R (%)
MW 40W	5	80	46	100
MW 40−20W	15	80	**50**	100
Δ	5	80	47	100
	15	80	47	100

[a] MW = microwave exposure. Δ = classical heating.

Conclusion

We have taken advantage of the complementarity of two recent and eco-friendly technologies, enzymatic catalysis using immobilized enzymes in dry media and microwave activation in solvent-free conditions, to enhance both reactivity and selectivity of enzymatic reactions. The specificity of microwave effects as compared to classical heating have also been evidenced, as already described for several classical organic reactions.[17] Such specificity may result from an improvement of irreversibility of the reaction due to more expedited removal of water or ethanol molecules and (or) to a decrease in activation parameters $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ as demonstrated by Lewis for the imidization of polyamic acid.[18]

Experimental Section

General. All chemicals, including supports, were purchased from Fluka and used without further purification. *P. cepacia* lipase (LP) was obtained from Amano Pharmaceutical Co. and Novozym 435 (SP 435) from Novo Nordisk Co.

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Microwave Reactor. Reactions were performed in a monomode microwave reactor (Synthewave 402 from Prolabo), fitted with a stirring system and an IR temperature detector which indicates the surface temperature.[9] Reaction conditions were controlled using the algorithm *tout ou peu* which allows temperature control at the given value during the reaction time by varying the power between an adequate value and 20 W (to operate under electromagnetic field all along the reaction).

Typical Procedure. Into a pyrex tube (12 cm^3) were introduced 1 g of SP 435, 2 mL of an ethereal solution of **1** (244 mg, 2 mmol), and 3 equiv (6 mmol) of octanoic acid (862 mg) or 1 g of LP/HSC (250 mg, 750 mg), 2 mL of an ethereal solution of **1** (122 mg, 1 mmol), and 4 equiv (4 mmol) of isopropenyl acetate (400 mg).

The mixture was heated as indicated in the tables. After the solution was cooled to rt, the solid was transferred on a fritted funnel and the product eluted with diethyl ether. After concentration under reduced pressure, the remaining alcohol and the ester were purified by silica gel chromatography, using a pentane/ethyl acetate gradient. Conversions and enantiomeric excess were determinated by GC capillary chiral column (Cydex B) with ethyl benzoate as an internal standard (GC Carlo Erba Fractovap 2900: oven temperature 105 °C, gas carrier pressure 1 kPa, retention times alcohol R = 7.80 min, alcohol S = 8.33 min).

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