Compensation Effect between Differential Activation Enthalpy and Entropy in Subtilisin-Catalyzed Kinetic Resolutions of Secondary Alcohols

Tadashi Ema,* Kunihiro Yamaguchi, Yuji Wakasa, Nobuaki Tanaka, Masanori Utaka, and Takashi Sakai*
Department of Applied Chemistry, Faculty of Engineering, Okayama University, Tsushima, Okayama 700-8530

(Received April 19, 2000; CL-000371)

Thermodynamic parameters for subtilisin-catalyzed kinetic resolutions of secondary alcohols were determined, and a compensation effect between $\Delta\Delta H^{\ddagger}$ and $\Delta\Delta S^{\ddagger}$ was found. These data are explained in terms of the transition-state model.

Lipases are abnormal enzymes in that they show high enantioselectivity and broad substrate specificity simultaneously unlike other enzymes.[1] In order to rationalize this abnormal feature observed in the lipase-catalyzed kinetic resolutions of secondary alcohols, we have proposed a stereo-sensing mechanism (Figure 1b).[2] In this transition-state model the enantioselectivity is explained by the conformational requirements and repulsive interactions, and any attractive binding interaction between enzyme's pockets and substrate's substituents is not involved. In this sense lipases are considered to be "chemical reagent-like".[3] Further study has revealed that such a concept can be applied to subtilisin Carlsberg (Figure 1c).[4] These transition-state models can rationalize the opposite empirical rules[4,5] as shown in Figure 1a. These transition-state models have been derived on the basis of MO calculations[2] and kinetic study,[2,4] and have been supported by the successful kinetic resolutions of a very large secondary alcohol having tetraphenylporphyrin as a substituent.[4,6] In this paper, we present the results of the thermodynamic study to gain a deeper insight into the origin of the enantioselectivity.

It has been reported that the thermodynamic parameters, $\Delta\Delta H^{\ddagger}$ and $\Delta\Delta S^{\ddagger}$, can be estimated according to equation 1,[7–9] where $\Delta\Delta H^{\ddagger} = \Delta H^{\ddagger}_{\text{fast}} - \Delta H^{\ddagger}_{\text{slow}}$ and $\Delta\Delta S^{\ddagger} = \Delta S^{\ddagger}_{\text{fast}} - \Delta S^{\ddagger}_{\text{slow}}$.

$$\ln E = -\Delta\Delta H^{\ddagger}/(RT) + \Delta\Delta S^{\ddagger}/R \qquad (1)$$

In the case of the lipase-catalyzed kinetic resolution of secondary alcohols, the E values[10] are too high in many cases, which is inappropriate for this purpose. By contrast, subtilisin shows low to moderate enantioselectivities,[4] which allows us to determine the reliable E values at a range of temperatures. Therefore, we employed subtilisin Carlsberg (ChiroCLEC-BL, Altus Biologics Inc.) as a biocatalyst, and carried out the kinetic resolutions of **1**–**10** (Chart 1) with vinyl acetate in dry *i*-Pr_2O at 10–40 °C. In all cases except for **10**, the (*S*)-enantiomers reacted faster. The thermodynamic parameters calculated according to equation 1 are listed in Table 1. The accuracy of the data is relatively high despite the heterogeneous reactions because the E values were determined under the competitive reaction conditions.

In most cases, the $\Delta\Delta H^{\ddagger}$ value contributes predominantly to the E value. In other words, enantiomer discrimination is enthalpy-driven. There is also a tendency that the $\Delta\Delta H^{\ddagger}$ value decreases as the two substituents of the secondary alcohols are more unbalanced in bulkiness (Table 1). In terms of the transition-state models, the $\Delta\Delta H^{\ddagger}$ value can be regarded as the difference in degree of repulsive interactions with the enzyme

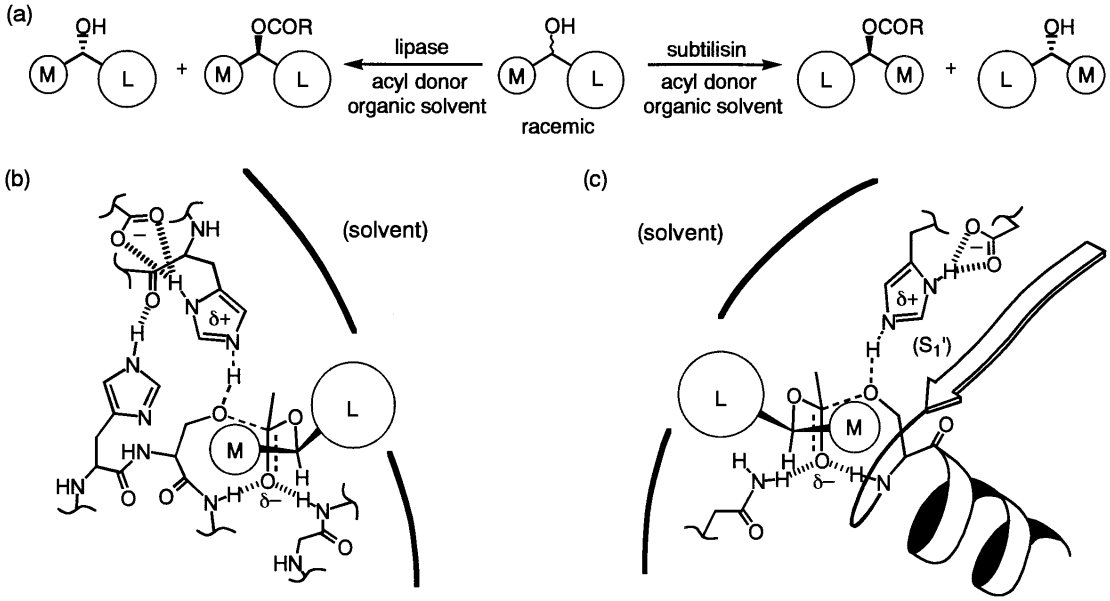

Figure 1. (a) Empirical rules for the lipase- and subtilisin-catalyzed kinetic resolutions of secondary alcohols. (b) Transition-state model for the lipase-catalyzed kinetic resolutions of secondary alcohols, where the faster-reacting enantiomer is shown. For details, see Ref. 2, 6. (c) Transition-state model for subtilisins. For details, see Ref. 4.

Chart 1.

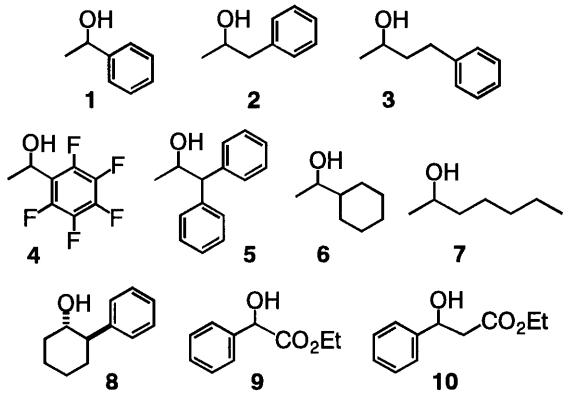

Table 1. Thermodynamic parameters for subtilisin-catalyzed kinetic resolutions of **1–10** in *i*-Pr_2O

Alcohol	$\Delta\Delta H^{\ddagger}$ [a]	$\Delta\Delta S^{\ddagger}$ [b]	$\Delta\Delta G^{\ddagger}$ [c]
1	–0.21 ± 0.33	+4.0 ± 1.1	–1.4
2	–3.0 ± 0.5	–2.4 ± 1.7	–2.3
3	–3.9 ± 0.3	–6.2 ± 0.9	–2.0
4	–5.2 ± 0.6	–9.3 ± 2.1	–2.4
5	–2.9 ± 0.4	–6.0 ± 1.4	–1.1
6	–0.50 ± 0.30	+2.4 ± 1.0	–1.2
7	–1.5 ± 0.1	+0.32 ± 0.44	–1.6
8	–6.3 ± 1.3	–14 ± 4	–2.1
9	–2.4 ± 0.6	–4.2 ± 2.1	–1.1
10	–1.2 ± 0.5	–2.1 ± 1.7	–0.6

[a]In kcal mol^{-1}. [b]In cal K^{-1} mol^{-1}. [c]In kcal mol^{-1}. Calculated from $\Delta\Delta G^{\ddagger} = \Delta\Delta H^{\ddagger} - 303\Delta\Delta S^{\ddagger}$.

between the enantiomers. Therefore, that tendency observed for the $\Delta\Delta H^{\ddagger}$ values is reasonable because the difference in degree of repulsive interactions between the enantiomers will increase as the two substituents of the secondary alcohols are more unbalanced in bulkiness.

Interestingly, we found a linear relationship between the $\Delta\Delta H^{\ddagger}$ and $\Delta\Delta S^{\ddagger}$ values for **1**–**10** (Figure 2). As the $\Delta\Delta H^{\ddagger}$ value becomes negatively large, the $\Delta\Delta S^{\ddagger}$ value also becomes negatively large.[11] Equation 1 indicates that a negative $\Delta\Delta H^{\ddagger}$ value contributes to an increase in *E* value whereas a negative $\Delta\Delta S^{\ddagger}$ value contributes to a decrease in *E* value. This partial compensation effect can be understood by considering repulsive interactions between the slower–reacting enantiomer and the enzyme. In general, although repulsive interactions are unfavorable in terms of enthalpy, they are favorable in terms of entropy because the degree of disorder (or freedom) increases. Because the degree of disorder of the amino acid residues and the substrate moiety will increase with an increase in degree of repulsive interactions, the entropy gain will increase proportionally with an increase in enthalpy loss. This opposite but proportional relationship between enthalpy and entropy can remain even after the $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values for the slower-reacting enantiomer are subtracted from the corresponding values for the faster-reacting enantiomer. Accordingly, the observed compensation effect can be explained by the transition-state model, and the thermodynamic data reported here help us understand what

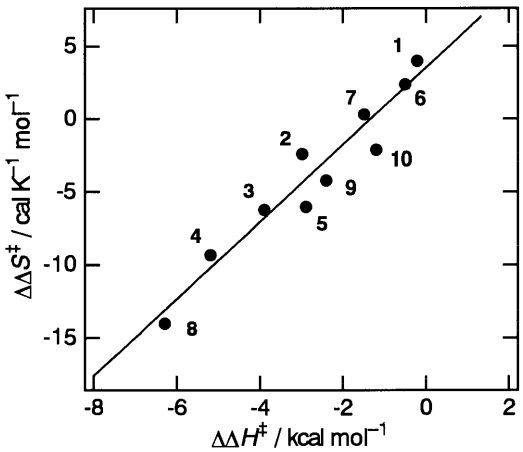

Figure 2. Correlation plot for $\Delta\Delta H^{\ddagger}$ and $\Delta\Delta S^{\ddagger}$ values of **1–10**.

is going on in the transition state.

We are grateful to the SC-NMR Laboratory of Okayama University for the measurement of the NMR spectra. This work was financially supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

References and Notes

1 For example: a) "Enzymatic Reactions in Organic Media," ed. by A. M. P. Koskinen and A. M. Klibanov, Blackie Academic, Glasgow (1996). b) U. T. Bornscheuer and R. J. Kazlauskas, "Hydrolases in Organic Synthesis," Wiley-VCH, Weinheim (1999).

2 T. Ema, J. Kobayashi, S. Maeno, T. Sakai, and M. Utaka, *Bull. Chem. Soc. Jpn.*, **71**, 443 (1998).

3 In the field of synthetic organic chemistry, many transition-state models have been proposed for various stereoselective chemical reactions. R. E. Gawley and J. Aubé "Principles of Asymmetric Synthesis," Elsevier, Oxford (1996).

4 T. Ema, R. Okada, M. Fukumoto, M. Jittani, M. Ishida, K. Furuie, K. Yamaguchi, T. Sakai, and M. Utaka, *Tetrahedron Lett.*, **40**, 4367 (1999).

5 R. J. Kazlauskas, A. N. E. Weissfloch, A. T. Rappaport, and L. A. Cuccia, *J. Org. Chem.*, **56**, 2656 (1991).

6 T. Ema, M. Jittani, T. Sakai, and M. Utaka, *Tetrahedron Lett.*, **39**, 6311 (1998).

7 R. S. Phillips, *Trends Biotechnol.*, **14**, 13 (1996).

8 T. Sakai, I. Kawabata, T. Kishimoto, T. Ema, and M. Utaka, *J. Org. Chem.*, **62**, 4906 (1997).

9 T. Sakai, T. Kishimoto, Y. Tanaka, T. Ema, and M. Utaka, *Tetrahedron Lett.*, **39**, 7881 (1998).

10 C.-S. Chen, Y. Fujimoto, G. Girdaukas, and C. J. Sih, *J. Am. Chem. Soc.*, **104**, 7294 (1982).

11 Similar linear relationships have been observed for chiral recognition and asymmetric photochemical reactions. a) K. Naemura, K. Matsunaga, J. Fuji, K. Ogasahara, Y. Nishikawa, K. Hirose, and Y. Tobe, *Anal. Sci.*, **14**, 175 (1998). b) R. Hoffmann and Y. Inoue, *J. Am. Chem. Soc.*, **121**, 10702 (1999).