Solvent Effects in Lipase-Catalysed Transesterification Reactions

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Kanerva, L. T., Vihanto, J., Halme, M. H., Loponen, J. M. and Euranto, E. K., 1990. Solvent Effects in Lipase-Catalysed Transesterification Reactions. – Acta Chem. Scand. 44: 1032–1035.

Porcine pancreatic lipase-catalysed transesterifications of 2,2,2-trifluoroethyl butyrate with racemic 2-octanol and 1-phenylethanol have been studied in different organic solvents. Solvent hydrophobicity (log P −1.1 to 3.3) has only a minor effect on the reaction rate. Independently of the solvent used as the reaction medium, both (*R*)-2-octyl and (*R*)-1-phenylethyl butyrates were obtained in high optical purity (ee >90 %). *Candida cylindracea* lipase is active only in the most hydrophobic solvents studied.

There are many potential advantages in employing enzymes in organic solvents rather than in aqueous media to catalyse organic reactions. Among them are increased solubility of non-polar substrates, shifting of thermodynamic equilibria to favour synthesis and ease of product recovery from organic solvents. Because enzymes are insoluble in common organic solvents enzyme recovery can easily be performed by filtration. Furthermore, most commercially available enzymes can be used directly as received, although some enzymes (e.g., subtilisin and chymotrypsin) must first be lyophilised at an appropriate pH.[1-4]

Porcine pancreatic lipase (PPL) used in organic solvents has proved to be a useful catalyst in many acylation reactions as well as in the resolution of racemic alcohols, carboxylic acids and esters because of its wide substrate specificity and low price. In our last paper we studied the structural effects of the R group of alkyl butyrates (PrCOOR) on the PPL-catalysed resolution of 2-octanol in ether.[5] In the cases studied, (*R*)-2-octyl butyrate was obtained in high optical purity, but the reaction rates depended considerably on the polar and steric effects of the R group. The highest reactivity was observed when 2,2,2-trifluoroethyl butyrate was used as the starting ester. The aim of this work was to study the solvent effects observed on the PPL-ctalysed transesterification of 2,2,2-trifluoroethyl butyrate with racemic 2-octanol and 1-phenylethanol in a wide variety of organic solvents. The applicability of *Candida cylindracea* lipase for the acylation of 1-phenylethanol in organic solvents was also tested.

Experimental

Materials. The enzymes were a crude porcine pancreatic lipase (Type II) and the lipase from *C. cylindracea* (Type VII) purchased from Sigma Chemical Co.

2,2,2-Trifluoroethyl butyrate was prepared by the reaction of 2,2,2-trifluoroethanol with butyryl chloride. Racemic alcohols as well as their pure enantiomers were purchased from Aldrich. The organic solvents were of the best analytical grade and were dried over molecular sieves overnight before use.

Transesterification reactions. Initial rate (v_0) measurements and the resolution of racemic 2-octanol and 1-phenylethanol were performed as previously described.[5] PPL (4.0 g) was weighed into a reaction vessel, and 0.04 mol of racemic 2-octanol and a 20 % molar excess of 2,2,2-trifluoroethyl butyrate in 28 ml of a dried solvent were added. For the resolution of 1-phenylethanol, 8 mmol of both the alcohol and the starting ester were added to 3.2 g of PPL in 40 ml of an organic solvent. For the *C. cylindracea* lipase-catalysed transesterification, 1 ml of the solution of racemic or (*R*)- or (*S*)-1-phenylethanol (0.1 M) and 2,2,2-trifluoroethyl butyrate (0.2 M) in an organic solvent were added to 100 mg of the enzyme. After sonication the mixture was shaken at room temperature throughout the course of the reaction. The reaction was stopped by filtering off the enzyme after about 45 % conversion except in the case of 1-phenylethanolysis of 2,2,2-trifluoroethyl butyrate in THF where the reaction was very slow. The reaction mixture was washed with water and $NaHCO_3$ solution and dried with magnesium sulfate.

In the resolution of 2-octanol, the optical purity of the remaining (+)-(*S*)-2-octanol was analysed by GLC using a Chrompack chiral column (XE-60-S-VAL; column temperature 150 °C for 20 min) by the method described by Gerlach *et al.*[6] The applicability of the method was tested using authentic (*R*)- and (*S*)-2-octanols. The enantiomeric excesses for the remaining (+)-(*S*)-2-octanol were obtained by means of eqn. (1) where A refers to the areas of (*R*)- and (*S*)-isocyanate derivatives, respectively. The optical purity of (−)-(*R*)-2-octyl butyrate formed was calculated by means of eqn. (2) where c is the conversion of the reaction and ee_P and ee_S are the enantiomeric excesses of the product fraction and that of the remaining substrate, respectively.[7]

$$ee = (A_{(S)} - A_{(R)})/(A_{(S)} + A_{(R)}) \quad (1)$$

$$c = ee_S/(ee_S + ee_P) \quad (2)$$

In the resolution of 1-phenylethanol, (+)-(*R*)-1-phenylethyl butyrate was formed and the remaining (−)-(*S*)-1-phenylethanol were separated by chromatography on silica gel eluting with hexane–dichloromethane (3:9). The purities of the products were determined by GLC. The specific rotations, $[\alpha]_D^{25}$, of the compounds were obtained by means of a JASCO Model DIP-360 digital polarimeter. The enantiomeric excesses were obtained by means of eqn. (3). The specific rotation for (−)-(*S*)-1-phenylethanol is known only for the neat alcohol.[8] We measured the value −55.15° (*c* 10, diethyl ether, 25 °C) for commercially available (−)-(*S*)-1-phenylethanol. Specific rotations obtained after the resolution experiments for (+)-(*S*)-1-phenylethanol gave the ee values (Table 2, see later) which, when fitted to eqn. (2), lead to the ee values for (+)-(*R*)-1-phenylethyl butyrate. These are in accordance with those obtained for (+)-(*R*)-1-phenylethyl butyrate (Table 2) using the value $[\alpha]_{lit}$ +103° (*c* 10, diethyl ether, 25 °C) calculated from the data of Kirchner *et al.*[2] and vice versa.

$$ee = [\alpha]_{obs}/[\alpha]_{lit} \quad (3)$$

The initial rates and the progress of the reactions were determined by taking samples from the reaction mixture at intervals and analysing them by GLC by following the formation of 2-octyl and 1-phenylethyl butyrates for the 2-octanolysis and 1-phenylethanolysis of 2,2,2-trifluoromethyl butyrate, respectively. In the case of 2-octanolysis, a gas chromatograph equipped with 25 m SE-30 (column temperature 80 °C for 3 min, then increased by 15 °C min^{-1} initially to 150 °C for 3 min and finally to 200 °C) and, in the case of 1-phenylethanolysis, with 25 m OV-1 (column temperature 70 °C for 2 min, then increased by 20 °C min^{-1} to 200 °C for 3 min) capillary columns were used. The results are given in Tables 1 and 2 and in Fig. 1.

The yields in the case of the 1-phenylethanolysis of 2,2,2-trifluoroethyl butyrate were calculated by assuming that only half of the racemic alcohol reacts by the enzyme-catalysed reaction. No reaction in the absence of the enzyme was detected. As in the case of the 2-octanolysis reactions the resolution products were not isolated, the yields are not included in Table 1. On the basis of our previous results for the PPL-catalysed 2-octanolysis of

Table 1. The formation of (−)-(*R*)-2-octyl butyrate and (+)-(*S*)-2-octanol from racemic 2-octanol and 2,2,2-trifluoroethyl butyrate catalysed by porcine pancreatic lipase in different organic solvents at room temperature.

Solvent[a]	v_0/μmol min^{-1} g^{-1}	Reaction time/h	Degree of conversion / %	ee / %	
				(*R*)-Ester	(*S*)-Alcohol
Hexane	14.1	120	47	88	78
Toluene	18.9	93	47	90	80
Diisopropyl ether	16.0	20	45	93	76
Diethyl ether	19.2	24	45	98	80
Diethyl ether[b]	13	70	46	100	
Diethyl ether[c]		130	47	95	90
Acetone	11.9	45	46	90	77
Acetonitrile	14.7	30	45	89	73

[a]Solvents are in the order of decreasing log *P* values; [b]Ref. 5; [c]Ref. 2, acylating agent 2,2,2-trichloroethyl butyrate.

Table 2. The formation of (+)-(*R*)-1-phenylethyl butyrate and (−)-(*S*)-1-phenylethanol from racemic 1-phenylethanol and 2,2,2-trifluoroethyl butyrate catalysed by porcine pancreatic lipase in different organic solvents at room temperature.

Solvent[a]	v_0/μmol min^{-1} g^{-1}	Reaction time/h	Degree of conversion / %	(*R*)-ester		(*S*)-alcohol	
				Yield / %	ee / %	Yield / %	ee / %
Dibutyl ether	1.74	40	48	73	97	78	65
Toluene	1.20	66	41	72	94	51	65
Diethyl ether[b]		133	45	75	95	56	90
Pyridine	0.900	94	43	70	99	82	75
Tetrahydrofuran	0.264	117	17	16	87	–	–
Acetonitrile	1.02	118	48	56	93	70	86

[a]Solvents are in the order of decreasing log *P* values; [b]Ref. 2, acylating agent 2,2,2-trichloroethyl butyrate.

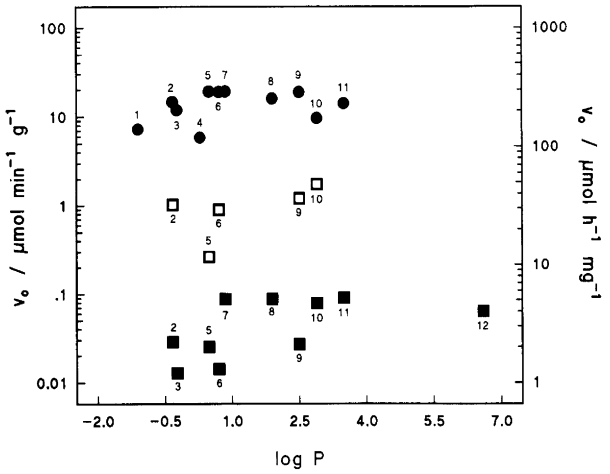

Fig. 1. The dependence of initial rates (v_0) on the hydrophobicity of the solvent for the PPL-catalysed transesterification of 2,2,2-trifluoroethyl butyrate (left-hand scale) with 2-octanol (●) and 1-phenylethanol (□) and that of tributyrin (right-hand scale) with 1-heptanol[3] (■). Solvents: 1, 1,4-dioxane; 2, acetonitrile; 3, acetone; 4, 2-butanone; 5, tetrahydrofuran; 6, pyridine; 7, diethyl ether; 8, diisopropyl ether; 9, toluene; 10, dibutyl ether; 11, hexane; 12, dodecane.

2,2,2-trifluoroethyl butyrate in diethyl ether[5] the yields in the present work are supposed to be of the same order of magnitude as those in Table 2.

Discussion

When non-aqueous enzymology is used for a given reaction, the first requirement is to find an appropriate reaction medium. Both the starting materials and the products must be soluble in the solvent chosen, and the solvent must be inert to the reaction. The enzyme should be insoluble in the reaction mixture because for the enzyme to be active in an organic solvent its native conformation must stay 'frozen' in a state similar to that of the active enzyme in water. Thus, e.g., enzymes are inactive in dimethyl sulphoxide presumably because it is a unique organic solvent which dissolves proteins.[3,9]

As far as enzymatic activity in organic solvents is concerned, the nature of the solvent may be critical. Most studies concerning solvent effects on the enzymatic activity of hydrolytic enzymes in organic solvents are based on chymotrypsin or subtilisin catalysis.[1,4,10] In these studies, relatively good correlations between the parameters of the Michaelis–Menten kinetics (K_m, V_{max}/K_m) and log *P* values of the solvent (the logarithm of the partition coefficient of the solvent between octanol and water[11]) were obtained. It seems that more hydrophobic solvents are preferable to less hydrophobic ones because hydrophilic solvents absorb some of the water essential to the enzyme activity.[1,3,4] In the case of protease catalysis in organic solvents, the loss of reactivity with decreasing hydrophobicity of a solvent is considerable. Thus, for the chymotrypsin-catalysed propanolysis of *N*-acetyl-L-phenylalanine ethyl ester the rate decreases by a factor of 10^4 when hexadecane is replaced by acetonitrile as the solvent.[4] For PPL catalysis in organic solvents the essential water is very tightly bound to the enzyme.[3] In accordance with this, the initial rates, v_0, for the PPL-catalysed transesterification of 2-octanol and 1-phenylethanol with 2,2,2-trifluoroethyl butyrate and that of tributyrin with 1-heptanol[3] show only a slight rate decrease with decreasing log *P* values of the solvents (Fig. 1). That is at least a partial explanation for the poor correlation between the values of v_0 and log *P* compared with those observed in protease catalysis in organic media.[1,4,10] It seems that aliphatic ethers are exceptionally good solvents for PPL catalysis in organic solvents.

The present results (Tables 1 and 2) clearly demonstrate that the resolutions of both racemic 2-octanol and 1-phenylethanol can be easily performed by PPL-catalysed transesterification of the alcohol with 2,2,2-trifluoroethyl butyrate in a wide variety of organic solvents. The resolution reactions were almost halted at about 50% conversion. The enantiomeric purity of the (*R*)-ester obtained after about 45% conversion is very high and practically independent of the chemical and of the physical properties of the solvent. The enantiomeric purity is similarly independent if the nucleophile is an aliphatic or aromatic alcohol; only the reaction time is somewhat longer for the resolution of 1-phenylethanol compared with that of 2-octanol. As we have previously shown, the optical purity of 2-octyl butyrate obtained from the PPL-catalysed transesterification between racemic 2-octanol and alkyl butyrates in diethyl ether is also independent of the structure of the alkyl butyrate, PrCOOR.[5] The optical purity of the (*S*)-alcohol can be increased if the reaction is allowed to exceed 50% conversion.

In conclusion, porcine pancreatic lipase is an excellent catalyst for the enzymatic resolution of racemic alcohols. Not only is the enzyme cheap and commercially available, but both hydrophobic and hydrophilic solvents can be used as reaction media for resolution purposes (Tables 1 and 2). Other lipases such as the lipase from *C. cylindracea* are also effective in the resolutions described. Thus, for the resolution of racemic 1-phenylethanol the enantiospecificity ratios, v_R/v_S, are 20 and 14 for the PPL- and *C. cylindracea* lipase-catalysed 1-phenylethanolyses of 2,2,2-trifluoroethyl butyrate in dibutyl ether, respectively. However, the lipase from *C. cylindracea* catalyses the alcoholysis of 2,2,2-trifluoroethyl butyrate effectively only in the most hydrophobic solvents (octane and dibutyl ether), and for resolution purposes the yeast lipase is practically inactive in the solvents of log *P* values lower than 1. This is in accordance with the data for the *C. cylindracea* lipase-catalysed transesterification of tributyrin with 1-heptanol in different organic solvents.[3] The essential water is apparently bound more loosely to the yeast lipase than to PPL.

Acknowledgements. Thanks are due to the Technology Development Centre (TEKES) for financial support.

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Received March 19, 1990.