Available online at www.sciencedirect.com
ScienceDirect
Journal of Molecular Catalysis B: Enzymatic 52–53 (2008) 121–127
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Exploring the possibility of predicting CALB activity in liquid organic medium, with the aid of intrinsic kinetic parameters and intrinsic solvent effect data obtained in solid/gaz reactor

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Available online 4 November 2007

Abstract

In organic solvents the activity of enzyme is often order of magnitude lower than in water solutions and varies significantly with the solvent used. Many attempts to quantify and model the decrease of enzyme catalytic efficiency in solvents have been performed, but still there is no general method for predicting enzyme activity in a particular solvent in absolute terms.

In this paper, a new method is proposed to predict initial reaction rates in different anhydrous monophasic liquid organic media, in the case of CALB-catalyzed alcoholysis reaction. The experimental tool used for this method is a solid/gas bioreactor, which allows to obtain enzyme intrinsic kinetic parameters and intrinsic effect of organic solvents on enzyme activity. It is shown that, once corrections for solvation of substrates have been performed, the effect of solvent molecules consists mainly on binding to enzyme active site, leading to competitive inhibition of solvent towards the first substrate ester. The intrinsic parameters obtained in solid/gas reactor are used to predict activity of different batches of CALB, in organic media of different polarities, based on a single rate measurement in hexane. The result is a right prediction of solvent order with regard to CALB activity, a proper approximation of initial rate for weakly inhibiting solvents and overestimation for solvents showing a marked inhibitor character.

Keywords: Lipase B from *Candida antarctica*; Alcoholysis; Organic solvent; Solid/gas biocatalysis; Competitive inhibition

1. Introduction

Enzyme catalysis in organic anhydrous solvents has been a topic of research which has been extensively explored over the last decades [1–4]. For a large number of enzymes and especially for lipases, these solvents possess great advantages: increased solubility of hydrophobic substrates, increased thermostability of the enzyme and shift of the thermodynamic equilibrium in favor of synthesis over hydrolysis.

However in organic solvents the activity of enzyme is often order of magnitude lower than in water solutions and varies significantly with the solvent used. Many studies have been performed in organic liquid media to explain this loss of activity, leading to well established different causes such as unfavorable energetics of desolvation of substrates and active site [2], transition state destabilization, loss of conformational mobility of the enzyme [5] and competition of solvent molecules with substrate for binding [6]. This last effect has often been considered as a major cause of reduced activity of enzymes in organic liquid media [6–8]. Attempts to quantify and predict the decrease of enzyme catalytic efficiency in solvents include, for example, correction of substrates and kinetic parameters for solvation [7], prediction of the enzymatic activation energy depending on solvent polarity [9], or addition of an initial equilibrium step in the catalytic pathway [10]. In these methods, kinetic and thermodynamic parameters are determined in a particular solvent (in general a polar solvent) for an enzyme catalyzed reaction and the differences likely to be obtained for these parameters between another solvent and this particular solvent, are predicted from dissimilarities of physico-chemical properties between the two solvents, like saturation solubility or partitioning between the two solvents for a substrate. Consequently these methods do not permit to predict enzyme activity in a particular solvent in absolute terms.

In the present paper, a way of determining the intrinsic effect of solvent in the case of an immobilized lipase-catalyzed

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doi:10.1016/j.molcatb.2007.11.001

alcoholysis reaction is proposed. The experimental tool used to determine this parameter is a solid/gas bioreactor. In this system, the immobilized enzyme forms a packed bed, percolated by nitrogen as carrier gas, which simultaneously carries gaseous substrate to the enzyme and removes reaction products. This type of reactor allows a precise control of the enzyme microenvironment and permits determination of the sole role of the addition of an organic compound [11–13]. It was previously shown that kinetic parameters obtained by using this kind of reactor, can be considered as "intrinsic" kinetic parameters, as enzyme activities are measured in absence of both solvent and diffusional limitations [12,13]. In the following work, we tested the possibility of predicting in absolute terms, the enzymatic reaction rates, in a totally anhydrous monophasic liquid organic medium, thanks to the kinetic intrinsic parameters and the intrinsic solvent effect data obtained in solid/gas reactor.

2. Theoretical analysis

In this study, the effect of monophasic organic anhydrous solvents on enzyme catalysis is considered to arise from two different causes: (1) changes in solubility and desolvation of free substrates and products; (2) changes of solvation of enzyme species (free or as enzyme-substrate complex) and in particular binding of solvent molecules on enzyme active site. The possibility of either quantifying or canceling these effects thanks to the use of the solid/gas enzymatic system is explained in the following section.

The ability of solvents to change enzyme conformation and/or flexibility [5,14] is not considered here. Indeed, in the case of the enzyme chosen for testing our model, i.e. *Candida antarctica* lipase B (CALB), no significant conformational change was displayed when studying lipase structure in different solvents (2-pentanone, 2-methyl-2-pentanol and 2-methyl pentane) by molecular modeling [15]. It is also assumed that solvents have no effect on the catalytic mechanism of the enzyme. This hypothesis was supported by kinetic results obtained in different kind of non-conventional media for CALB, which could all be modelized by the same mechanistic model [12,16].

2.1. Effect of solvents on free substrates and products solubility and desolvation

One of the main effects of solvents on enzyme catalysis arises from solvent interaction with substrates and products, by solvation and desolvation phenomena, which modifies substrate and products availability for the enzyme [17,18]. Different methods have been proposed for correcting these effects [8,19–22]. One of them consists in expressing the quantities of substrate and the kinetic constant in terms of thermodynamic activities instead of concentrations [21,22]. To perform such corrections, activity coefficients (γ-values) of substrates are needed; they are either found from values in data bases, determined experimentally or estimated by using the UNIFAC group contribution method [23]. Nevertheless, authors frequently call UNIFAC predictions into question, as sources of inaccurate γ-values, being the cause of differences in enzyme performance observed in various solvents [6,7,21]. Deviations in the UNIFAC calculations up to a factor of 2 have been reported [21]. Moreover, in most of the experiments, γ-values are determined for one set of substrates and solvent quantities and considered to be constant in the range of concentrations used. These approximations lead to potentially cumulative errors on kinetic constant determination.

Consequently it appears that correction for solvation of substrates is far from being straightforward [19,22]. Therefore determination of intrinsic kinetic parameter of enzymes, i.e. independent of solvent used, is not possible.

To circumvent this problem, the use of enzymes at the solid/gas interface appears concurrent to liquid processes and presents some very interesting features. Indeed, contrary to the classical solid/organic liquid system, the solid/gas process offers the possibility to perfectly control and adjust thermodynamic activities of both reaction species and non-reactant components. A complete explanation of the design of solid/gas reactor and of the theoretical background for thermodynamic activities control has been presented previously [11]. In this system, the thermodynamic activity of each compound is determined by calculating the ratio of the partial pressure of the compound in the gas to its saturation vapor pressure at the working temperature, assuming that the poorly charged inert inlet gas can be considered as an ideal gas. The partial pressure is obtained from molar composition of the inlet gas and the total pressure. As molar fraction of the different species in the inlet gas can be chosen independently, thermodynamic activities of these species can be fixed independently very easily. This constitutes a significant advantage over solid–liquid system, in which reaction species and solvent molar fractions are linked together and for which totally inert liquid solvents do not exist.

2.2. Effect of solvents on the energy of desolvation of the active site, on the activation energy of enzyme catalysis and on the extent of solvation of the enzyme species (free or as enzyme-substrate complex)

The ability of solvent molecules to bind in the active site of free enzymes and to the covalent reaction intermediates, has been studied by many authors, in order to explain why enzymatic activity is often much reduced in organic solvents compared with water.

As an example, Schmitke *et al*. [24] have compared X-ray crystal structures of an acyl-enzyme intermediate of subtilisin Carlsberg formed in anhydrous acetonitrile and water. It appears that the structures of this covalent intermediate (*trans*-cinnamyl-subtilisin) did not change upon formation in either acetonitrile or water, and that the free enzyme active site structure remained unchanged when using water or acetonitrile. Therefore the solvent would not change the activity of subtilisin by causing a conformational change in the active site or by affecting the mode of binding of the substrate. However the locations of bound solvent molecules in the active site of the acyl- and free enzyme forms in acetonitrile and in water are distinct, leading to different energies required to displace these solvent molecules to allow substrate binding and catalysis. Such differences may contribute