Research Journal of Applied Sciences 2 (2): 185-187, 2007

Lipase-Catalyzed Hydrolysis of Esters in Presence of Organic Co-Solvents: A Multiparametric Correlation of the Effect of Co-Solvent on the Enzyme Activity

[1]F. Benamia, [1]R. Blida, [2]A. Tahar and [1]Z. Djeghaba
[1]Laboratoire de Chimie Organique Appliquée,
[2]Laboratoire de Biostatistique, Université Badji Mokhtar, Annaba, BP 12 Annaba 23000, Algeria

Abstract: Porcine Pancreatic Lipase (PPL) was used for the hydrolysis of three esters: ethyl butyrate, ethyl acrylate and ethyl acetoacetate, in 25 different organic co-solvents. In this study, we have used a multiple linear regression (stepwise method analysis) in order to interpret the enzyme activity as function of the initial rate of reaction. The selected models, illustrating the enzymatic activity according to several descriptors of organic solvents, show that the activity increases when the solvent polarity decreases. Such correlation between the activity and solvent polarity in terms of partition coefficient (log P), refractive index (n_D) and the Hildebrand solubility (δ) is significant when the substrate is more polar and more soluble in water.

Key words: Lipase, hydrolysis, co-solvent, modeling, multiple linear regression

INTRODUCTION

Biotransformations with enzymes, in both aqueous and non-aqueous environment, have been actively studied as a way to produce natural substances relevant to various industrial scopes such as cosmetics, fine chemicals, pharmaceuticals and food ingredients (Bazbradica *et al*., 2005). Among those enzymes, lipases (glycerol ester hydrolases E.C.3.1.1.3) are widely used as versatile biocatalysts for hydrolysis of carboxylic esters and acyl transfer onto hydroxy and amino groups with the formation of carboxylic esters and amides, respectively (Faber, 2000). Lipase catalyzed reactions take place at the interface between the aqueous phase containing the enzyme and the organic phase containing the substrate. This binding not only places the lipase close to the substrate but also dramatically increases enzyme activity. This interfacial activation is explained by some structural rearrangement of the enzyme at the water/organic phase interface which facilitate the access of the substrate to the active site (Otero *et al*., 2005; Cajal *et al*., 2000). Thereby, the interface area, which is affected by the characteristics of the organic phase, influences the catalytic performance of the enzyme, particularly in terms of conversion and selectivity (Otero *et al*., 2005; Cajal *et al*., 2000; Mezzetti *et al*., 2003). For instance, in ester hydrolysis, the nature of the organic co-solvent has been found to have an influence on both reaction rate and selectivity of lipases (Nini *et al*., 2001; Wang *et al*., 2005).

In a reaction medium, solvent action is the result of different types of interactions between the solvent and entities present in this medium, especially in reactions catalyzed by enzymes. These interactions concern at the same time the substrate and the biocatalyst. In the study of these types of interactions, log P (P: 1-octanol/water partition coefficient) is the most frequently used parameter (Chua and Sarmidi, 2005). In such complex system, one physico-chemical parameter alone cannot describe the whole properties of a solvent and the effects it can have on the progress of the reaction. The objective of this study is to correlate the nature of the solvent and the enzymatic activity, using a multi parametric approach.

MATERIALS AND METHODS

The Hydrolysis by the Porcine Pancreatic Lipase (PPL) of three esters is undertaken. The esters and their relative solubilities in water are given in Table 1. The enzyme activity is interpreted using the stepwise method analysis by establishing a mathematical model between initial rate of reaction and some physico-chemical descriptors of organic solvents.

Chemicals and enzyme: Porcine Pancreatic Lipase (PPL) (300 units g^{-1}) was purchased from Sigma-Aldrich. Esters and all organic solvents were purchased from Aldrich chemical and were of the highest purity available.

Table 1: Solubilities of the esters considered in this study

Ester	Solubility in water (% v/v)
Ethyl butyrate	0.49
Ethyl acrylate	1.20
Ethyl acetoacetate	12.00

Corresponding Author: Z. Djeghaba, Laboratoire de Chimie Organique Appliquée, Université Badji Mokhtar, Annaba, BP 12 Annaba 23000, Algeria

Physical properties of solvents: The following physical properties were considered as potential solvent descriptors: coefficient of partition between n-octanol and water (log P), Hildebrand solubility (δ), refractive index (n_D) and the normalized Reichardt-Demroth parameter (E_T^N) (Reichardt, 2004; Carlson,2000; CRC, 1998).

Hydrolysis reaction: Enzyme powder (1g) was added to 2 mL of organic solvent containing 5 mM ester and 30 mL of phosphate buffer (pH = 8; 0.2 M). Therafter, the reaction mixture was shaked (300 rpm) at 40°C. The reaction rates were determined by following the formation of carboxylic acid. The initial pH value was recorded using a pH-stat (PHM 290 METER LAB™ Autoburette ABU 901). The amount of yielded carboxylic acid was neutralized by adding NaOH solution (1N). Initial reaction rates were determined from the slopes of linear regression plots of carboxylic acid formation vs. time. Twenty five solvents are used in this study (Table 2).

Data analysis: The stepwise method is based on an algorithm that chooses the predictor that has the most elevated correlation with the dependent variable (initial reaction rate). Then, it examines every other predictor to see the one that combined with the first predictor gives the smallest value of the Mean Square of Errors (MSE).

Table 2: Lipase activity of PPL determined in hydrolysis of ethyl butyrate, ethyl acrylate and ethyl acetoacetate ester in different organic solvents

	Initial rates (mM/min/g)		
Solvent	Ethyl acrylate	Ethyl acetoacetate	Ethyl butyrate
None	0	0.047	0.256
DMF(N,N-dimethylformamide)	0.374	0.168	0.158
acetonitrile	0.033	0.046	0.159
1,4-dioxane	0.052	0.046	0.260
acetone	0.060	0.442	0.238
2-butanone	-	0.053	0.030
THF(tetrahydrofuran)	0.046	0.037	1.097
diethyl ether	0.051	0.059	0.239
dichloromethane	0.035	0.035	0.029
chlroform	0.032	0.701	0.032
benzene	0.028	0.321	0.096
fluorobenzene	0.016	0.264	0.022
1-chlorbutane(n-butyl chloride)	0.037	0.063	0.373
toluene	0.019	0.044	0.101
CCl_4(carbon tetrachloride)	0.020	0.052	0.144
chlorobenzene	0.023	0.032	0.094
bromobenzene	0.017	0.027	0.081
ethylbenzene	0.019	0.047	0.106
di-n-butyl ether	0.029	0.235	0.123
C_2Cl_4(tetrachloroethylene)	0.015	0.035	0.116
cyclohexane	0.024	0.061	0.209
n-hexane	0.032	0.476	0.163
n-heptane	0.027	0.005	0.265
n-octane	0.029	0.076	0.165
n-nonane	0.025	0.049	0.171
n-decane	0.019	0.057	0.023

This process is reiterated; however to every stage, it tests the significance of the predictor newly introduced and don't add it to the model if it is not meaningful to a level of 5% (Draper and Smith, 1998).

RESULTS AND DISCUSSION

The hydrolytic activity of the PPL, in term of initial rate, has been measured for each of the three esters in 25 different organic co-solvents (Table 2). The processing of the experimentally obtained data has been carried out using the MINITAB software by taking as dependant variable the initial rate of the reaction (y) and as independent variables the four physico-chemical parameters characterizing each solvent mentioned above. This statistical study using stepwise method led to the models presented in Table 3.

The search for linear models expressing the enzymatic activity according to several descriptors characterizing organic solvents allowed to detect and to eliminate all the solvents which have parameters that do not correlate. Then, the stepwise method gave models with satisfactory predictive quality for a significance level of 0.001. These models were validated using both the internal and external validation.

The comparison of the 3 models of Table 3 shows t hat the model calculated for the third substrate ($y = 0.367\text{-}0.149\ n_D\text{-}0.187\ \delta$) is the best. This can be confirmed by the values of the Fisher's variate (F_{obs}) which are meaningful for the 3 models but increase from 4.43 for the first model to 626.41 for the third one. The goodness of the third model is also confirmed by the increase of the determination coefficient, R^2, which jumps from 18% to 99.6% as well as the adjusted determination coefficient (R^2_{adj}) that reaches 99.4% and the determination coefficient of prediction (R^2_{pred}) which is computed between the observed and adjusted value for the different models. The decrease of both the error of estimation, $S_{y.x}$, from 7% to 0.1% and of the Mean Square of Errors (MSE), from 0.0056 to $9.94\text{x}10^{-7}$ is other parameter which support that the third model is the best. In order to assess the predictive quality of the above models, the quadratic averages of the Predicted Residual Sum of Squares (PRESS) have also been calculated using MINITAB and as it can be seen in Table 3, the lowest value is obtained for the third model (2.02×10^{-5}).

The models show that the activity increases when the solvent polarity decreases. However, it is observed that the model in the case of ethyl butyrate is not of good quality when compared to those calculated for the two others esters (Table 3). This can be explained by the partial solubility in water of ethyl acrylate and ethyl

Table 3: Statistical parameters of fitting and validation of the models

		Models fitting					Validation of models		
Substrate	Model	R^2%	c_t%	F_{obs}	Probability of significant correlation	S_{yx}	R^2_{predi}%	Press	MSE
CH_3-$(CH_2)_2$-COO-C_2H_5	$y = 1.02\text{-}0.620\ n_D$	18.1	14.0	4.43	0.048*	0.072	5.63	0.12	0.0056
CH_2=CH- COO-C_2H_5	$y = 0.239\text{-}0.0046\ \log P\text{-}0.134\ n_D$	98.9	98.6	314.33	0.000***	0.00174	98.01	0.000038	3.03×10^{-6}
CH_3-CO-CH_2- COO-C_2H_5	$y = 0.367\text{-}0.149\ n_D\text{-}0.187$ ä	99.6	99.4	626.41	0.000***	0.00099	98.38	0.0000202	9.94×10^{-7}

*: Significative correlation at level $\alpha = 0.05$ ($p \leq 0.05$). **: High significative correlation at level $\alpha = 0.01$ ($p \leq 0.01$). ***: Very high significative correlation at level $\alpha = 0.001$ ($p \leq 0.001$)

acetoacetate. Addition of an organic solvent is going to extract the substrate (ester) from the aqueous phase and will place it at the interface. As a result, the interface surface is increased and so far the enzymatic activity. On the other hand, this explanation is confirmed by the behavior of ethyl butyrate. The latter being insoluble in water, it is noticed that addition of a co-solvent does not have a great effect on the enzymatic activity because the surface of the interface does not change considerably. These results are in perfect agreement with the action of the lipases which takes place at the water-lipid interface (Mezzetti *et al*., 2003; Cernia *et al*., 2004).

CONCLUSION

The utilization of a multiparametric approach in the simulation of the enzymatic activity by means of solvent nature is a good method to describe the enzymatic hydrolysis reaction in the presence of a co-solvent. For instance, the obtained model for ethyl acetoacetate case indicates that less polar solvents (low refractive index) with high solvatation power (low Hildebrand solubility) are the best. These two qualities allow, indeed, the co-solvent to form an important interface with the water while increasing the concentration of the substrate in the organic phase by solubilisation of its part initially present in the aqueous phase.

REFERENCES

Bezbradica, D., D. Mijin, S. Siler-Marinkov and Z. Knezevic, 2005. The *Candida rugosa* lipase catalyzed synthesis of amyl isobutyrate in organic solvent and solvent-free system: a kinetic study. J. Mol. Catal. B: Enzym., 38: 11-16.

Cajal, Y., A. Svendsen, J. De Bolos, S.A. Patkar and M.A. Alsina , 2000. Effect of the lipid interface on the catalytic activity and spectroscopic properties of a fungal lipase. Biochimie, 82: 1053-1061.

Carlson, R., 2000. Design and optimization in organic synthesis, Data Handling in Sciences and Tech., 8: 536.

Cernia E., C. Palocci, E. Ricchiuto and S. Soro, 2004. Monolayer technique for the study of the hydrolysis of dilaurin isomers bicatalysed by different lipases. Colloids and Surfaces B: Biointerfaces, 33: 7-13.

Chua, L.S. and M.R. Sarmidi, 2005. Effect of solvent and initial water content on (R, S)-1-phenylethanol resolution. Enzyme Microb. Tech., 38: 551- 556.

CRC Handbook of Chemistry and Physics, 1998. Edited by David R. Lide, Former Director, Standard Reference Data, National Institute of Standards and Technology, CRC Press, Boca Raton, New York.

Draper N.R. and H. Smith, 1998. Applied regression analysis. Wiley, New York, pp:805.

Faber, K., 2000. Biotransformations in Organic Chemistry. 4th Edn., Springer, Berlin, pp: 94.

Mezzetti, A., C. Keith and R.J. Kaslaukas, 2003. Highly enantioselective kinetic resolution of primary alcohols of the type Ph-X-CH (CH3)-CH2OH by *Pseudomonas cepacia* lipase: Effect of acyl chain length and solvent. Tetrahedron: Asymmetry, 14: 3917-3924.

Nini, L., L. Sarda, L.C. Comeau, E. Boitard, J.P. Dubes and H. Chahinian, 2001. Lipase-catalysed hydrolysis of short-chain substrates in solution and in emulsion: A kinetic study. Biochimica Biophysica Acta, 1534: 34-44.

Otero, C., M. Fernandez-Pérez and J. Pérez-Gil, 2005. Effects of interactions with micellar interfaces on the activity and structure of different lipolytic isoenzymes from *Candida rugosa*. Enzyme Microb. Tech., 37: 695-703.

Reichardt, C., 2004. Solvents and Solvent Effects in Organic Chemistry, 3rd (Edn.), Updated and Enlarged Ed., Elsevier, pp: 162-317.

Wang, P.Y. and S.W. Tsai, 2005. Hydrolytic resolution of (R,S)-ethyl mandelate in biphasic media via *Klebsiella oxytoca* hydrolase. Enzyme Microb. Tech., 37: 266-271.