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# Calculation of Enantiomer Ratio and Equilibrium Constants in Biocatalytic Ping-Pong Bi-Bi Resolutions

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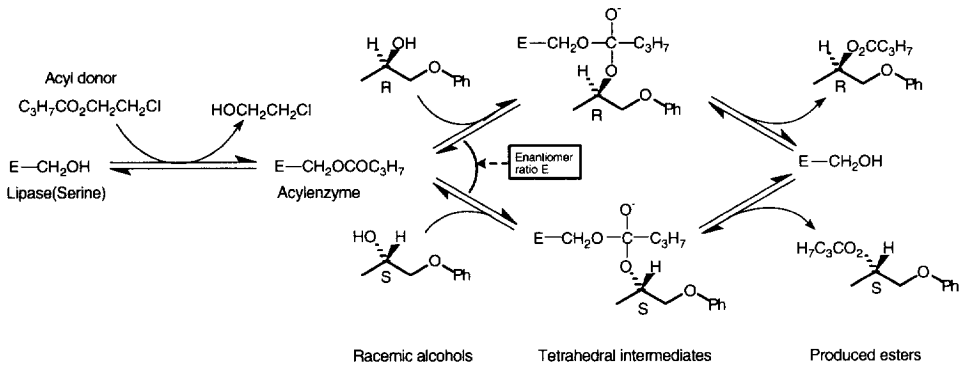
***Abstract:*** A computer program for the determination of kinetic and thermodynamic parameters in biocatalytic ping-pong bi-bi resolutions has been developed. The program uses enantiomeric excesses of both product *($ee_P$)* and remaining substrate *($ee_S$)* measured at more than one conversion*($\xi$)*, and determines both the equilibrium constant $K_{eq}$, the enantiomer ratio $E$ and the selectivity factor $\alpha$. The program has been tested for transesterification of 1-phenoxy-2-propanol using different excesses of acyl donor.


## INTRODUCTION

The quality of the product of a racemate resolution is characterised by the enantiomeric excess, *ee*, however, the important parameter for a resolution process is the enantiomer ratio $E$. A high $E$-value for a given substrate-enzyme pair is crucial for the success of a kinetic resolution, since a high $E$-value ensures not only a high *ee*, but also a high yield. In principle, for an irreversible reaction it is always possible to achieve a high $ee_S$ if a low yield can be accepted. This is one major difference between resolution and asymmetric synthesis which has to be considered when choosing between these two methods in order to provide enantiomerically pure compounds. If the resolution reaction is reversible, also the equilibrium constant $K$ has to be taken into account. In order to calculate $E$ and $K$ it has been common to use the equations developed by Chen *et al.* [2] by which $E$ may be calculated from a determination either of $ee_P$ or $ee_S$ at one conversion, $\xi$. Recently we have developed a method for calculation of $E$ and $K$ by fitting datapoints measured at several conversions("E&K Calculator", version 1.01).[3]

The above mentioned method of treating resolutions relies on a so-called uni-uni mechanism, *i.e.* one substrate and one product. Lipase catalyzed hydrolysis or transesterification does not follow this mechanism, but rather what is known as a ping-pong bi-bi mechanism.[4] Transesterification reactions include two steps with two substrates and two products (Scheme 1). The equilibrium constant as defined by Chen *et al.* is $K$ = [Substr. alc.]/[Prod. est.] while the equilibrium constant for a bi-bi reaction is $K_{eq}$ = [Produced ester] [leaving alcohol]/[Acyl donor][Substrate alcohol].




*Scheme 1.* Lipase catalyzed transesterification of 1-phenoxy-2-propanol using 2-chloroethyl butanoate as acyl donor. Example used for demonstration of the present computer program.

The rate equation for several types of such bi-bi reactions has been derived by Straathof *et al.*[4] Equation 1 is the differential equation describing the rate of the reaction outlined in Scheme 1, $E$ is the enantiomer ratio, $K_{eq}$ is the equilibrium constant and $\alpha^R$ is the selectivity factor, the ratio of specificity constants for the acylation of free enzyme with acyl donor and the $R$-ester.[5]

$$\frac{dc_Q^R}{dc_Q^S} = \frac{c_B^R\left(Ec_A + \frac{c_Q^S}{\alpha^R}\right) - c_Q^R\left(\frac{Ec_P}{K_{eq}} + \frac{c_B^S}{\alpha^R}\right)}{c_B^S\left(c_A + \frac{c_Q^R}{\alpha^R}\right) - c_Q^S\left(\frac{c_P}{K_{eq}} + \frac{c_B^R}{\alpha^R}\right)} \qquad 1)$$

In contrast to the uni-uni case[2] it is not possible to separate the variables and integrate this equation analytically. Integration of Equation 1 must therefore be done numerically. Previously these parameters have been determined by initial rate experiments at different substrate and product concentrations.[5] The equilibrium constant may be determined by measuring the substrate and product concentration at equilibrium.

## RESULTS AND DISCUSSION

The first step of the new method is to calculate $ee_S$ and $ee_P$ from the rate equation for any allowed combination of the parameters. By inspection of Equation 1 it may be seen that it is not possible to separate the variables in order to integrate the equation analytically. Hence, in order to calculate $ee_S$ and $ee_P$ for different values of $\xi$, $E$, $K_{eq}$, $\alpha^R$ and excess of acyl donor, numerical integration of Equation 1 must be performed. This integration yields the simulated concentrations of $c_Q^R$ and $c_Q^S$ for a given set of $E$, $K_{eq}$, $\alpha^R$ . From the values of $c_Q^R$ and $c_Q^S$ it is in turn possible to calculate $\xi$, $ee_S$ and $ee_P$.

As the integration proceeds a table of evenly spaced ($\xi$, $ee_S$) points are stored. To evaluate the integrated equation at $\xi$ values between these stored points, cubic spline interpolation was used. This procedure makes it possible to evaluate both $ee_S$ and $ee_P$ as a function of $\xi[ee_S = f(\xi)E, K_{eq}, \alpha^R$ and $ee_P = f(\xi)E, K_{eq}, \alpha^R]$.

The problem of fitting a set of experimental data to the rate equation is by this method reduced to an ordinary minimisation in three dimensions, assuming that the excess of acyl donor is known. We have done this by the method of simplex minimisation[6] by reducing the least square error of a penalty function similar to the penalty function described earlier.[3]

In order to illustrate the use of the program, we have chosen transesterification of 1-phenoxy-2-propanol in hexane using 2-chloroethyl butanoate as acyl donor and lipase B from *Candida antartica* as catalyst. To obtain a precise determination of $E$, $K_{eq}$, $\alpha^R$ reactions with different amounts of acyl donor excess (1.5, 3, 5, 7 and 10 times excess) were carried out. Using the new program we obtained consistent results for $E$, and $K_{eq}$.(Table 1) The corresponding *ee vs.* conversion curves (Figure 1) clearly shows that increasing amounts of acyl donor increases both the enantiomeric excess of the substrate and the yield.

*Table 1* Results obtained by applying ping-pong bi-bi mechanism to the reactions shown in Figure 1.

| Acyl donor excess | $E$ | $K_{eq}$ | $\alpha^R$ |
|---|---|---|---|
| 1.5 | 151 | 0.366 | 480 |
| 3.0 | 259 | 0.317 | 940 |
| 5.0 | 130 | 0.358 | 0.6 |
| 7.0 | 142 | 0.259 | 1060 |
| 10.0 | 133 | 0.259 | 740 |
| Mean±std.dev | 139±10* | 0.32±0.05 | ** |

*$E$ value of 259 not included, **not determined.

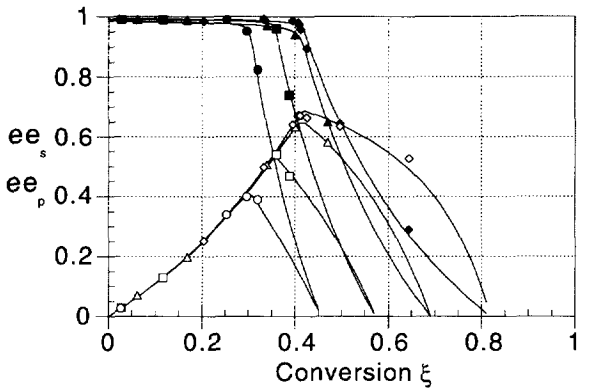



*Figure 1.* Resolution of 1-phenoxy-2-propanol with varying acyl doner concentrations. From left to right, 1.5(O), 3.0(□), 5.0(Δ), and 10.0(◊) times excess of acyl donor.

Performing experiments with different acyl donor concentrations makes it possible to calculate the error of the parameters. For the present example: $E$ = 139±10, $K_{eq}$ = 0.32±0.05 (Table 1). The value of $\alpha^R$ changes unsystematically over the series, from a maximum value of 1060 to a minimum value of 0.6. Simulated curves drawn with $E$ = 139, $K_{eq}$ = 0.32 and varying $\alpha^R$ values revealed that the curves changed only minutely when $\alpha^R$ was varyed over the observed ranged. Only when $\alpha^R$ was below 0.1 a change of the curve was observed. Further simulations with varying excess of acyl donor and magnitude of $E$ respectively showed that change of $\alpha^R$ influenced the curves more when either $E$ or the excess of acyl donor was low. With the new program it is simple to evaluate the effect of changes in all parameters involved in the resolution process.

In order to demonstrate the difference of the curves based on the two different mechanisms, we calculated theoretical curves based on the results obtained above. With varying amounts of acyl donor, 1.5, 3 and 5 times excess, quite different $ee_S$ *vs.* conversion curves were obtained when the two different mechansims were applied.(Figure 2) The equilibrium constant calculated for the three cases shows consistent values only when the bi-bi mechanism is used. (Table 2) Resolutions with different excess of acyl donor should ideally give the same $E$, $K_{eq}$ and $\alpha^R$.

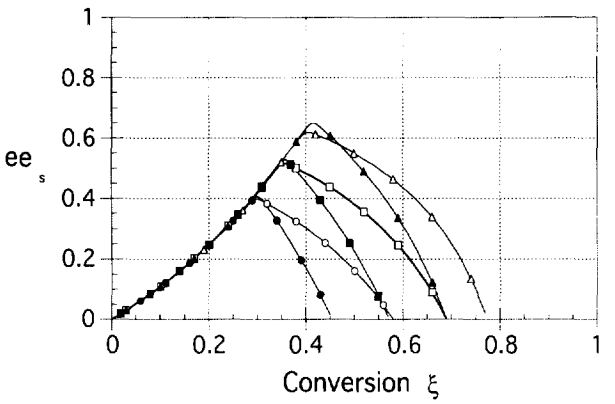



*Figure 2* Curves based on data generated for resolution of 1-phenoxy-2-propanol with 2-chloroethyl butanoate as acyl donor with three different acyl doner concentrations, 1.5(O), 3(□) and 5(Δ) times excess respectively, filled symbols bi-bi mechanism, open symbols uni-uni mechanism.

*Table 2* $K_{eq}$ calculated by the bi-bi and the uni-uni model for different concentrations of acyl donor.

| Kinetic model | Acyl donor excess | $K_{eq}$ |
|---|---|---|
| bi-bi | 1.5 | 0.37 |
| uni-uni | 1.5 | 0.90 |
| bi-bi | 3.0 | 0.32 |
| uni-uni | 3.0 | 0.64 |
| bi-bi | 5.0 | 0.36 |
| uni-uni | 5.0 | 0.63 |

We then used the new program in order to quantify the difference between $E$ based on the uni-uni equation and $E$ calculated from the rate equation (1) of the ping-pong bi-bi reaction. Keeping $E_{bi\text{-}bi}$ at a constant value of 100 and $\alpha^R$ at 1000 we found that $E_{uni\text{-}uni}$ increased from 100 to 108 when $K_{eq}$ was reduced from 100 to 0.1. Moreover, using $E_{bi\text{-}bi}$ = 100, $K_{eq}$ = 100 and changing $\alpha^R$ from 1000 to 0.1, $E_{uni\text{-}uni}$ was reduced from 100 to 76.

## EXPERIMENTAL

Transesterification, analyses of enantiomeric excess and synthesis of 1-phenoxy-2-propanol have been described earlier.[3,7] The enzyme catalyzed reaction gave *(R)*-butanoate as the product and *(S)*-alcohol, $[\alpha]_D^{20}$ -2.7 (c 1.80, EtOH). The absolute configuration was identified by comparison with authentic material.[8] The program for calculation of $E$, $K_{eq}$ and $\alpha^R$ was written in C++ and compiled for Power Macintosh with CodeWarrior Academic compilator,

version 9. Values of $K_{eq}$, $E$ and $\alpha^R$ that describe a given ($\xi$, $ee_S$) and ($\xi$, $ee_P$) data set were calculated by minimisation of a penalty function. All minimisation runs were started with the initial values $E$ = 10, $K_{eq}$ = 100 and $\alpha^R$ = 1000. After 350 steps the program was restarted with the currently best values until stable values were found. The penalty function was dependent on the least square error between experimental points and calculated points as described earlier.[3] Fitting of data to the uni-uni equations was performed with the computer program "E&K Calculator", version 1.01.[9] The new program for ping-pong bi-bi resolutions is "E&K Calculator", version 2.01.[9] Visualisation and drawing of the curves were performed by Kaleidagraph™ 3.0.

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## REFERENCES

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2. Chen, C.-S.; Wu, S.-H.; Girdaukas, G.; Sih, C.J. *J. Am. Chem. Soc.* **1987**, *109*, 2812-2817.
3. Anthonsen, H.W.; Hoff, B.H.; Anthonsen, T. *Tetrahedron: Asymmetry* **1995**, *6*, 3015-3022.
4. Straathof, A.J.J.; Rakels, J.L.L.; Heijnen, J.J. *Biocatalysis* **1992**, *7*, 13-27.
5. van Tol, J.B.A.; Kraayveld, D.E.; Jongejan, J.A.; Duine, J.A. *Biocatal. Biotransform.* **1995**, *12*, 119-136.
6. Press, W.H.; Teukolsky, S.A.; Vetterling, W.T.; Flannery, B.P. *Numerical Recipes in C: The Art of Scientific Computing.* Cambridge University Press, 1992.
7. Hansen,T.V.; Waagen, V.; Partali, V.; Anthonsen, H.W.; Anthonsen, T. *Tetrahedron: Asymmetry* **1995**, *6*, 499-504.
8. Waagen, V.; Partali, V.; Hollingsæter, I.; Soon-Huang, M.-S.; Anthonsen, T. *Acta Chem. Scand.* **1994**, *48*, 506 - 510.
9. Anthonsen, H.W. http://bendik.mnfak.unit.no

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