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A Convenient Method for Evaluation of the Enantiomeric Ratio of Kinetic Resolutions

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Abstract : A simple method for evaluation of the enantiomeric ratio E of kinetic resolutions by using only the extent of substrate conversion c has been developed and verified experimentally.

Non-racemic chiral molecules play important role in context of biological activity.[1] The interaction of an enantiomerically pure material with a receptor may manifest itself as a difference in biological activity. Increased understanding of pharmacokinetics and mechanism of action of these chemicals in biological systems has led to the development of drugs,[2] agrochemicals and food additives in enantiomerically pure form. According to a recent report,[2b] more than 50% of the commercial drugs available worldwide have stereogenic centers. The kinetic resolution of racemates is often the method of choice for the enantiomerically pure compounds.[3] Although it has been used extensively,[4] there is a need for methods for quantitative analysis of the enantioselectivity of kinetic resolutions that will allow chemists to make useful predications. In 1982, Sih and his co-workers[5] have described three important parameters and their quantitative relationship for kinetic resolutions. The three parameters, the extent of conversion of racemates c, the enantiomeric excess ee of the product ee(P) or the remaining substrate ee(S) and the enantiomeric ratio E, have been used widely in the study of enzymatical catalytic resolutions.

In a kinetic resolution, the enantiomeric excess of the substrate or product varies with c. However, the enantiomeric ratio E for a kinetic resolution is a constant independent of time and substrate concentrations, which dictates the enantioselectivity of the kinetic resolution.

$$E = \frac{\ln[(1-c)(1-ee(S))]}{\ln[(1-c)(1+ee(S))]} = \frac{\ln[1-c(1+ee(P))]}{\ln[1-c(1-ee(P))]} \qquad \text{Eq. 1}$$

It is evident that a conventional method for the evaluation of E with Eq. 1 needs the knowledge of the other two variables (c and ee).[5] Generally, E is calculated after determination of c of a resolution and ee values of isolated product or substrate. Although c can be obtained conveniently, the determination of ee(P) or ee(S) would be relatively difficult. Thus, there is a limitation for analysis of enantioselectivity of the kinetic resolution by Eq. 1. This limitation can, however, be overcome if it is possible to use only c instead of both c and ee for evaluation of E. This communication deals with the relationship between the c-t curve (the curve of conversion c as a function of time t) and the enantioselectivity of kinetic resolutions, and deals with the evaluation of E with only c.

An important kinetic parameter, c, expresses the percentage of the product converted from substrate. The rate of change of c in the resolution and c-t curve show the intrinsic property of the kinetic resolution system. When the enzymatic catalytic reaction presented by curve (a) slows down obviously, c is near 100% (see Fig.1). All substrates (both enantiomers) have been converted into products. Thus, there is no enantioselectivity for this reaction. An ideal resolution reaction presented by curve (c) stops at 50% conversion that indicates that only half amount of substrates (one enantiomer) reacts. The slope of curve (b) changes obviously near 50% conversion and c would pass 50%. The reaction presented by the curve (b) is a partial resolution and during the reaction both enantiomers react with different velocities.

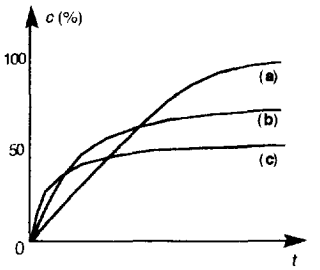


Fig.1 Three typical c-t cuvres for enzymatically catalytic reaction

Generally, the obvious change of slope for c-t curve near 50% conversion could be used as an index for determination of the enantioselectivity of the kinetic resolution of racemates. In the literature, several qualitative analyses of enantioselectivity by this approach have been reported.[6] However, this approach has some drawbacks such as: (1) Drawing a precise c-t curve, which needs a series of measurements to be carried out, may be laborious; (2) Giving a quantitative index by the change of c-t curve slope is not easy; (3) Comparing the slope of c-t curves among several resolutions is difficult since the slope is dependent on both E and rate constant k, and in general the two unknown parameters in every resolution are different respectively. The aforementioned facts prompted us to develop a method of quantitative treatment of c data of kinetic resolution for evaluation of E.

The basic starting point is the definition of enantiomeric ratio E[5]. Suppose that A and B are the fast- and slow-reacting enantiomers that compete for the same site on the enzyme. For a simple irreversible kinetic resolution, the enantiomeric ratio E may be shown to be

$$E = \frac{\ln(A/A_0)}{\ln(B/B_0)} \qquad \text{Eq. 2}$$

, which indicates that the discrimination between two competing enantiomers (A and B) by the enzyme is equal to the constant E. Eq. 2 can be written as

$$\frac{\ln(A/A_0)}{\ln(B/B_0)} = \frac{-kt}{-kt/E} \qquad \text{Eq. 3}$$

Eq. 3 reveals that during the kinetic resolution the concentrations of fast- and slow-reacting enantiomers A and B are governed respectively by

$$A = A_0 e^{-kt} \qquad \text{Eq. 4}$$

$$B = B_0 e^{-kt/E} \qquad \text{Eq. 5}$$

where A_0 and B_0 denote initial concentrations of the fast- and slow-reacting enantiomers and k is the rate constant for the fast-reacting enantiomer. We make the following comments on Eqs. 4 and 5: (1) the rate constant for the fast-reacting enantiomer (k) is E times larger than that of the slow-reacting one (k/E); (2) for an

ideal resolution reaction, $1/E = 0$; (3) for a reaction with no enantioselectivity, $1/E = 1$; (4) for most kinetic resolutions, which are partial resolution, we find $0<1/E<1$. The substrate S[7] includes both enantiomers A and B (that is $S=A+B$ and $S_0=A_0+B_0$), which are indistinguishable from each other unless the analysis is carried out under asymmetric conditions (for example: HPLC with a chiral column, NMR with a chiral reagent, etc.). Experimentally, the analysis should be based on the concentration of the substrate S instead of A and B. For a resolution of racemates ($0.5S_0 = A_0 = B_0$), we can write

$$2S/S_0 = e^{-kt} + e^{-kt/E} \qquad \text{Eq. 6}$$

If the first resolution reaction stops at $t=t_1$, we have $S=S_1$. Then we substitute t_1 and S_1 along with the fact that $S_0 = 100\%$ into Eq. 6 and we can write

$$1/E = \ln(2S_1 - e^{-kt_1})/-kt_1 \qquad \text{Eq. 7}$$

If we substitute Eq. 7 into Eq. 6, then we find

$$2S = e^{kt} + (2S_1 - e^{-kt_1})^{t/t_1} \qquad \text{Eq. 8}$$

Stopping the second resolution at $t=2t_1$, we have $S=S_2$. Substituting $2t_1$ and S_2 into Eq. 8, using $x=e^{-kt_1}$ and rearranging it give

$$x^2 - 2S_1 x + (2S_1^2 - S_2) = 0 \qquad \text{Eq. 9}$$

One of the roots of this equation is x_1. We have

$$e^{-kt_1} = x_1 \quad \text{and} \quad -kt_1 = \ln x_1$$

We substitute these results back into Eq. 7, then we have Eq. 10 in which E is only unknown variable. Eq. 10 is in form more compatible with experimental data.

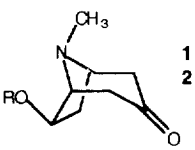
$$1/E = \ln(2S_1 - x_1) / \ln x_1 \qquad \text{Eq. 10}$$

In brief, our approach includes (1) determining respectively S_1 and S_2 of a kinetic resolution at t_1 and at $2t_1$; (2) evaluating Eq. 9 and choosing the right root x_1; (3) substituting x_1 into Eq. 10 then obtaining E.[8]

Using this approach, we have analyzed the lipasic transesterification of racemic 6β-acetoxytropinone **1** in heptane in the presence of *n*-butanol[9] (see Table 1). The reaction is monitored by NMR and TLC with modified method of Büchi.[10]

Table 1 Transesterification of (±)-6β-acetoxytropinone **1**

Time (hours)	0	8	16	48
S (%)	100	60	36	10



1 R= Ac
2 R= H

Let 8 hours as t_1 and we have $2t_1 = 16$ hours. $S_1(=0.60)$ and $S_2(=0.36)$ were substituted into Eq. 9, then we have

$$x^2 - 1.20x + 0.36 = 0$$

We substitute the only root $x_1 = 0.6$ and S_1 into Eq. 10 and have $1/E = 1$. This result implies no enantioselectivity for this enzymatic catalytic reaction that is in good agreement with other experimental results: (1) when this reaction stopped at 50% conversion, there is no obvious optical rotation for the recovered substrate and product; (2)the isolated product, 6β-hydroxytropinone **2**, is with mp=120-121°C by comparing 120-121°C for authentic racemate and 129-130°C for the optically pure enantiomer.[11] Using the data in Table 1, we can plot a curve by ln*S* as a function of time *t* with good linearity,[12] which proved not only that in this reaction E equals indeed 1 but also that the fast- and slow-reacting enantiomers are governed by Eqs. 4 and 5 respectively.

The other example is the enzymatic catalytic esterification of racemic 2-octanol with vinyl acetate in the presence of PPL (lipase from *porcine pancreas*, Sigma). The reaction is monitored by GC. We have S_1=0.715 at t_1 (16 hours) and S_2=0.579 at $2t_1$ (32 hours), which were substituted into Eq. 9, then we have

$$x^2 - 1.430x + 0.443 = 0$$

Solving this equation we choose right root x_1=0.454. Substituting x_1 and S_1 into Eq. 10 gives E=32.5. The E-value calculated according to traditional Sih's method of this kinetic resolution is 28.2.[13]

We have developed a simple method for determination of enantiomeric ratio E of kinetic resolution only using c. It may be considered as an interesting alternative to evaluation of E. Since c can be readily determined by several means, from the experimental standpoint our method is easily to perform and it is very practical. We have successfully applied our approach to analysis of enantioselectivity of two lipasic reactions. The results are in good agreement with those of other experiments.

Note that the present method is not applicable to optically labile substrates and that the validity of application of this theory to practical systems should be proved further, which is under our current investigation.

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7. In order to discribe our method simply, we use the concentration of substrate $S[=(1-c)S_0]$ instead of c in this communication.
8. The present method can be simplified to two steps: (1) determining respectively S_1 and S_2 of a kinetic resolution at t_1 and at $2t_1$; (2) evaluating E-value by the equation:
$$E=\ln[S_1(1-(S_2/S_1^2-1)^{1/2})]/\ln[S_1(1+(S_2/S_1^2-1)^{1/2})]$$
9. To a solution of 50mg of (±)-**1** in 3mL heptane, in the presence of 50μL of *n*-BuOH, 25mg of CCL (*Candida cylindracea* lipase, Sigma) are added and the mixture is stirred at room temperature.
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12. Substituting $1/E$=1 and S_0=100% into Eq. 6 gives $\ln S=-kt$ which is a linear equation.
13. (±)-2-octanol is subjected to the lipasic esterification as discribed above (see ref. 9) for 2-octanol (5.2g), vinyl acetate (8mL), PPL (5g) and heptane (80mL). When the reaction is stopped at 36.6% conversion (monitored by GC), the recovered 2-octanol is with $[\alpha]_{546}^{20}$ = 5.90 (*c* 1, EtOH). The ee value (51.3%) of (+)-2-octanol is determined on the basis of the comparison of the observed rotation with the value of optically pure authentic sample (Fluka) $[\alpha]_{546}^{20}$ = 11.5 (*c* 1, EtOH).

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