Tetrahedron: Asymmetry Vol. 2, No. 10, pp. 1011-1014, 1991
Printed in Great Britain

0957-4166/91 $3.00+.00
Pergamon Press plc

Influences of reaction conditions on the enantioselective transesterification using *Pseudomonas cepacia* lipase

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(*Received* 14 *June* 1991)

Abstract: The influences of different reaction conditions on *Pseudomonas cepacia* lipase (PCL) catalyzed esterification of several chiral allylic alcohols (2-(1-hydroxyethyl)acrylonitrile and some closely related compounds) have been investigated. These compounds are possible precursors in the synthesis of the antibiotic Nyccomycin. An increase in the reaction temperature led to an increased final conversion, decreased enantioselectivity of the reaction, and a reduction of the residual activity of the lipase. PCL is thermostable and catalytically active for more than 100 hours at 100 °C. Variation of the chain length of the acyl group of the esters used as cosubstrates had only a small influence on the conversion. As expected, the alcohol generated during the synthesis had a strong negative influence on the progress of the reaction. PCL showed strong substrate selectivity for some of the tested allylic alcohols. The enantioselectivity changed from substrate to substrate.

Introduction

The synthesis of enantiomerically pure, pharmaceutically active substances often represents a chemical and technical problem. Enzymes, especially lipases and esterases, offer great potential for enantioselective syntheses. These enzymes possess a broad substrate specificity and a high stereoselectivity. They do not need cofactors and are stable and catalytically active in a wide range of organic solvents[1,2]. It has been found that lipase activity in organic media requires a small amount of water[2,3]. Organic solvents afford several potential advantages, like easier product and enzyme recovery, better solubility of reactants, and greater stability of the enzyme.

Efficient planning of a synthesis reaction requires knowledge of the stereochemical behavior of the biocatalyst. Therefore, it is desirable to know the shape of the active site of the enzyme. This information can be obtained in two ways: The enzyme structure can be determined by x-ray diffraction, or the substrate structure can be varied (using closely related compounds). Analysis of the reaction behavior and consideration of the substrates molecular shapes allow one to deduce the general configuration of the active site, analogous to the making of plaster casts of a footprint[4-8]. We have followed this principle by using different allylic alcohols to provide some information about the substrate specificity of the active site of *Pseudomonas cepacia* lipase (PCL).

The choice of the optimal reaction temperature and appropriate cosubstrate are very important factors for enzymatic activity. In this work, the influences of reaction temperature and type of acyl donor (cosubstrate) on the conversion and the enantioselectivity in the transesterification of different allylic alcohols with PCL (Figure 1) were studied.

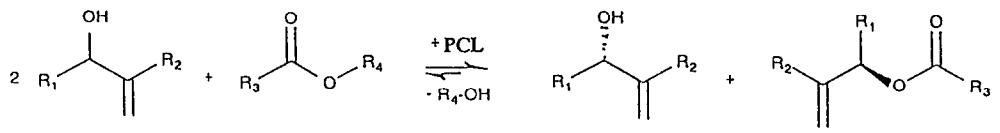


Figure 1: Asymmetric transesterification of allylic alcohols (R_1 and R_2 are identified in Table 1) with different esters (see Figure 3) as acyl donors.

Materials and methods

The lipase from *Pseudomonas cepacia* (PCL), (LPS A001526, earlier specification: *Pseudomonas fluorescens*, Amano) was used as received. Racemic mixtures of the allylic alcohols shown in Table 1 and Figure 1 were used as substrates. The pure, racemic substrates were a gift from Prof. H.M.R. Hoffmann (Institute of Organic Chemistry, University of Hannover). The determination of the conversion and enantiomeric excess were performed by gas chromatography using a chiral stationary phase (Lipodex A^R and Lipodex E^R, Macherey & Nagel) and ^1H NMR spectroscopy with (+) or (-) $Eu(hfc)_3$ as chiral auxiliary. The activity of the enzyme was measured in a Metrohm autotitration system by hydrolysis of 3 g Tricaprylin (Fluka) in 50 ml 0.05 M potassium phosphate buffer (pH 8) and 10 µl of Emulan P emulsifier (BASF). The solution was stirred vigorously and the temperature maintained at 25 °C. The specific rotation of the allylic alcohols was determined by polarimetry (Perkin-Elmer model 241) in methyl tert-butyl ether (MTBE) (Merck).

Results and discussion

Influence of the temperature

Up to a certain point, increasing the temperature normally leads to an increase of the enzymatic reaction rate. At the same time, the enantioselectivity often decreases and a loss of enzyme stability can be observed[9]. To verify these predictions, a series of tests conducted at temperatures from 30 to 100 °C was carried out. For these tests, 50 µl of the racemic alcohol 1 (see Table 1) as the substrate, 2 ml of cyclohexyl acetate (Aldrich) as the acyl donor and 250 mg of PCL were used. The magnetic stirrer speed was kept at 400 rpm in all experiments.

The time courses of the conversions and the enantiomeric excesses of the substrate are demonstrated in Figure 2. Increasing the temperature clearly resulted in an increased reaction velocity and a higher final conversion. In the beginning of each reaction, conversion and enantiomeric excess were in good accordance. As expected, one enantiomer was converted faster than the other. After about 20 to 50 hours the conversion increased further while the enantiomeric excess plateaued or decreased. The enantioselectivity of the reaction seemed to decrease. Thus, for the synthesis of products with high enantiomeric purity it would be necessary to stop the reaction after a few hours. At 100 °C, the enzyme is active with acceptable conversions and the greatest reaction velocity in this series of tests. However, the stability of PCL is lowest at 100 °C: after 72.5 h, the residual activity (relative to the initial level) is only 36 % for enzyme maintained at 100 °C, while enzyme kept at 30 °C has a residual activity of 99 %.

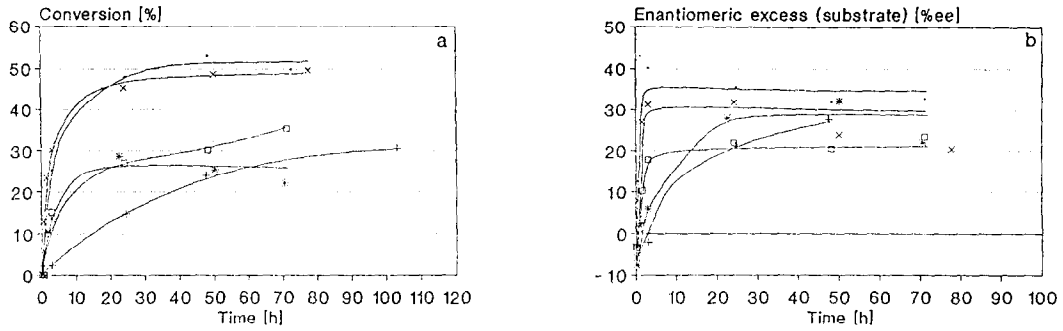


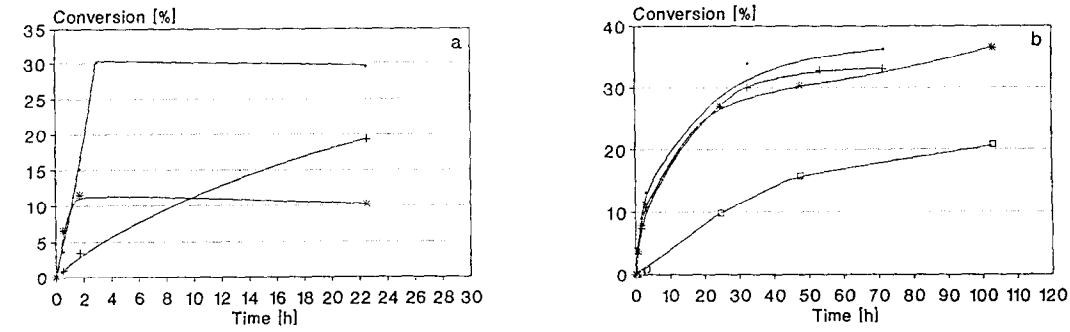
Fig. 2: Time courses of the conversion (a) and enantiomeric excesses (b) in the transesterification of 2-(1-hydroxy-ethyl)acrylonitrile using cyclohexyl acetate and PCL at different temperatures (+) 30 °C, (*) 40 °C, (□) 60 °C, (x) 80 °C, (·) 100 °C. The deviation of all data were 5 %.

Influence of the acyl donor

Lipase-catalyzed transesterifications are two-step mechanisms. In the first step, the acyl group of the ester is transferred to the enzyme, forming an acyl-enzyme complex. In the second step, one of the competing enantiomers of the chiral alcohol will be preferentially esterified.

The type of acyl donor chosen plays an important role in this transesterification reaction since it influences the thermodynamic equilibrium. In addition the acyl group of the ester has steric and stereoelectronic influences on the process of deacylization[10].

In this study, both the acyl group and the alcohol group of the ester have been varied. All reactions were carried out at 40 °C and 400 rpm with 50 µl of the racemic alcohol **3** (see Table 1) as the substrate, 2 ml of ester as the acyl donor and 250 mg of PCL for 94.5 hours. The results of these tests are shown in Figure 3. Changing the structure of the acyl group led to lower final conversions in the following order: cyclohexyl acetate (32 %) > n-butyl acetate (20 %) > ethyl acetate (10 %). The lowest reaction velocity was measured with n-butyl acetate.



Figures 3a and 3b: Time course of the conversion using (a) different acetates, {(·) cyclohexyl acetate, (+) n-butyl acetate, (*) ethyl acetate} and (b) ethyl esters {(·) ethyl valerate, (+) ethyl butyrate, (*) ethyl propionate, (□) ethyl pivalate} for the trans-esterification reaction with PCL and 2-(1-hydroxy-n-butyl)acrylonitrile. The deviation of all data were 5 %.

In general, varying the structure of the acyl group of the ester had a relatively small influence on the final conversion and reaction rate. The use of ethyl valerate, ethyl butyrate, and ethyl propionate resulted in nearly the same final conversion. In the case of ethyl pivalate, however, the conversion was significantly lower, probably because of the bulky t-butyl group (Fig 3b).

Influence of the alcohol component

To provide some informations about the substrate specificity, transesterification reactions with different allylic alcohol compounds were carried out at 40 C and 400 rpm. Further reaction conditions are shown in Table 1.

substrate			conversion	enantiomeric excess		residual activity	time
				substrate	product*	of the enzyme	
No.	R_1	R_2	[%]	[%ee]	[%ee]	[%]	[h]
1	Me	CN	27	11	98 (-)	66	474
2	Et	CN	42	5	63 (+)	52	213
3	n-Pr	CN	22	21	75 (+)	52	848
4	Cyh	CN	56	9	n.m. (+)	78	346
5	Ph	CN	9	8	76 (+)	59	848
6	Me	COMe	31	25	86 (-)	53	416

Table 1: Conversions and enantiomeric excesses of the six allylic alcohols (determined by GC with Lipodex E^R), residual activity of the lipase and reaction time (Me = Methyl, Et = Ethyl, n-Pr = n-Propyl, Cyh = Cyclohexyl, Ph = Phenyl, CN = Cyano, COMe = Methoxy, * = specific rotation (determined by polarimetry), n.m. = not measured, R_1, R_2 see Figure 1); reaction conditions: substrate No. [mg], cyclohexyl acetate [ml], PCL [mg]: **1** [580], [2], [800]; **2** [500], [0,8], [300]; **3** [430], [0,6], [900]; **4** [250], [2,6], [800]; **5** [600], [1,2], [900]; **6** [370], [2], [800]

By comparing the results of the transesterification of the six allylic alcohols (Table 1), some interesting conclusions can be reached. First, it is apparent that the lipase from *Pseudomonas cepacia* preferred the (-) enantiomer of the alcohol in the cases of substrates **1** and **6** and the (+) enantiomer in all other cases. Second, in the case of substrate **5**, the bulky phenyl group seemed to prevent interaction with the active site of the lipase and led to low conversions. Finally the residual activity was greater than 50 % in all cases after 213 to 848 hours of reaction time.

Summary

An enantioselective synthesis using the allylic alcohols described in this report seems to be possible, but it is very difficult to predict the reaction behavior that will result in high conversions and high enantiomeric excesses. Currently the optimization of enzymatic transesterification processes is based on mostly empirical results. Reactivity of the substrate and enantioselectivity of the reaction are strongly dependent upon reaction conditions such as temperature, type of ester, and reaction time.

Acknowledgement

The authors thank Prof. H.M.R. Hoffmann and his co-workers from the Institute of Organic Chemistry of the University of Hannover for providing us with the allylic alcohols and Prof. W.A. König from the Institute of Organic Chemistry of the University of Hamburg for helping us to develop the chiral analysis.

References

1 A. L. Margolin, *Biol. Chem. Hoppe Seyler*, 370 (9), 984 (1989)
2 A. M. Klibanov, *Chem. Tech*, 354-359 (1986)
3 P. J. Halling, DECHEMA Biotechnology Conferences 1, 35-40, (1988)
4 K. Laumen, Ph. D. thesis, University of Wuppertal (1987)
5 E. J. Toone, M. J. Werth, J. B. Jones, *J. Am. Chem. Soc.*, **112**, 4946-4952, (1990)
6 Z.F. Xie, H. Suemune, K. Sakai, *Tetrahedron Asymmetry* **1** (2), 395-402 (1990)
7 W. Freist, *Chemie in unserer Zeit* 3, 143-50 (1990)
8 S. Schapöhler, Ph. D. thesis, University of Hannover (1990)
9 W. Lokotsch, K. Fritsche, Ch. Syldatk, *Appl. Microbiol. Biotechnol.* **31**, 467-72 (1989)
10 Ch.-Sh. Chen, Ch. J. Sih, *Angew. Chemie* **101**, 711-24 (1989)