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Kinetic modelling of lipase-catalyzed remote resolution of citalopram intermediate in solvent-free system

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ABSTRACT

The kinetic modelling of *Candida antarctica* lipase B catalyzed remote resolution of a tertiary alcohol, citalopram intermediate (diol), was studied using vinyl acetate as acylating agent in solvent-free system. The diffusion limitation and enzyme deactivation were proved to be negligible. A kinetic model based on ping-pong bi–bi mechanism with competitive substrates using King–Altman method was proposed. The substrate inhibition by each enantiomer of diol and the spontaneous transesterification were also considered. Following model discrimination and the application of Haldane equations to reduce the degree of freedom in parameter estimation, the 11 free parameters were successfully identified. Kinetic parameters were estimated using time–concentration curves of different diol concentrations and the simulated values fitted the experimental values well with an average relative error of 12.8%. Furthermore, the developed model was used to investigate the effect of diol concentration on reaction enantioselectivity by the prediction of the typical plot of enantiomeric excess versus conversion of substrate. The average relative error between the results predicted and experimental data was 13.1%.

1. Introduction

Chiral alcohols are useful building blocks for the synthesis of a wide range of pharmaceutical, agricultural and fine chemicals. Lipase-catalyzed kinetic resolution is one of the most important approaches of enantiopure alcohol production for its high efficiency and mild reaction conditions [1]. While the lipase-catalyzed kinetic resolution already succeeded in many primary and secondary alcohols [2–8], the application of this method to tertiary alcohols is more difficult to achieve [9]. Due to the adverse steric hindrance caused by tertiary alcohols, the enantioselectivity of the lipase-catalyzed reactions was relatively low [10,11]. To improve the interpretation of tertiary alcohols kinetic resolution, modelling and simulation can be applied as efficient tools. However, few papers dealing with kinetic study of enzymatic resolution of tertiary alcohol have been published [12–14].

Citalopram is a highly selective inhibitor of serotonin (5-HT) reuptake which has been proved to be an efficient antidepressant. It contains a quaternary chiral center and almost the entire inhibitory activity resides in the *S*-enantiomer of citalopram [15]. The *S*-4-[(4-dimethylamino)-1-(4′-fluorophenyl)-1-hydroxy-1-butyl]-3-(hydroxylmethyl)-benzonitrile (diol), a tertiary alcohol, is a useful intermediate in the synthesis of *S*-citalopram [16]. Although only a few lipases were shown to be active on tertiary alcohols, the lipase-catalyzed remote resolution of diol was accomplished by *Candida antarctica* lipase B and lipoprotein lipase *Pseudomonas* sp. [17,18]. The remote chiral discrimination was achieved by the lipase-catalyzed transesterification of the primary hydroxyl group which was four bonds away from the centre. However, none kinetic model dealing with remote resolution of tertiary alcohols has been developed.

This study focused on the kinetic study of remote kinetic resolution of diol, a tertiary alcohol, by Novozym 435 catalyzed transesterification using vinyl acetate as acylating agent in solvent-free system (Scheme 1). A kinetic model was developed based on ping-pong bi–bi mechanism with competitive substrate inhibition. The substrate inhibition by each enantiomer of diol as well as the spontaneous transesterification was also considered. The developed model was used to study the effect of diol concentration on reaction enantioselectivity by the simulation of the typical plot of enantiomeric excess of substrate and product versus conversion.

2. Materials and methods

2.1. Chemicals

The 4-[(4-dimethylamino)-1-(4′-fluorophenyl)-1-hydroxy-1-butyl]-3-(hydroxyl-methyl)-benzonitrile (diol) was prepared and

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Nomenclature

Notation

E	enantiomeric ratio value
ee_S	enantiomeric excess of substrate
ee_P	enantiomeric excess of product
D	effective diffusion coefficient ($m^2\ s^{-1}$)
C_0	initial concentration of the diffusion substance in the liquid bulk ($mol\ L^{-1}$)
C_L	concentration of the diffusion substance in the liquid bulk ($mol\ L^{-1}$)
R_P	radius of the immobilized lipase particle (m)
q^n	dimensionless variable
Bi	Biot numbers
A_p	outer surface area of the immobilized lipase particle (m^2)
V_p	volume of the immobilized lipase particle (m^3)
V_0	initial apparent rate ($mol\ L^{-1}\ s^{-1}$)
S_0	substrate concentration at outer surface of the immobilized lipase particle ($mol\ L^{-1}$)
k_L	the mass transfer coefficient ($m\ s^{-1}$)
A	substrate, vinyl acetate
B	substrate, diol
E	enzyme
F	acyl-enzyme intermediate
k_i	rate constant, $i = \pm1, \pm2, \pm3, \pm4$
P	product, enol
Q	product, diol acetate
k_t	rate constant of the spontaneous transesterification ($L/(mmol\ h)$)
V^f	maximum rate of forward reaction ($mmol\ (L\ h)^{-1}$)
K_i	K_j inhibition constant (mmol/L)
K_I	the dissociation constant of EB (mmol/L)
K_m	affinity constant (mmol/L)

Greek variables

α	the ratio of liquid volume to solid volume
Φ	Thiele modulus

Subscripts

R, S	R- or S-enantiomer

purified as described in the patent [19]. Novozym 435 (*C. antarctica* lipase B immobilized on acrylic resin) was provided by Novozymes. High-performance liquid chromatography (HPLC) grade *n*-hexane and ethanol were supplied by Tedia. Other materials were of analytical grade and commercially available.

2.2. Analytical method

Samples were analyzed by normal phase HPLC (Agilent Technologies Series 1100 instrument), equipped with UV–vis detector (Agilent, USA), using a CHIRALPAK AD-H column (250 mm × 4.6 mm i.d.) and Agilent ChemStation for LC. The mobile phase was *n*-hexane: ethanol (90:10, v/v) at a flow rate of 1 mL/min. The wavelength of UV–vis detector was 236 nm. Retention times: *R*-diol acetate, 8.8 min; *S*-diol acetate, 9.7 min; *R*-diol, 17.5 min; *S*-diol, 22.5 min. Enantioselectivity was expressed as E value and calculated as follows: $E = \ln[(1-C)\times(1-ee_s)]/\ln[(1-C)\times(1+ee_s)]$, where C denoted conversion of substrate and ee_s denoted enantiomeric excess of substrate. The *ee* value was calculated as $[(R-S)/(R+S)]\times 100\%$, where R and S were the chromatographic peak areas of the *R*- and *S*-enantiomers, respectively.

2.3. General methods for kinetic study

Time-course measurements of the transesterification reaction were performed at various initial diol concentrations in a 10 mL batch reactor using 4 mL vinyl acetate as reactant and solvent. The enzymatic reaction was started by the addition of 10 mg/mL lipase. The reactor was placed in a shaker where the rotation speed and temperature was controlled as 30 °C and 250 rpm. The spontaneous reactions were carried out under the same conditions with enzymatic reactions. For the product inhibition study, the reactions were carried out with original diol acetate concentration of 0, 30, 45, 60, 90 120 mmol/L, respectively, under the condition of 120 mmol/L diol, 10 mg/mL Novozym 435, 30 °C. A 20 μl of well-stirred reaction mixture was taken at intervals for HPLC analysis. Diol and vinyl acetate were dehydrated with anhydrous $CaCl_2$ at room temperature under reduced pressure for 2–3 days before used. The lipases were dehydrated under reduced pressure at 4 °C.

2.4. Measurement of effective diffusion coefficient (D) of diol in the reaction system

The measurement was carried out in a beaker in water bath which was kept at 30 ± 0.2 °C by thermostatic water bath. Stirrer was used to ensure the solution in the flask be mixed thoroughly. At the beginning, 3.5 g lipase and 25 mL vinyl acetate solution (diol concentration 150 mmol/L) were added into the beaker. Since the diol molecule would diffuse into the immobilized lipase particle gradually, diol concentration in bulk liquid (C_L) must decrease with the time increasing. Therefore, when samples were taken in a definite time period and the concentration of diol in the bulk liquid was determined, a curve of C_L–t could be obtained.

2.5. Study of lipase stability

After completion of a resolution reaction (ee_s > 98%), the lipase was filtered off, washed with vinyl acetate, dried at room temperature under vacuum for 10 min. The lipase was subsequently used as a catalyst to the batch reactor according to the procedure described in the previous section.

3. Results and discussion

3.1. Substrate and product inhibition

The effect of concentration of diol was studied over the range of 30–300 mmol/L. The concentration of vinyl acetate was excessive and was considered constant. Fig. 1 showed that with the increase of the diol concentration, the rate of reaction increased and reached a maximum at the concentration of 200 mmol/L. An increase of diol concentration decreased the initial rate, which indicated that diol at high concentration could inhibit the enzyme [20]. For the product inhibition study, the initial rate was almost unchanged with the increase of original diol acetate concentration, which indicated that the inhibition of diol acetate was negligible.

3.2. Effect of internal and external diffusion

Due to the use of an immobilized enzyme, the possible influence of external diffusion limitation and internal diffusion limitation on the kinetics was investigated. The effect of external diffusion limitation was studied by carrying out several experiments at different rotating speeds from 50 to 250 rpm. The dependence of the