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Measurement and prediction of solvent effect on enzymatic esterification reactions

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ABSTRACT

The solvent effect on the equilibrium position of the esterification reaction of tyrosol with (*R*)-(+)-α-lipoic acid (RLA) catalyzed by Novozym 435® (*Candida antarctica* lipase B immobilized on macroporous acrylic resin), in organic solvents and ionic liquids of various polarities has been experimentally examined. The solubilities of tyrosol in these organic solvents and ionic liquids have been also measured. It is shown that there is a correlation between the solubility of tyrosol in the reaction medium and the conversion yield of the reaction. Actually, high substrate solubility leads to a low conversion yield, with the higher conversion yields observed for the hydrophobic ionic liquids where tyrosol solubility is lower. Furthermore, tyrosol solubilites in the organic solvents and ionic liquids used for the enzymatic reactions of tyrosol with RLA have been measured, while the application of COSMO-RS model resulted to satisfactory predictions. Finally, the prediction of the solvent effect on the equilibrium position of esterification reactions has been investigated. The COSMO-RS model was used to calculate the activity coefficients of the chemical species involved in various reactions, carried out in different solvents. When applicable, the UNIFAC model has been also employed for comparison purposes. It is shown that the COSMO-RS method can be successfully applied for solvent selection with general applicability both for organic solvents and ionic liquids as well as in any kind of substrates.

1. Introduction

Biocatalysis is becoming an important tool for the production of fine chemicals, mainly from the environmental and long-term sustainability point of view. Attractive features of biocatalysts include versatility, substrate selectivity, regio-, chemo-, and enantioselectivity, limited use of hazardous reagents, reduction of by products, and catalysis at moderate temperatures and pressures.

The use of enzymes in organic media has increased dramatically in recent years and has been the subject of intensive basic application-orientated research [1–4]. In organic media, hydrolytic enzymes such as lipases can be employed to usefully carry out synthetic reactions (e.g., esterification and transesterification reactions), since the equilibrium position of the reaction is shifted sufficiently to give a high yield of the synthesis product [5–7]. A novel class of non-conventional media that has extensively used in the last decades in the field of biocatalysis constitutes the so called ionic liquids (ILs). ILs are organic salts consists exclusively of ions that are liquid at or near room temperature. Due to their unique physicochemical properties such as negligible vapor pressure, excellent chemical and thermal stability, ILs usually referred to as "green" solvents compared to traditional volatile organic compounds [8,9]. The use of ILs as media for biocatalytic reactions has been extensively studied the last decade revealed that in such media enzymes maintained and in some cases display enhanced activity, stability and selectivity [10–13].

Many enzymatic reactions operate under conditions where the position of the chemical equilibrium may control the final yield in contrast to chemical synthesis that are usually based on strongly thermodynamically favored reaction paths. Furthermore, the equilibrium state concentrations for a given enzymatic reaction depend strongly upon the solvent. Since for a given enzymatic reaction there are hundreds of solvents and many more solvent mixtures that can be considered, the optimum solvent choice by experimental determination is an expensive and time consuming procedure. So, it is clear that a reliable theoretical technique for predicting the reaction thermodynamics would be a valuable tool

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for biocatalysis in non-conventional media. Correct predictions of the activity coefficients of each reactant in each solvent lead to prediction of the solvent dependence of the equilibrium constant, thus allowing the proper choice of the best solvent for a given biocatalytic synthesis.

Group contribution methods (GCMs), especially UNIFAC, have been applied in the prediction of solvent effects on chemical equilibria [14–16]. The basis of UNIFAC method is to consider molecules as an aggregation of functional groups. This approach offers the great advantage of being able to describe a virtually infinite number of different compounds with a small number of estimated parameters, though often with questionable accuracy [17,18]. UNIFAC predictions are independent of the location of the functional groups and cannot account for the effect that one group has to another if they are close in the molecule (proximity effect). As a result, the identification of chemical groups is independent of the intramolecular environment. Moreover, any kind of contact between two groups in UNIFAC is associated with the same energy, independent of their individual locations and directions. Despite these drawbacks UNIFAC is widely applied due to its broad parameterization and its high calculation speed.

Klamt and co-workers proposed an alternative to GCMs, theoretical method, the COSMO-RS model [19–21]. This is based upon the approximated continuum description of solvent, in particular on the "conductor-like screening model", which has proven to be a powerful model for the prediction of thermophysical properties of mixtures. Unlike UNIFAC, COSMO-RS is not based on an analytical form of the excess Gibbs energy and it contains only a few adjustable parameters at the atomistic level. As a result COSMO-RS does not depend on experimental data or any parametrization for the solvent so it efficiently enables the calculation of the chemical potential of almost any solute is almost any solvent. Fermeglia et al. [22] have evaluated COCMO-RS and UNIFAC in the prediction of solvent effect on the equilibrium position for a series of lipase-catalyzed esterification reactions concluded that COSMO-RS method is very accurate and superior to UNIFAC.

In the first part of this paper, the effect of the solvent on the equilibrium position for the esterification reaction of tyrosol with RLA in organic solvents and ionic liquids of various polarities has been experimentally examined. The above biocatalytic process could lead to the preparation of hybrid molecules which could combine the beneficial biological properties of their parent molecules and possess improved biological activities and physicochemical properties [13]. Moreover, the solubilities of tyrosol in these organic solvents and ionic liquids have been measured.

In the second part the prediction of the solvent effect on the equilibrium position of esterification reactions with the COSMO-RS model and, when applicable, the UNIFAC model is investigated. Both the esterification reactions of tyrosol with RLA as well as other lipase-catalyzes esterification reactions from the literature have been considered. Finally, the COSMO-RS model is applied in the prediction of tyrosol solubilities in the organic solvents and ionic liquids used for the esterification of tyrosol with RLA.

2. Experimental

2.1. Materials

Novozym 435® (*Candida* antarctica lipase B immobilized on macroporous acrylic resin) was obtained from Novo Nordisk A/S. Tyrosol and organic solvents, i.e., 2-methyl-propanol (*tert*-butanol), 2-methyl-2-butanol (*tert*-pentanol), acetonitrile and *n*-hexane, were purchased from Sigma and were of the highest available purity. RLA was a generous gift from GeroNova Research Inc. (USA). Ionic liquids methyltrioctyl-ammonium bis(trifluoromethylsulfonyl) imide ((mtoa)NTf_2), 1-butyl-2,3-dimethylimidazolium tetrafluoroborate ((bdmim)BF_4), 1-ethyl-3-methylimidazolium tetrafluoroborate ((emim)BF_4), 1-butyl-3-methylimidazolium tetrafluoroborate ((bmim)BF_4) or hexafluorophosphate ((bmim)PF_6), 1-octyl-3-methylimidazolium tetrafluoroborate ((omim)BF_4) or hexafluorophosphate ((omim)PF_6) were purchased from Fluka, Alfa Aesar and Solvent Innovation. The source and purities of the chemicals used are listed in Table 1.

2.2. Enzymatic reactions

The enzymatic acylation of tyrosol by RLA in organic solvents or in ionic liquids was carried out in stirred flasks. In a typical acylation reaction tyrosol (20 mM) and RLA were added in the reaction solvent (1 mL) previously dehydrated with 4 Å molecular sieves. The molar ratio of substrates (tyrosol to RLA) was 1:1. In all cases studied, the flasks were incubated in an orbital shaker at 600 rpm at 318.15 or 333.15 K in the case of ionic liquids. The

Table 1
Materials description.

Chemical name	Source	Initial mole fraction purity	Purification method	Final mole fraction purity	Analysis method
Tyrosol	Sigma–Aldrich	98%	None	–	–
(*R*)-(+)-α-lipoic acid	GeroNova Research	99.8%	None	–	–
Novozyme 435®	Novo Nordisk A/S	1–10% protein/mg solid	None	–	–
2-Methyl-2-propanol	Fluka	≥99%	None	–	–
2-Methyl-2-butanol	Fluka	≥96%	None	–	–
Acetonitrile	Fisher Scientific	HPLC gradient Grade	None	–	–
n-Hexane	Lab-Scan	95%	None	–	–
(mtoa)NTf_2[a]	Sigma–Aldrich	≥99%	None	–	–
(bdmim)BF_4[b]	Solvent Innovation	99%	None	–	–
(emim)BF_4[c]	Merck	High purity	None	–	–
(bmim)BF_4[d]	Sigma–Aldrich	≥97%	None	–	–
(bmim)PF_6[e]	Alfa Aesar	98+%	None	–	–
(omim)BF_4[f]	Solvent Innovation	99%	None	–	–
(omim)PF_6[g]	Solvent Innovation	99%	None	–	–

[a] Methyltrioctyl-ammonium bis(trifluoromethylsulfonyl) imide.
[b] 1-Butyl-2,3-dimethylimidazolium tetrafluoroborate.
[c] 1-Ethyl-3-methylimidazolium tetrafluoroborate.
[d] 1-Butyl-3-methylimidazolium tetrafluoroborate.
[e] 1-Butyl-3-methylimidazolium hexafluorophosphate.
[f] 1-Octyl-3-methylimidazolium tetrafluoroborate.
[g] 1-Octyl-3-methylimidazolium hexafluorophosphate.