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Presumption of Kinetic Constants by Simulation of A Production Process of Optical Isomers under Esterase Reaction

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We performed a site-specific and asymmetric hydrolysis of prochiral 2-phenyl-1,3-propanediol diacetate by a bacterial esterase. Several kinetic constants were presumed from the production experiment of *S*-form isomer and *R*-form isomer. Reaction rate constant (k) and K_m value (K^D_m) were 13.7 mole/g/h and 1.92 x 10^{-2} mole/l, respectively, from the experimental data. In this experiment, the hydrolysis by esterase followed first order reaction in order that the initial substrate concentration was very low. Reaction rate constants for *R*-form isomer production (k^R_{+1}) and *S*-form isomer production (k^S_{+2}) were 6.85 mole/g/h and 6.85 mole/g/h, respectively. When K_m values for hydrolysis of *R*-form isomer (K^R_m) and that of *S*-form isomer (K^S_m) were assumed to be 1.92 x 10^{-2} mole/l each, reaction rate constants for hydrolysis of *S*-form isomer (k^S_{+4}) and that of *R*-form isomer (k^R_{+3}) were 2.95 mole/g/h and 7.75 mole/g/h, respectively.

Keywords: Presumption of kinetic constants; Optical isomers; *S*-form; *R*-form; Esterase reaction.

Introduction

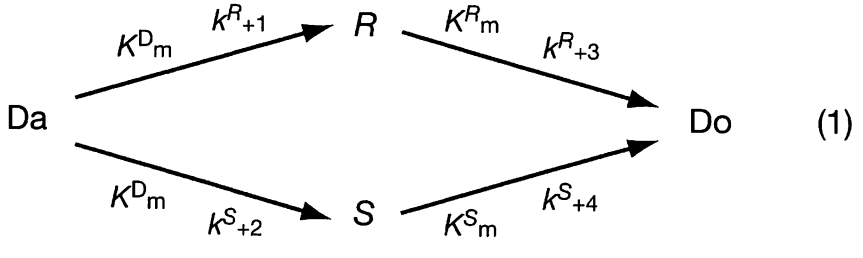
Optically pure 2-substituted-1,3-propanediol derivatives are useful chiral building blocks which are required for the synthesis of bioactive compounds. Therefore, we performed a site-specific and asymmetric hydrolysis of prochiral 2-phenyl-1,3-propanediol diacetate by a bacterial esterase (Ref. 1). And also, several kinetic constants were presumed from the production experiment of *S*-form and *R*-form.

1. Experimental

The hydrolysis by esterase was carried out as described previously (Ref. 1).

2. Theoretical consideration

The hydrolysis process to *R*-form (*R*) and *S*-form (*S*) as isomers and then to diol (Do) of diacetate (Da) by esterase assumes as follows.



(1)

For simplification, if each step of this reaction (Eq. (1)) is Michaelis-Menten type, and substrate and product inhibitions do not occur, the enzyme reaction rate is generally obtained from the following equation.

$$v = kE_0Ss/(K_m + Ss) \quad (2)$$

Where, E_0 is initial enzyme concentration, k is reaction rate constant, K_m is Michaelis constant, and Ss is

substrate concentration.

If the substrate concentration (Da) is very low compared with K_m value (K^D_m), Eq. (2) is expressed with Eq. (3).

$$v \fallingdotseq kE_0Ss/K_m \fallingdotseq kE_0Da/K_m \quad (3)$$

That is, this enzyme reaction proceeds under a first order reaction rate for the substrate. Therefore, reaction rate equation of each step is expressed as follows.

$$-dDa/dt = (k^R_{+1}/K^D_m + k^S_{+2}/K^D_m)E_0Da \quad (4)$$

$$dR/dt = k^R_{+1}E_0Da/K^D_m - k^R_{+3}E_0R/K^R_m \quad (5)$$

$$dS/dt = k^S_{+2}/K^D_mDa - k^S_{+4}E_0S/K^S_m \quad (6)$$

And also,

$$Do = (Da,o - Da) - (R + S) \quad (7)$$

Where, Da,o is initial substrate concentration.

When the equation (4), (5) and (6) are integrated sequentially, the following equations are obtained.

$$Da = Da,oExp(-(k^R_{+1} + k^S_{+2})E_0t/K^D_m) \quad (8)$$

$$R = (k^R_{+1}E_0^2Da,o/K^D_m)(k^R_{+3}/K^R_m - (k^R_{+1} + k^S_{+2})/K^D_m)Exp(-(k^R_{+1} + k^S_{+2})E_0t/K^D_m)$$
$$(1 - Exp(-(k^R_{+3}/K^R_m - (k^R_{+1} + k^S_{+2})/K^D_m)E_0t)) \quad (9)$$

$$S = (k^S_{+2}E_0^2Da,o/K^D_m)(k^S_{+4}/K^S_m - (k^R_{+1} + k^S_{+2})/K^D_m)Exp(-(k^R_{+1} + k^S_{+2})E_0t/K^D_m)$$
$$(1 - Exp(-(k^S_{+4}/K^S_m - (k^R_{+1} + k^S_{+2})/K^D_m)E_0t)) \quad (10)$$

3. Presumption of kinetic constants by simulation of experimental values

First, from the experimental data of substrate hydrolysis (Da), the hydrolysis rate constant (k) and Michaelis constant (K^D_m) must be presumed. Therefore, Eq. (2) was integrated, and the following equation was obtained.

$$V_m = kE_0 = (K_m/t)\ln(Ss/Ss,o) + (Ss - Ss,o)/t \quad (11)$$

Where, $k = k^R_{+1} + k^S_{+2}$, $k^R_{+1} = k^S_{+2}$, $K_m = K^D_m$, Ss,o = Da,o and Ss = Da. And E_0 and Da,o were 2.5 x 10^{-3} g/l and 0.01 mole/l (=10 mM), respectively, from the experimental data (Ref. 1).

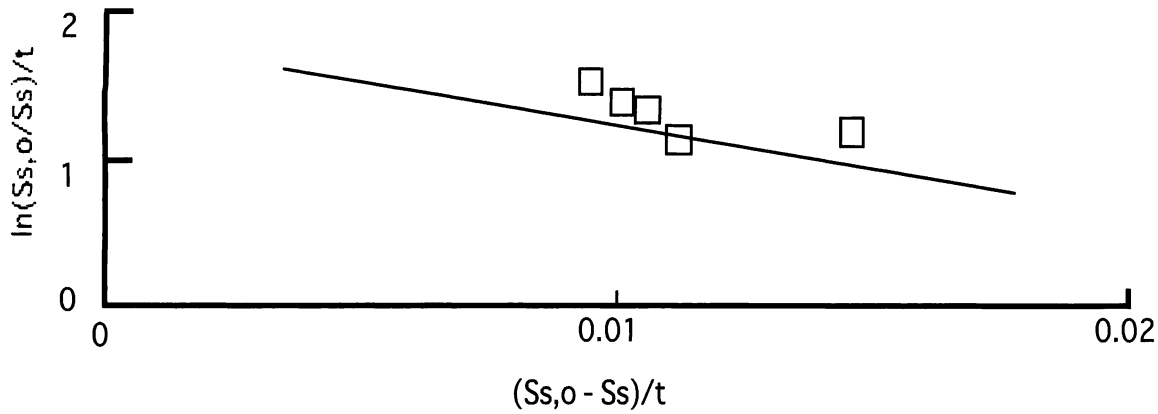


Fig. 1. Decision of kinetic constants (k and K_m (= K^D_m)) for hydrolysis of diacetate (Da).

As shown in **Figure 1**, from the relationship of ln(Sso/S)/t and (Ss,o - S)/t, k and K_m (= K^D_m) were about 13.7 mole/g/h and 1.92 x 10^{-2} mole/l, respectively.

Next, by using these values obtained experimentally, another kinetic constants must be presumed. For simplicity, Michaelis constants for *S*-form isomer (K^S_m) and *R*-form isomer (K^R_m) were assumed as equal to Michaelis constant for substrate (K^D_m). Hydrolysis rate constants for *S*-form isomer (k^S_{+4}) and *R*-form isomer (k^R_{+3}) were presumed as 2.95 mole/g/h and 7.75 mole/g/h, respectively.

Figure 2 is the result simulated as K^D_m = 1.92 x 10^{-2} mole/l, $k^R_{+1} = k^S_{+2}$ = 6.85 mole/g/h, and $K^R_m = K^S_m$ = 1.92 x 10^{-2} mole/l, k^R_{+3} = 7.75 mole/g/h and k^S_{+4} = 2.95 mole/g/h. Where, E_0 = 2.5 x 10^{-3} g/l, Da,o = 10 mM.

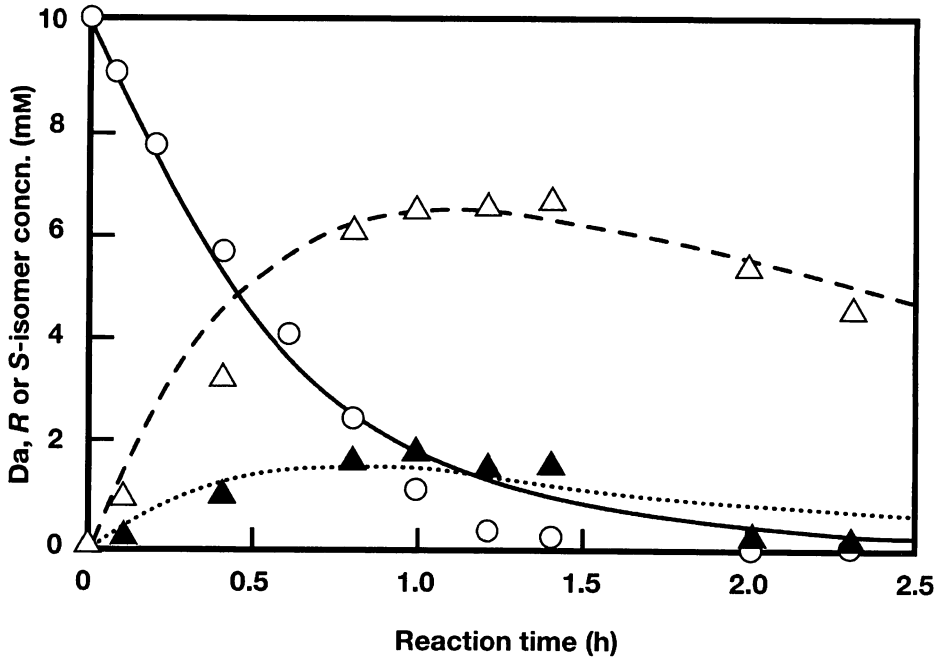


Fig. 2. Comparison with experimental values and result of simulation. Experimental values: ○; Concn. of substrate (Diacetate), △; Concn. of *S*-form isomer, ▲; Concn. of *R*-form isomer, Calculation result: Solid line; Concn. of Da, Broken line; Concn. of *S*-form isomer, Dotted line; Concn. of *R*-form isomer.

4. Discussion

In the case of $k^R_{+1} = k^S_{+2} = k^R_{+3} = k^S_{+4}$ = 6.85 mole/g/h, that is, all of the reaction rate constants are equal, if Michaelis constant for *R*-form isomer is 1.70 x 10^{-2} mole/l, and that for *S*-form isomer is 4.45 x 10^{-2} mole/l, similar simulation result can be obtained. Apparently from Eq. (2), if the reaction rate constant is the same value each other as the above case, in the case that Michaelis constant is lower, the reaction velocity must be higher. That is, the hydrolysis velocity of *S*-form isomer is lower than that of *R*-form isomer. This result shows that the affinity to *R*-form isomer of the esterase used is higher than that to *S*-form isomer. Those values seem to be suitable. Moreover, those Michaelis constants are not in conflict with the assumption of Eq. (3). Consequently, from data shown in Figure 1, those kinetic constants with a higher accuracy may not be obtained.

That is, the values of k^R_{+3}, k^S_{+4}, K^R_m and K^S_m are not decided unequivocally in order to the lack of experimental data. However, the values of k^R_{+3}/K^R_m and k^S_{+4}/K^S_m are, respectively, 4.04 x 10^2 l/g/h and 1.54 x 10^2 l/g/h unequivocally. Incidentally, k/K^D_m = 7.13 x 10^2 l/g/h.

Conclusions

The result of simulation is relatively good compatible with the experimental values. Therefore, esterase reaction in this experiment is first order reaction, and from the above theoretical consideration, kinetic constants of esterase used in experiment could be presumed. That is, Michaelis constant for hydrolysis of substrate (K_m= K^D_m) is 1.92 x 10^{-2} mole/l, reaction rate constant for hydrolysis of substrate (k) is 13.7 mole/g/h, $k^R_{+1} = k^S_{+2}$ = a half of k, Michaelis constant for hydrolysis of *R*-form isomer (K^R_m) and that of *S*-form isomer (K^S_m) is 1.92 x 10^{-2} mole/l, and reaction rate constant for hydrolysis of *R*-form isomer and that of *S*-form isomer is 7.75 mole/g/h and 2.95 mole/g/h, respectively.

Notation

Da ; Substrate concentration at arbitrary reaction time (mole/l).
Da,o ; Initial substrate concentration (mole/l)
Do ; Diol concentration at arbitrary reaction time (mole/l)
Eo ; Initial enzyme concentration (g/l)
Ss,o ; Initial substrate concentration (mole/l)
Ss ; Substrate concentration at arbitrary reaction time (mole/l)
t ; Time of hydrolysis (h)
R ; *R*- form isomer concentration at arbitrary time (mole/l)
S ; *S*- form isomer concentration at arbitrary time (mole/l)
v; Reaction velocity (mole/l/h)
V_m ; Maximum velocity of enzyme reaction (= kE_0) (mole/l/h)
K_m ; Michaelis constant (mole/l)
K^D_m ; Michaelis constant for hydrolysis of substrate (diacetate) (mole/l)
K^R_m ; Michaelis constant for hydrolysis of *R*- form isomer (mole/l)
K^S_m ; Michaelis constant for hydrolysis of *S*- form isomer (mole/l)
k ; Hydrolysis rate constant of substrate (mole/g/h)
k^R_{+1} ; Hydrolysis rate constant to *R*- form isomer (mole/g/h)
k^R_{+3} ; Hydrolysis rate constant to diol (mole/g/h)
k^S_{+2} ; Hydrolysis rate constant to *S*- form isomer (mole/g/h)
k^S_{+4} ; Hydrolysis rate constant to diol (mole/g/h)

References

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