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Theoretical Prediction and Experimental Verification of ee_s Versus Time in Biocatalytic Resolution and its Application in a Bioresolution-inversion Process

Yi Xu* & Dan Zhou
School of Chemical and Environmental Engineering, Shanghai Institute of Technology, Shanghai, China

Jianbo Chen
College of Life and Environment Science, Shanghai Normal University, Shanghai, China

ABSTRACT: A systematic theoretical derivation of bioresolution-inversion process was made. An equation was derived between the maximum ee value of final product ($ee_{f(\max)}$) and enantiomeric ratio (E) of a reaction. The corresponding equations of $conv._{(\max)}$, $ee_{p(\max)}$, $ee_{s(\max)}$) versus E-value were also derivative and the interrelationships among $ee_{f(\max)}$, $conv._{(\max)}$, $ee_{p(\max)}$ and $ee_{s(\max)}$ were deduced. Furthermore, a simple equation was developed to predict the enantiomeric excess of substrate (ee_s) at any other time of the whole reaction course based on the ee_s value which was determined at a certain reaction time. This equation of ee_s versus time was verified by three different experiments. Based on the equation of ee_s versus time, a new equation for predicting the time ($t_{(\max)}$) needed to reach the maximum enantiomeric excess of the final product ($ee_{f\ (\max)}$) after the resolution-inversion was developed.

Keywords: theoretical prediction; bioresolution-inversion; epoxide hydrolase; lipase

1 INTRODUCTION

Preparation methods of optically active compounds are classified into two broad categories: the optical resolution of racemic compounds and the asymmetrization of prochiral compounds. Biocatalysts are widely used in both cases. When the starting material is a racemic mixture, the most popular enzymatic approach to obtaining the optically active compounds is kinetic resolution. However, the maximum theoretical yield is limited to 50% and the tedious procedures for the separation of the recovered starting material and the product are inevitable and half of the starting material (or product) has the wrong absolute configuration for certain purposes.

To overcome these drawbacks, several methods have been offered, such as the dynamic kinetic resolution. Another method is the inversion of the stereogenic centre of the substrate (or product) after a biocatalytic resolution. For example, the lipase/Mitsunobu process of secondary alcohol [1-11] or an acid hydrolysis/inversion of the remaining epoxide in the epoxide hydrolase-catalysed enantiomeric hydrolysis of epoxide [12-18]. By these methods, one can obtain the chiral compounds with high optical purity at 100% theoretical yield. Although pioneer works had been made before1 or 10, the derivations were incomplete. In the following paragraph, a complete derivation was made. Moreover, the possibility for predicting the time (t_{max}) which is needed to reach the maximum enantiomeric excess of the final product (ee_f) was firstly explored.

2 EXPERIMENT

2.1 *Generalization*

All the chemicals and reagent were commercially obtained and of analytical grade.

2.2 *Enantioselective hydrolysis of 3-(2-nitrophenoxy) propylene oxide (1a) by Trichosporon loubierii ECU1040*

Lyophilized yeast cells (3 g) were rehydrated in sodium phosphate buffer (90 ml, 100 mm, pH 7.0) for 30 min on a shaker (160 rpm, 30 °C). Then 10 ml DMSO containing 500 mg of the substrate was added and the mixture was agitated at 30 °C. Samples were taken at different time. The ee value of epoxide was directly determined by HPLC analysis through using Chiralcel OD column. The mobile phase was hexane/ isopropanol (90/10, v/v) at a flow rate of 1.0 ml/min and detected at 254 nm.

2.3 *Enantioselective hydrolysis of trans-3-(4-methoxyphenyl)glycidic acid methyl ester (MPGM) by Serratia sp. lipase*

Experiments were performed through using a substrate concentration of 50 mm in 10 ml toluene solution and 10 ml culture supernatant (the pH value was adjusted to 7.5 by Tris-HCl buffer). The reactions were carried out at 30 °C and 160 rpm in 100 ml flasks equipped

*Corresponding author: xuyi@sit.edu.cn

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with tight plugs. Samples were taken at different time for the determination of ee value of MPGM. The ee value was determined by HPLC with a chiral column (Chiralcel OJ, 25×4.6 cm, Daicel Chemical Industries, Tokyo, Japan) and elution by hexane/isopropanol (60: 40, v/v; 0.8 ml/min) and detection at 254 nm. The retention time was respectively 13.5 and 15.7 min for (2S, 3R)-MPGM and (2R, 3S)-MPGM.

2.4 *Enzymatic transesterification of (R, S)-4-hydroxy-3-methyl-2-(2-propenyl)-2-cyclopentn-1-one) [HMPC] with vinyl acetate catalyzed by Lipase PS*

50 mm (R, S)-HMPC dissolved in vinyl acetate was added to the lipase PS, and the reaction was conducted at 30 °C, 160 rpm. Samples were taken at different time for the determination of ee value HMPC. The enantiomeric excess of substrate (ee_s) and product (ee_p) was determined by GLC using β-DEXTM 120 column (oven temperature, 150 °C; injector and detector temperature, 280 ◦C). The retention time was respectively 15.4, 16.1, 20.6 and 21.2 min for (R)-HMPC acetate, (S)-HMPC acetate, (S)-HMPC and (R)-HMPC.

3 DERIVATION OF EQUATIONS

3.1 *Derivation of equations for resolution-inversion process*

If we define the enantiomeric excess of the final product (after the biocatalytic resolution and inversion) as ee_f, the value of ee_f would be directly dependent upon the conversion ratio and the enantioselectivity of biocatalyst, E-value. For a simple irreversible biocatalytic kinetic resolution-inversion process, supposing that no racemization occurred in the whole course, we can obtain equations 1~5 [19]:

$$E = \frac{\ln\left(A/A_0\right)}{\ln\left(B/B_0\right)} \tag{1}$$

$$ee_s = \frac{B-A}{B+A} \tag{2}$$

$$ee_p = \frac{P-Q}{P+Q} \tag{3}$$

$$C = \frac{P+Q}{A_0+B_0} = \frac{ee_s}{ee_s+ee_p} \tag{4}$$

$$ee_f = \frac{(B+P)-(Q+A)}{(B+P+Q+A)} = \frac{2ee_s ee_p}{ee_s+ee_p} \tag{5}$$

In this equation, A and B refer to the fast- and slow-reacting enantiomers of the substrate; P and Q refer to the corresponding enantiomers of the product; ee_s and ee_p are respectively the enatiomeric excess of the substrate and the product; E is the enantiomeric ratio.

If we define

$$B/B_0 = x \tag{6}$$

Then,

$$A/A_0 = x^E \tag{7}$$

By substituting equations (6) and (7) into equations 2~5, we can obtain as follows:

$$C = 1 - x/2 - x^E/2 \tag{8}$$

$$ee_s = \frac{x-x^E}{x+x^E} \tag{9}$$

$$ee_p = \frac{x-x^E}{2-x-x^E} \tag{10}$$

$$ee_f = x - x^E \tag{11}$$

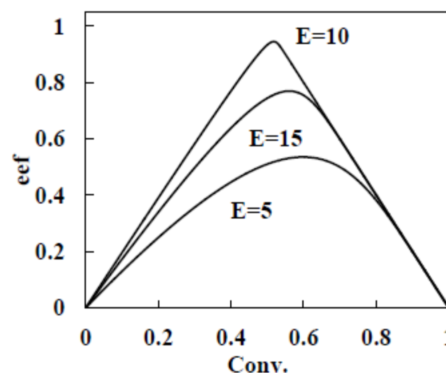

Figure 1. Graphic plots of *Conv.* versus ee_f at different *E-values*

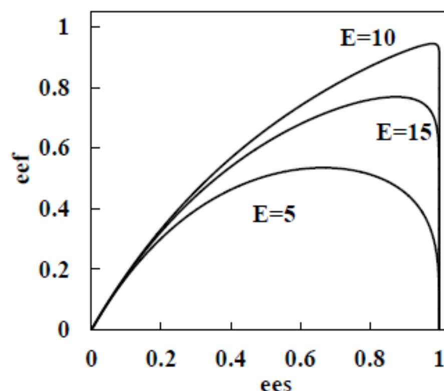

Figure 2. Graphic plots of ee_s versus ee_f at different *E-values*

By combination of equations 8~11, we can get plots of C versus ee_f (Figure 1), ee_s versus ee_f (Figure 2) and ee_p versus ee_f (Figure 3). From Figures 1~3, we can see that there is a maximum ee_f value ($ee_{f(max)}$) for a fix '*E*' and the $ee_{f(max)}$ varies with the change of *E-value*.

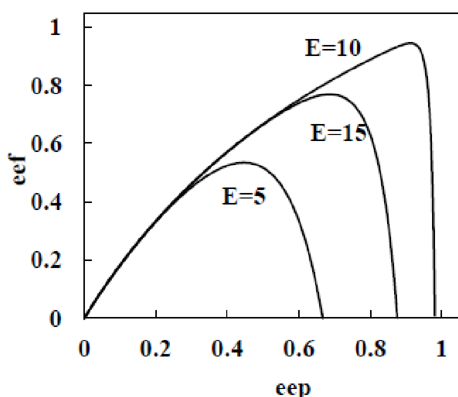

Figure 3. Graphic plots of ee_p versus ee_f at different *E-values*

It is obvious that if

$$ee_f\big|_x' = 0,$$

That is:

$$x = \left(\frac{1}{E}\right)^{\frac{1}{(E-1)}}$$

One can get the maximal ee_f value: ee_f (max) and the corresponding conv., ee_p, ee_s are defined as conv. (max), ee_p (max), ee_s (max). By substituting the x value into equations 8~11, we can get the following equations:

$$ee_{f(\max)} = \left(\frac{1}{E}\right)^{\frac{1}{(E-1)}} - \left(\frac{1}{E}\right)^{\frac{E}{(E-1)}} \quad (12)$$

$$Conv._{(\max)} = 1 - \frac{\left(\frac{1}{E}\right)^{\frac{1}{(E-1)}} + \left(\frac{1}{E}\right)^{\frac{E}{(E-1)}}}{2} \quad (13)$$

$$ee_{p(\max)} = \frac{\left(\frac{1}{E}\right)^{\frac{1}{(E-1)}} - \left(\frac{1}{E}\right)^{\frac{E}{(E-1)}}}{2 - \left(\frac{1}{E}\right)^{\frac{1}{(E-1)}} - \left(\frac{1}{E}\right)^{\frac{E}{(E-1)}}} \quad (14)$$

$$ee_{s(\max)} = \frac{\left(\frac{1}{E}\right)^{\frac{1}{(E-1)}} - \left(\frac{1}{E}\right)^{\frac{E}{(E-1)}}}{\left(\frac{1}{E}\right)^{\frac{1}{(E-1)}} + \left(\frac{1}{E}\right)^{\frac{E}{(E-1)}}} = \frac{E-1}{E+1} \quad (15)$$

From equations 12~15, we can clearly know the maximum ee_f and corresponding *conv.*, ee_p and ee_s.

For example, if *E-value* equals 200, we can get the maximum ee_f value 96.9% at 51.1% conversion or at 94.9% ee_p or at 99.0% ee_s. In practical process, the chemical inversion (*y*) is not always 100%, perhaps 90% or others in some cases. Considering the above mentioned condition, some modifications should be made for an incompletely chemical inversion. In fact, only equation 12 should be changed to equation 16 and others are kept unchanged (*y* is the efficiency of chemical inversion):

$$ee_{f(\max)} = y\left(\left(\frac{1}{E}\right)^{\frac{1}{(E-1)}} - \left(\frac{1}{E}\right)^{\frac{E}{(E-1)}}\right) \quad (16)$$

Figure 4 shows the curves of $ee_{f(max)}$, $ee_{s(max)}$, $ee_{p(max)}$ and $Conv._{(max)}$ vs. *E*. It is interesting to see that the $ee_{f(max)}$ value is always larger than $ee_{s(max)}$ value, but smaller than $ee_{p(max)}$ value.

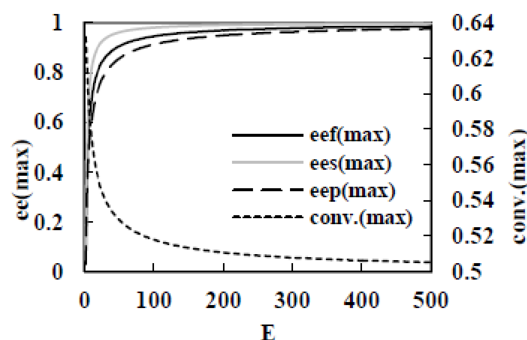

Figure 4. Theoretical plots of $ee_{f\,(max)}$, $ee_{s(max)}$, $ee_{p(max)}$ and $conv_{(max)}$ as a function of *E* according to equations (12) - (15)

Now, a question arises. How to predict the time for the reaction to stop at appropriate moment to reach $ee_{f(max)}$?

3.2 *Prediction of the time-dependant changes in enantiomeric excess of substrate ($ee_s \sim t$)*

According to Chen *et al.* [20] and Lu *et al.* [21], for a simple irreversible kinetic resolution, the *E-value* is shown as follows:

$$E = \frac{\ln\left(\frac{A}{A_0}\right)}{\ln\left(\frac{B}{B_0}\right)}$$

This indicates that the distinction between two competing enantiomers (A and B) by an enzyme is equal to a constant E. Equation 1 can be re-written as follows:

$$\frac{\ln\left(A/A_0\right)}{\ln\left(B/B_0\right)}=\frac{-kt}{-kt/E} \quad (17)$$

Then the equation 17 can be derived to:

$$A = A_0 \exp(-kt) \quad (18)$$

$$B = B_0 \exp(-kt/E) \quad (19)$$

It is at a low initial substrate concentration according to Lu's derivation and Michaelis-Menten equation (if the substrate concentration is low enough and relative to Km, the reaction is the first order). Here, A_0 and B_0 are initial concentrations of the fast- and slow-reacting enantiomers, k is the rate constant for the fast-reacting enantiomer. For the kinetic resolution of a racemate (A_0=B_0=0.5S_0), it is known that:

$$ee_s = \frac{B-A}{B+A}$$

By substituting equation 18 and equation 19 into equation 2, we can write as follows:

$$\ln(B/A) = k(1-1/E)t = \ln\left(\frac{1+ee_s}{1-ee_s}\right) \quad (20)$$

Considering that both k and E are constants, we can acquire as follows:

$$\frac{1}{t_1}\ln\left(\frac{1+ee_{s1}}{1-ee_{s1}}\right)=\frac{1}{t_2}\ln\left(\frac{1+ee_{s2}}{1-ee_{s2}}\right) \quad (21)$$

ee_{s1} and ee_{s2} are respectively ee_s values at t_1 and t_2. Equation 21 can be written as follows:

$$ee_{s2}=\frac{\left(\frac{1+ee_{s1}}{1-ee_{s1}}\right)^{t2/t1}-1}{\left(\frac{1+ee_{s1}}{1-ee_{s1}}\right)^{t2/t1}+1} \quad (22)$$

It can be concluded from equation 22 that if we know ee_{s1} at t_1, then we can theoretically predict the ee_s value at another time (t_2) in the same reaction mixture.

By substituting equation 15 into equation 22, we can get:

$$t_{(\max)} = t_1\frac{\ln E}{\ln\left(\frac{1+ee_{s1}}{1-ee_{s1}}\right)} \quad (23)$$

If one knows the ee_s value at t_1 and the *E-value*, he can calculate the time which is needed to reach the maximum ee_f according to equation 23.

4 EXPERIMENTAL VERIFICATION OF TIME-DEPENDANT CHANGES IN ENANTIOMERIC EXCESS OF SUBSTRATE (EES ~ T)

The equation 22 was verified by three different biocatalytic kinetic resolution experiments.

It can be seen from Figure 5 that for the first example, , both of the theoretical curves (the curves were respectively plotted according to equation 22 and ee_s values at 30 min and 60 min) fit the experimental data quite well in the resolution of 3-(2-nitrophenoxy) propylene oxide by epoxide hydrolase of *Trichosporon loubierii* ECU1040 [22]. This enables one to stop the reaction at a proper time (e.g. ee_s > 98%) to get both high optical purity and high yield of the epoxide. And this will also simplify the work of measurement.

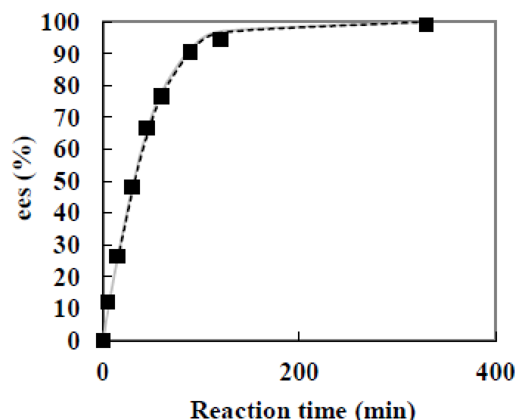

Figure 5 Variation of ee_s in the resolution of racemic 1 by lyophilized cells of *Trichosporn loubierii* ECU1040 (100 g/L). Symbols: ■ Measured; — ---- Calculated with the ee_s at t = 30 min (10 mM) and 60 min (10 mM), respectively.

The second example is related to enzymatic resolution of MPGM. (2*R*, 3*S*)-MPGM, a very important intermediate in the synthesis of Diltiazem Hydrochloride, can be prepared according to enantioselective hydrolysis of the racemic MPGM catalyzed by *Serratia* sp. Lipase [23]. So it is necessary to stop the reaction when the ee_s value was enough high so that we can get (2*R*, 3*S*)-MPGM at both high yield and optical purity. It can be seen from Figure 6 that the theoretical curves fit the experimental data quite well. This enables one to stop the reaction at a proper time (e.g. $ee_s \geq$ 98%) to get both high optical purity and high yield of the MPGM.

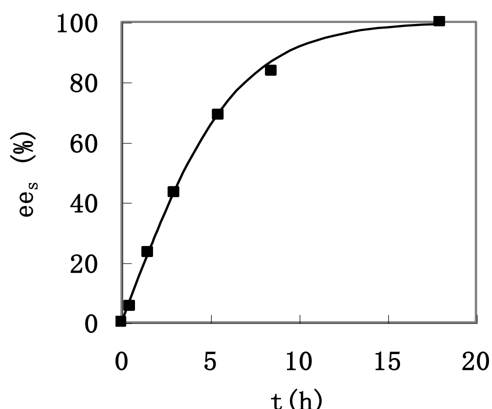

Figure 6. Variation of ee_s in the resolution of racemic MPGM by by Serratia sp. lipase. Symbols: ■ Measured; — Calculated with the ee_s at t = 1.5 h.

Chiral HMPG and its ester are very important agricultural intermediates. Figure 7 showed the time course of ee_s value in transesterification of (*R*, *S*)-HMPC with vinyl acetate which is catalyzed by the Lipase PS. The theoretical curve was plotted based on equation 22 and the ee_s at 3h. The theoretical curves fit the experimental data quite well. The $t_{(max)}$ value can be also calcultated from equation 23. This enables one to stop the reaction at a suitable time to obtain high ee value and yield of the substrate ((*S*)-HMPC) and product ((*R*)-HMPC acetate). And the highest yield and ee value of final product ((*R*)-HMPC acetate) can be obtained after the bioresolution/chemical inversion.

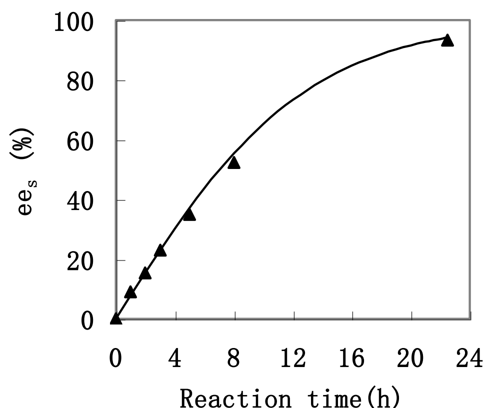

Figure 7. Time course of ee_s value in transesterification of (*R*, *S*)-HMPC with vinyl acetate catalyzed by Lipase PS. ▲ Measured; —Calculated with the ee_s at t = 3 h.

5 CONCLUSIONS

A systematic theoretical derivation of bioresolution-inversion process was made. An equation was derived between the $ee_{f(max)}$ and E-value of a reaction. The corresponding equations of $conv._{(max)}$, $ee_{p(max)}$, $ee_{s(max)}$ versus *E-value* were also derived and the interrelationships among $ee_{f(max)}$, $conv._{(max)}$, $ee_{p(max)}$ and $ee_{s(max)}$ were deduced. Furthermore, a simple equation was developed to predict the enantiomeric excess of substrate (ee_s) at any other time of the whole reaction course based on the ee_s value which was determined at a certain reaction time. This equation of ee_s versus time was verified by three different experiments. Based on the equation of ee_s versus time, a new equation for predicting the time ($t_{(max)}$) needed to reach the maximum enantiomeric excess of the final product ($ee_{f\ (max)}$) after the resolution-inversion which was developed. The current work will be beneficial to the biocatalytic resolution-inversion study.

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