Fluid Phase Equilibria 450 (2017) 126–132

Contents lists available at ScienceDirect

Fluid Phase Equilibria

journal homepage: www.elsevier.com/locate/fluid

Prediction of solvent effect on enzyme enantioselectivity

Alexandra V. Chatzikonstantinou [a], Giannis-Florjan Norra [b], Haralambos Stamatis [a], Epaminondas Voutsas [b, *]

[a] Laboratory of Biotechnology, Department of Biological Applications and Technologies, University of Ioannina, 45110 Ioannina, Greece
[b] Thermodynamics and Transport Phenomena Laboratory, Department of Chemical Engineering - Section II, National Technical University of Athens, Heroon Polytechniou 9, Zographos, GR-15780 Athens, Greece

ARTICLE INFO

Article history:
Received 18 April 2017
Received in revised form
25 July 2017
Accepted 25 July 2017
Available online 28 July 2017

Keywords:
Enantioselectivity
Enzyme biocatalysis
Lipase
Activity coefficient
COSMO-RS

ABSTRACT

Enantiomeric ratios for the esterification reaction of 2-phenylpropionic acid with 1-octanol catalyzed by immobilized *Candida antarctica* lipase B in sixteen organic solvents have been measured. Correlation equations that relate the enzyme enantioselectivity with the van der Waals volume (V_w), the van der Waals area (A_w), and the Hildebrand solubility parameters (δ^2) of the solvents were obtained, and they were compared with linear solvation energy relationship (LSER) correlation equations, which relates the enzyme enantioselectivity with polarity, acidity, basicity, and Hildebrand solubility parameters of the solvents. Statistical analysis of the experimental data for the esterification reaction investigated here as well as for seven other reactions taken from the literature showed that the pure solvent V_w, A_w and δ^2 parameters, sufficiently describe the dependence of enzyme enantioselectivity on the properties of the solvent, yielding better results than the LSER equations that use one more parameter. Furthermore, the enzyme enantioselectivity has been related to the infinite dilution activity coefficients of substrates in the solvent, which are predicted by the COSMO-RS model. It is shown that the predicted by COSMO-RS ratios of infinite dilution activity coefficients of the two enantiomers can be used to correlate satisfactorily the enantiomeric ratios.

1. Introduction

The demand for enantiopure pharmaceuticals and agrochemicals has been growing continuously, especially due to the fact that different enantiomers can cause different biological effects [1,2]. Thus, the preparation of optically active products has become a major research area particularly in pharmaceutical and fine-chemical industry [3–5].

The use of biocatalysts in nonaqueous media has been proved to be a useful procedure for the preparation of optically pure compounds [6,7]. The activity and the enantioselectivity of enzymes have been found to be strongly dependent on the nature of the solvent used [8–11]. Several experimental, theoretical and simulation studies have been devoted to investigate the mechanisms by which solvents affect enzyme enantioselectivity and to define principles that can best guide the selection of the solvent in order to rationalize the phenomenon [9] [12–20].

The solvent could affect the enzyme conformation [16–18], or the active site of enzyme by binding in it or near it and thus affecting the molecular recognition process between enzyme and substrate [16,21]. On the other hand, Klibanov and coworkers proposed that solvent effects are due to differences in the energies of solvation of the substrates in their enzyme-bound transition states [8,14,22]. Ke et al. [14] determined the desolvated part of the substrate in the transition state using molecular modelling and dynamic simulations based on the crystalline structure of the enzymes, γ-chymotrypsin and subtilisin, and then they used UNIFAC to predict the activity coefficients of the two enantiomers. Later, Colombo et al. [13] applied the same method in order to predict the solvent effect on the selectivity of lyophilized or cross-linked enzyme crystals of subtilisin in the resolution of secondary alcohols, but it was found that the theoretical model did not predict well the effect of the solvent.

No clear consensus has yet emergent on which physicochemical properties of the solvent to use to describe the effect of the solvent on enzyme enantioselectivity. The most studied relations are those with solvent hydrophobicity [10] [23–28], measured as logP (P is the octanol-water partition coefficient), dielectric constant and

* Corresponding author.
E-mail address: evoutsas@chemeng.ntua.gr (E. Voutsas).

http://dx.doi.org/10.1016/j.fluid.2017.07.016
0378-3812/

dipole moment [23,29], but the results are not always successful and, furthermore, no rationale for using them in a predictive way has been offered.

The size of the solvent molecules, expressed through the van der Waals volume parameter, has also been suggested as a one of the parameters that govern solvent effect on enantioselectivity [12,19]. Except for the molecule size, functional group, location and structure of the carbon chains of organic solvents seem to effect the enantioselectivity of the enzyme [27]. Lee [30] has studied the application of a linear solvation energy relationship (LSER) that relates the enzyme enantioselectivity with the polarity, acidity, basicity and Hildebrand solubility parameters of the solvent.

The objective of the present manuscript is two-fold. First, enantiomeric ratios for the esterification reaction of 2-phenylpropionic acid with 1-octanol catalyzed by immobilized Candida antarctica lipase B, a commonly used industrial lipase with a very broad substrate specificity, in sixteen organic solvents have been measured. The second is to investigate the capability of predicting the effect of solvent and substrate on enzyme enantioselectivity in non-aqueous organic media. To this purpose two approaches were followed. In the first approach, the effect of solvent on enzyme enantioselectivity has been studied. To this purpose, a multiparameter regression analysis has been performed for the esterification reaction measured in this work, as well as for seven transesterification reactions, catalyzed by different lipases and proteases in various organic solvents, taken from the literature. The solvent parameters studied are, the van der Waals volume (V_w), the van der Waals area (A_w) and the Hildebrand solubility parameter (δ^2). These parameters were chosen on the basis that the enzyme enantioselectivity is a phenomenon driven by both entropic (combinatorial) effects and enthalpic effects [12,31,32]. V_w and A_w take into account entropic (combinatorial) effects and δ^2 takes into account enthalpic effects. For comparison, LSER correlation equations that relate the enzyme enantioselectivity with the polarity, acidity, basicity and Hildebrand solubility parameters of the solvent have been developed using also multiparameter regression analysis. In the second approach, the effect of solvent and substrate on enzyme enantioselectivity was investigated. To this purpose, the enzyme enantioselectivity is related to the infinite dilution activity coefficients of substrates in the various solvents, which are predicted with the COSMO-RS method.

2. Materials and methods

2.1. Materials

Immobilized lipase B from *Candida antarctica* was purchased from c-LEcta GmbH, Germany. All chemicals, R-(−)-2-phenylpropionic acid, (S)-(+)-2-phenylpropionic acid, racemic 2-phenylpropionic acid, 1-hexanol and organic solvent used were purchased from Alfa Aesar, Fluka or Sigma-Aldrich and were of the highest available purity. The source and purities of the chemicals used are listed in Table 1.

2.2. Enzymatic reactions

The enzymatic esterification of 2-phenylpropionic acid with 1-octanol to octyl-2-phenylpropionate and water was carried out in sealed stirred round bottom flasks (10 mL) using various organic solvents. The reactants R-(−)-2-phenylpropionic acid or (S)-(+)-2-phenylpropionic acid (0.01 mmol) and 1-octanol (0.05 mmol) were dissolved in 2 mL of the organic solvent previously dried with 4 Å molecular sieves. The enzymatic esterification started by addition of 15 mg/mL of immobilized lipase. In all cases studied, the flasks were incubated in an orbital shaker at 240 rpm at 55 °C. Reactions were carried out in the presence of 20 mg/mL of 4 Å molecular sieves. Control experiments were conducted without enzyme.

Samples were withdrawn at various times and diluted with organic solvent. The reaction was terminated by removing the biocatalyst and the molecular sieves by filtration using a 0.45 μm nylon membrane filter. The reaction's progress was monitored by gas chromatography (GC). A Shimadzu 17A gas chromatographer was used, equipped with a flame ionization detector (FID) and a β-DEX 120 column (Supelco), 30 m × 0.25 mm x 0.25 μm. Helium was used as the carrier gas at a flow rate of 0.9 mL/min. The injector and detection port temperatures were set to 250 °C and 300 °C, respectively. The oven temperature was set at 150 °C for 5 min and linearly increased to 180 °C by 10 °C/min. For the analysis of the alcohol substrate and the ester products, appropriate standard curves were used using n-decane as external standard. Reaction rates were calculated from the slope of the linear portion of plots of product (ester) concentration versus time and expressed as mmol h^{-1} g^{-1} of enzymatic preparation. All experiments were repeated at least 3 times. In order to determine the enantioselectivity E without

Table 1
Materials description.

Chemical name	Source	Initial mole fraction purity	Purification method
Immobilized lipase B from *Candida antarctica*	c-LEcta GmbH	Specific activity:> 9000 PLU/g dry beads	None
Water	Fisher Scientific	HPLC gradient grade	None
R-(−)-2-phenylpropionic acid	Sigma -Aldrich	97%	None
(S)-(+)-2-phenylpropionic acid	Sigma -Aldrich	97%	None
(±)-2-Phenylpropionic acid	Sigma -Aldrich	97%	None
1-Hexanol (anhydrous)	Sigma -Aldrich	>99%	None
Acetonitrile (anhydrous)	Sigma -Aldrich	99.8%	None
Acetone	Sigma -Aldrich	HPLC gradient grade	None
2-Butanone	Sigma -Aldrich	>99%	None
Diethyl ether	Sigma -Aldrich	Analytical standard	None
2-Methyl-2-butanol	Alfa Aesar	98%	None
tert-Butyl alcohol	Alfa Aesar	99%	None
tert-Butyl methyl ether	Sigma -Aldrich	99.8%	None
Methylene chloride	Sigma -Aldrich	HPLC gradient grade	None
Di-isopropylether	Sigma -Aldrich	Analytical standard	None
Toluene	Sigma -Aldrich	99.8%	None
Cyclohexane	Sigma -Aldrich	99.5%	None
n-Hexane	Sigma -Aldrich	Analytical grade	None