are formed, all having remarkably similar stereochemistires. It is apparent therefore, that all of these reaction sequences are proceeding under thermodynamic control. The controlling factor is the formation of an intramolecular hydrogen bond between the N-H functionality and the coordinated halide moiety. Although the stereochemical control exerted by this type of hydrogen bonding is quite unusual, another result of this interaction is the stabilization of otherwise unstable metal hydrides. Thus the iridium(III) amine-trihydride complex **15** is stable in solution only when excess H_2 is present; loss of H_2 to generate the amide-dihydride $IrH_2[N(SiMe_2CH_2PPh_2)_2]$ occurs readily. However, upon substitution of one of the hydrides of **15** by a halide (eq 6), the resulting amine-dihydrides do not lose H_2 under vacuum even when heated to 80 °C. Even more remarkable is the corresponding rhodium chemistry. The rhodium(I) cyclooctene complex **12** is apparently unreactive to H_2 (1-4 atm) in aromatic or hydrocarbon solvents; after extended periods (several months), only decomposition to rhodium metal is observed. However, in chlorinated solvents, the reaction of H_2 with **12** generates the amine-dihydride complex **14**, which is stabilized by the presence of the intramolecular hydrogen bond.

All of these transformations involving H_2 are clearly quite complex; mechanistic investigations into these processes are currently in progress.

Acknowledgment. We thank NSERC and the Alfred P. Sloan Foundation for financial support. Johnson-Matthey is greatfully acknowledged for generous loans of $RhCl_3$ and $IrCl_3$. We also thank Professor James Trotter for the use of the diffractometer and structure solution programs.

Supplementary Material Available: Tables of calculated hydrogen coordinates and isotropic thermal parameters, final anisotropic thermal parameters, torsion angles, and intramolecular X···H interactions (14 pages); tables of observed and calculated structure factor amplitudes (102 pages). Ordering information is given on any current masthead page.

Quantitative Analyses of Biochemical Kinetic Resolution of Enantiomers. 2. Enzyme-Catalyzed Esterifications in Water-Organic Solvent Biphasic Systems

Ching-Shih Chen, Shih-Hsiung Wu, Gary Girdaukas, and Charles J. Sih*

Contribution from the School of Pharmacy, University of Wisconsin, Madison, Wisconsin 53706.
Received September 26, 1986

Abstract: New quantitative expressions, which relate the kinetic and thermodynamic parameters that govern the stereospecificity of enzyme-catalyzed resolution of enantiomers in biphasic aqueous-organic media, have been developed. The theoretical predictions have been verified experimentally, and the technical merit of this esterification methodology has been assessed in relation to the more conventional hydrolytic procedure.

Hydrolytic enzymes are valuable chiral catalysts for the resolution of racemic alcohols[1] and carboxylic acids[2] via enantiospecific hydrolyses of the corresponding esters. The reactions are usually conducted in aqueous media where the equilibrium position of the reaction is markedly shifted toward hydrolysis. Recently, the use of hydrolytic enzymes as catalysts for preparative ester synthesis has attracted considerable attention.[3] By replacing water with a biphasic aqueous-organic solvent reaction medium, the water activity is lowered and the thermodynamic equilibrium of the reaction is now shifted toward the synthetic direction.[4] Further, if the product(s) has good solubility in the organic phase and poor solubility in the aqueous phase while the reactants have the opposite solubility behavior, the reaction is shifted even further toward esterification. Several types of hydrolytic enzymes (proteases,[5] amidases,[6] lipases,[1,7] etc.) have now been shown to catalyze stereospecific condensation reactions in such aqueous-organic biphasic milieus. In particular, microbial lipases (EC 3.1.1.3) have been widely used for the resolution of racemic alcohols through enantiospecific esterifications.[1,7] These enzymes are relatively stable to nonpolar organic media and catalyze reactions efficiently at the lipid-water interface. However, the underlying physicochemical principles that govern enantiospecific esterification in biphasic systems have not yet been delineated.

In a previous paper,[8] we developed equations and useful graphs for the systematic treatment of kinetic resolution data of hydrolase-catalyzed irreversible reactions in water. As an extension of this work, we herein introduce new quantitative expressions to relate the kinetic and thermodynamic parameters that determine the stereospecificity of enzyme-catalyzed resolution of enantiomers in biphasic aqueous-organic media. Our theoretical predictions have been experimentally verified, and the technical merit of this esterification methodology can now be compared to the more conventional hydrolytic procedure.

Theory

In a normal hydrolytic reaction, water is also a substrate. Its high relative concentration (55.5 M) drives the reaction toward

(1) Langrand, G.; Baratti, J.; Buono, G.; Triantaphylides, C. *Tetrahedron Lett.* **1986**, *27*, 29.
(2) Gu, Q. M.; Chen, C. S.; Sih, C. J. *Tetrahedron Lett.* **1986**, *27*, 1763.
(3) (a) Kasche, V. *Enzyme Microb. Technol.* **1986**, *8*, 4. (b) Brink, L. E. S.; Tramper, J. *Biotech. Bioeng.* **1985**, *27*, 1258. (c) Halling, P. J. *Enzyme Microb. Technol.* **1984**, *6*, 513. (d) Carrea, G. *Trends Biotechnol.* **1984**, *2*, 102.
(4) Martinek, K.; Semenov, A. N.; Berezin, I. V. *Biochim. Biophys. Acta* **1981**, *658*, 76.
(5) (a) Fruton, J. S. *Adv. Enzymol. Rel. Areas Mol. Biol.* **1982**, *53*, 239. (b) Oyama, K.; Irino, S.; Harada, T.; Hagi, N. *Ann. N.Y. Acad. Sci.* **1984**, *434*, 95. (c) Markussen, J.; Vølund, A. In *Enzymes in Organic Synthesis*; Pitman: London, 1985; p 188.
(6) Kasche, V.; Haufler, U.; Riechmann, L. *Ann. N.Y. Acad. Sci.* **1984**, *434*, 99.
(7) (a) Langrand, G.; Secci, M.; Buono, G.; Baratti, J.; Triantaphylides, C. *Tetrahedron Lett.* **1985**, *26*, 1857. (b) Koshiro, S.; Sonomoto, K.; Tanaka, A.; Fukui, S. *J. Biotechnol.* **1985**, *2*, 47. (c) Kirchner, G.; Scollar, M. P.; Klibanov, A. M. *J. Am. Chem. Soc.* **1985**, *107*, 7072.
(8) Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294.

0002-7863/87/1509-2812$01.50/0

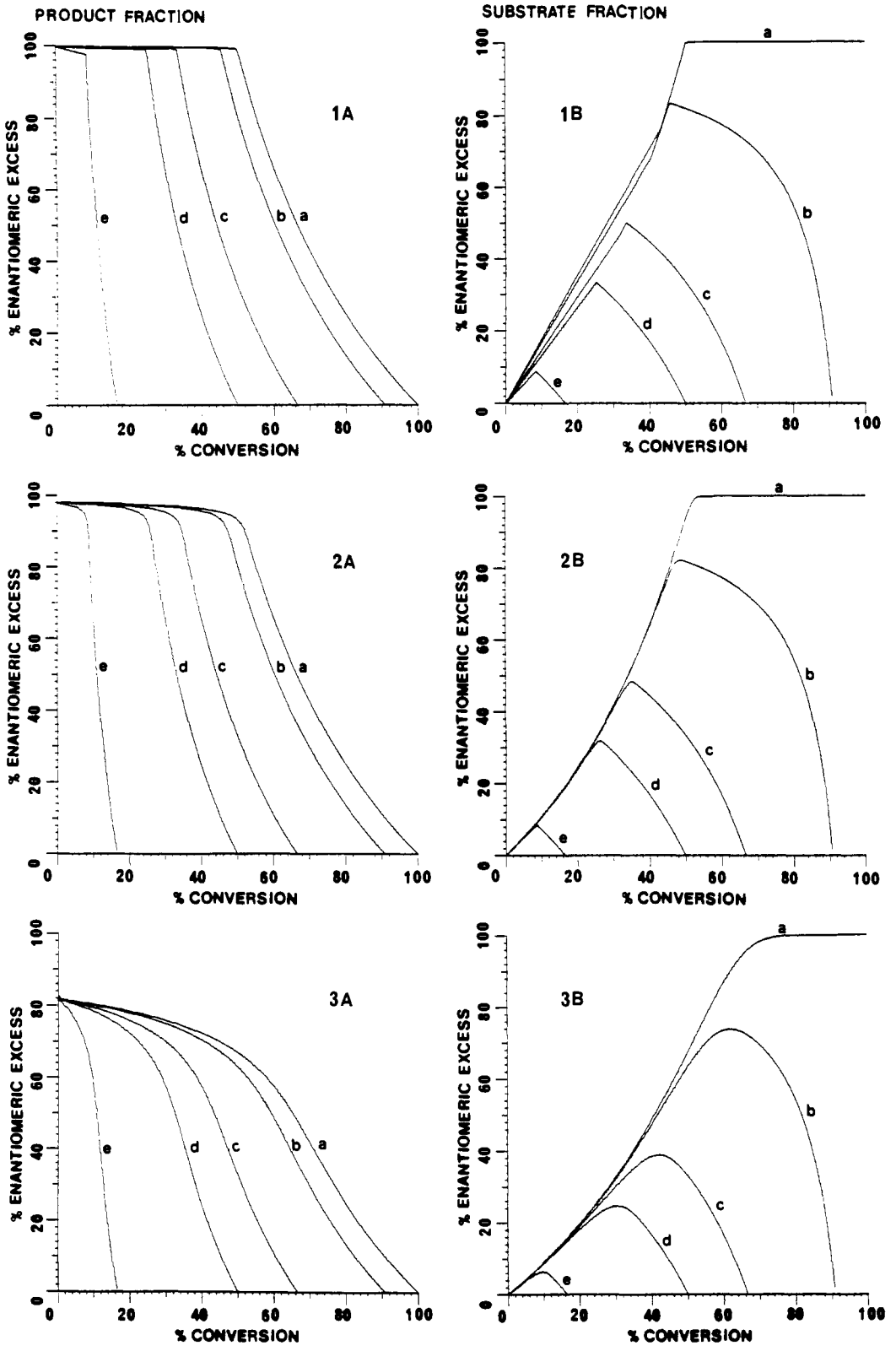


Figure 1. Expression of the percentage enantiomeric excess (ee) of product (A) and substrate (B) fractions as a function of the percentage conversion at different values of E and K. These curves were computer generated[10] from eq 7 and 8. The values of E were (1) 1000, (2) 100, and (3) 10. The values of K were (a) 0, (b) 0.1, (c) 0.5, (d) 1, and (e) 5.

completion at equilibrium. However, for enzyme-catalyzed condensation reactions in biphasic systems (water–water immiscible organic solvent), the reactions are reversible. Hence, the rate constants for both the forward and the reverse reactions must be considered. In the presence of an excess quantity of acyl donor or acceptor ($>10\ K_m$), the scheme for either esterification or transesterification (ester interchange) may be envisaged as follows:

$$\text{Enz} + \text{A} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{Enz} + \text{P}$$

$$\text{Enz} + \text{B} \underset{k_4}{\overset{k_3}{\rightleftharpoons}} \text{Enz} + \text{Q}$$

where A,B and P,Q are pairs of enantiomers; k_1, k_3 and k_2, k_4 denote the apparent pseudo-first-order rate constants (V_{max}/K_m)

Table I. Effect of Acyl Donors and Reaction Media on Enantiospecificity (E) and Equilibrium Constant (K) in the Enzyme-Catalyzed Resolution of (±)-**1**[a]

	reaction medium											
	isooctane						H_2O					
acyl donor	k_1	k_2	k_3	k_4	E	K	k_1	k_2	k_3	k_4	E	K
$CH_3(CH_2)_4CO_2H$	1.8	1	*b*	*b*	>100	0.56	*c*	*c*	*c*	*c*	*c*	*c*
$CH_3(CH_2)_{10}CO_2H$	108	3.6	1	0.035	108	0.035	22	6	1	0.26	22	0.26
$C_6H_5(CH_2)_4CO_2H$	5.3	1	*b*	*b*	>100	0.19	210	68	1	0.25	210	0.27

[a] k_1, k_2, k_3, and k_4 are the apparent relative rate constants for the forward and reverse reactions. [b] Undetectable. [c] Not determined.

for the forward and reverse reactions, respectively. As the enzyme only speeds up the attainment of equilibrium but does not change the position of equilibrium, the equilibrium constant (K) depends only on the initial and final states and is independent of the reaction pathway. Therefore, the equilibrium constants for the pair of enantiomers in achiral solvent media should be equal (eq 1). It is readily apparent from eq 1 that when $k_2 > k_4$, $k_1 > k_3$.

$$K = \frac{k_2}{k_1} = \frac{k_4}{k_3} = \frac{A}{P} = \frac{B}{Q} \tag{1}$$

This relationship reveals that *the sense of chirality for the forward (esterification) and reverse (hydrolytic) reactions is retained.* That is, if A is the fast reacting enantiomer in the forward reaction, P must be the fast reacting enantiomer in the reverse reaction.

The net rate for the two competing enantiomers, A and B, prior to attaining equilibrium is described by the following equations

$$-\frac{dA}{dt} = k_1A - k_2P = (k_1 + k_2)A - k_2A_0 \tag{2}$$

$$-\frac{dB}{dt} = k_3B - k_4Q = (k_3 + k_4)B - k_4B_0 \tag{3}$$

where $A_0 = A + P$; $B_0 = B + Q$. Combining eq 2 and 3 yields,

$$\frac{dA}{dB} = \frac{(k_1 + k_2)A - k_2A_0}{(k_3 + k_4)B - k_4B_0} \tag{4}$$

Integration of eq 4 affords eq 5, which may be reduced to eq 6.

$$\frac{\ln\left[1 - \left(1 + \frac{k_2}{k_1}\right)\left(1 - \frac{A}{A_0}\right)\right]}{\ln\left[1 - \left(1 + \frac{k_4}{k_3}\right)\left(1 - \frac{B}{B_0}\right)\right]} = \frac{k_1}{k_3}\left[\frac{1 + \frac{k_2}{k_1}}{1 + \frac{k_4}{k_3}}\right] \tag{5}$$

$$\frac{\ln\left[1 - (1 + K)\left(1 - \frac{A}{A_0}\right)\right]}{\ln\left[1 - (1 + K)\left(1 - \frac{B}{B_0}\right)\right]} = E \tag{6}$$

where

$$E = k_1/k_3;\ K = k_2/k_1 = k_4/k_3$$

When k_2 and $k_4 = 0$, eq 6 is reduced to the familiar homocompetitive eq 7 for the irreversible case;[8] the constant E is the enantiomeric ratio, which has been defined previously.

$$\ln\left(\frac{A}{A_0}\right) \Big/ \ln\left(\frac{B}{B_0}\right) = E \tag{7}$$

According to the principle of microscopic reversibility,[9] reversible reactions must proceed through the same transition state(s) in both directions. Consequently, *if the reaction conditions for the forward and reverse reactions are identical, the enantiomeric ratios or E values for the forward reaction* (k_1/k_3) *and the reverse reaction* (k_2/k_4) *must be the same*, in accordance with the predictions of eq 1. As shown in eq 6, when the reactions are reversible, the discrimination of the two competing enantiomers (A and B) by the enzyme is governed by the values of E and K. It is noteworthy that E is a kinetic parameter whose value will vary with different catalysts, whereas K is a thermodynamic function that is independent of the properties of enzymes. However, both E and K are sensitive to environmental changes such as the water content of the medium, species of acyl donor and acceptor, pH, temperature, etc. Because the enantiospecificity of enzyme-catalyzed synthesis depends on the complex interaction of both kinetic and thermodynamic functions, it is difficult to predict a priori the stereochemical behavior of such systems.

To correlate the extent of conversion (c) with the enantiomeric excess of substrate (ee_S) and product (ee_P) fractions, eq 6 was transformed into the following equations

$$\frac{\ln\left[1 - (1 + K)(c + ee_S\{1 - c\})\right]}{\ln\left[1 - (1 + K)(c - ee_S\{1 - c\})\right]} = E \tag{8}$$

$$\frac{\ln\left[1 - (1 + K)c(1 + ee_P)\right]}{\ln\left[1 - (1 + K)c(1 - ee_P)\right]} = E \tag{9}$$

where

$$c = 1 - \frac{A + B}{A_0 + B_0};\ ee_S = \frac{B - A}{A + B};\ ee_P = \frac{P - Q}{P + Q}$$

The theoretical curves[10] (Figure 1), generated from eq 8 and 9, provide a useful overview of the interrelationships between the variables c, ee_S, and ee_P for fixed values of E and K. These computer-generated graphs show that a small increase in the value of K has a very pronounced effect on the optical purity of the substrate and product fractions even for a system with a very high E value. For example, for an enzyme system with an E value of 1000 and a K value of 0.1 (Figure 1, 1Ab, 1Bb), maximal optical purity attainable for the substrate (ee_S) is 0.82 at 48% conversion. The values of ee_S and ee_P drop precipitously if the conversion is extended further. For a biochemical system with an E value of 100 and K values ranging from 0 to 1 (Figure 1, 2A, 2B), the ee_S and ee_P at 50% conversion are as follows: 0.95 ($K = 0$); 0.81 ($K = 0.1$); 0.33 ($K = 0.5$); and 0 ($K = 1$). It is evident that the optical purity of both fractions is inversely related to the magnitude of K.

For the reversible systems, the kinetics of enzymic resolution may be visualized to occur as follows: at the initial stages of the reaction, the enzyme preferentially attacks the fast reacting enantiomer A and transforms it to P. The high enantiospecificity results from a large difference in the net rates of the two competing reactions. As the reaction for the fast reacting enantiomer approaches equilibrium, the net rate (A ⇄ P) gradually diminishes, while the concentration of the slow reacting enantiomer, B, is still largely unchanged. When the equilibrium of the fast reacting enantiomer is established (net rate = 0), the optical purity of the substrate (ee_S) and the product (ee_P) fractions begin to fall due to the concentration changes of the slow-reacting enantiomer.

In enzymic kinetic resolution of enantiomers when the two competing reactions are irreversible, the optical purity of the substrate (ee_S) and product (ee_P) fractions is dependent on the

(9) Burwell, R. L., Jr.; Pearson, R. G. *J. Phys. Chem.* **1966**, *70*, 300.

(10) Figure 1 was computer generated by relating the variables c, ee_S, and ee_P in eq 7 and 8 to a function of x for values of $0 \le x < 1$; $c = (2 - 3x + x^E)/[2(1 + K)]$; $ee_S = (x - x^E)/(2K - 3x + x^E)$; $ee_P = (x^E - x)/(2 - 3x + x^E)$. Implicit functions (eq 7 and 8) were solved by parametric representations. See: Buck, R. C. In *Advanced Calculus*, 2nd ed.; McGraw-Hill: New York, 1965; p 313. Copies of the computer program are available upon request.

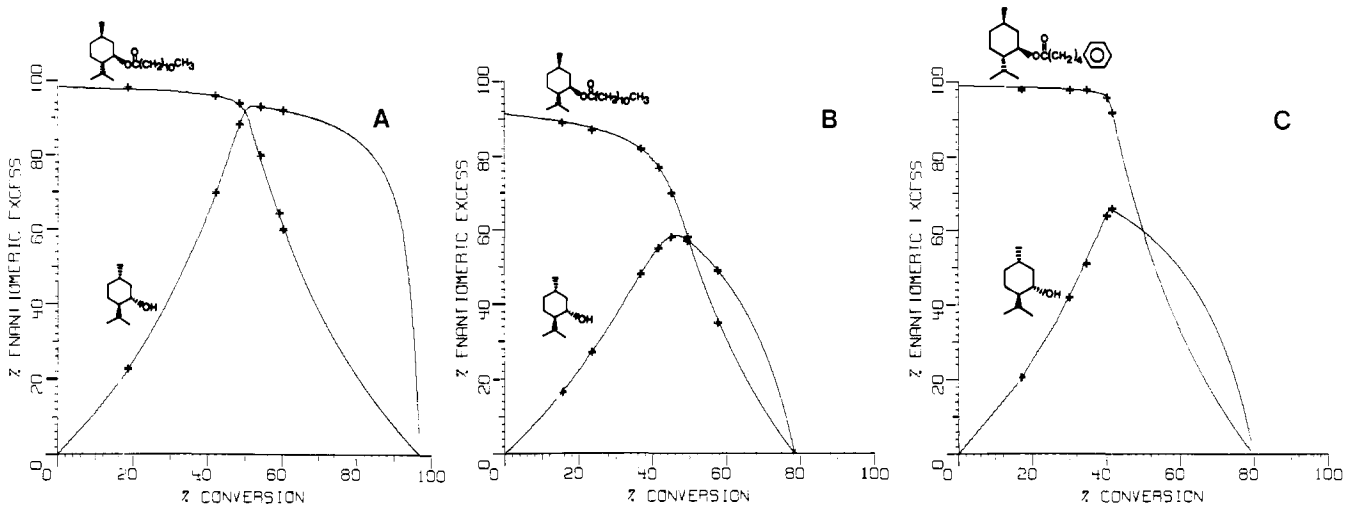


Figure 2. Enzyme-catalyzed enantiospecific synthesis of menthyl esters in different reaction media. # = experimentally determined values. The curves depicting the relationship between ee and c were computer generated from eq 7 (substrate) and 8 (product) with use of the apparent constants (E and K) listed in Table I. (A) Formation of menthyl laurate in isooctane; (B) formation of menthyl laurate in phosphate buffer; (C) formation of menthyl phenylvalerate in phosphate buffer.

Table II. Effect of Acyl Acceptors and Reaction Media on Enantiospecificity (E) and Equilibrium Constant (K) in the Enzyme-Catalyzed Resolution of (±)-**2**[a]

	reaction medium											
	isooctane						H_2O					
acyl acceptor	k_1	k_2	k_3	k_4	E	K	k_1	k_2	k_3	k_4	E	K
$CH_3(CH_2)_2CH_2OH$	12	2	1	0.15	12	0.15	1	4	*b*	*b*	>100	4
$C_6H_{11}OH$	80	1.6	1	0.02	80	0.02	1	1.8	*b*	*b*	>100	1.8

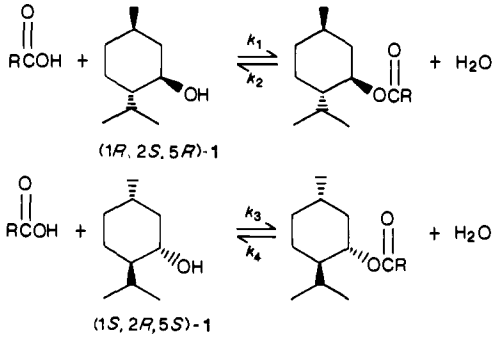
[a] k_1, k_2, k_3, and k_4 are the apparent relative rate constants for the forward and reverse reactions. [b] Undetectable.

extent of conversion, c, and the enantiomeric ratio, E. However, for reversible biocatalytic systems, ee_S and ee_P are dependent on an additional parameter, the equilibrium constant, K, besides c and E. Consequently, *high optical purity of the remaining substrate* ($ee_S \geq 0.98$) *cannot be attained by extending the conversion much beyond 50%* (compare curves, $K = 0$ vs. $K > 0$ in Figure 1). These graphical representations (Figure 1) allow one to predict precisely when to stop the kinetic resolution to maximize chemical and optical yields after the values of E and K have been defined. The optimum time to terminate the resolution is when the equilibrium of the fast-reacting enantiomer is reached. Obvioulsy, to obtain highly enantiospecific esterification, it is imperative to keep the water content in the organic medium to a minimum to diminish the reverse hydrolytic process.

Results

As the published experimental data on enzyme-catalyzed enantiospecific esterification in biphasic media are rather fragmentary, it was necessary to collect more quantitative data to verify our theoretical predictions. The results of our resolution studies using *dl*-menthol [(±)-**1**] and *dl*-2-(*p*-chlorophenoxy)propionic acid [(±)-**2**] as model substrates and a commercial *Candida cylindracea* lipase (free from contaminating hydrolases) as the biocatalyst in biphasic media are described.

Enantiospecific Esterification. (a) Kinetic Resolution of (±)-1.



Three acyl donors, hexanoic, lauric, and phenylvaleric acids, were chosen for this series of resolution experiments. Each of them was separately exposed to *C. cylindracea* lipase in the presence of (±)-**1** in two reaction media with varying amounts of water: phosphate buffer, pH 7.0, and isooctane saturated with phosphate buffer, pH 7.0. At various intervals, the values of ee_S and ee_P were determined from which c may be calculated by using the equation $c = ee_S/(ee_S + ee_P)$.[8] In turn, from the experimental values of c, ee_S, and ee_P, the kinetic (E) and thermodynamic (K) parameters were calculated and are listed in Table I. The (−)-1*R*,2*S*,5*R* isomer of **1** was preferentially attacked by this lipase. This observation and the experimental values are in good agreement with not only the data reported by other investigators[7a,b] but also the data coincided well with the theoretical curves generated for the values of E and K with use of eq 8 and 9 (Figure 2A–C).

The observed E values for the same system in the two reaction media are clearly different (Table I). This variance may be rationalized by the notion that the enzyme undergoes different conformational changes in different environments. As a consequence, the relative catalytic efficiencies (k_{cat}/K_m) of the enzyme toward competing substrates in different media will vary.

The optical purity and the yield of the ester formed depend markedly on the acyl donor used (Table I). It is to be expected that different rates of enzymatic acylation and deacylation are observed with different acyl donors.[7b] Further, the physical property of the acyl donor has a significant influence on the equilibrium constant, K. For example, when hexanoic acid was employed as the acyl donor in the isooctane–water medium, methyl hexanoate of high optical purity (ee = >0.98) was formed but the maximal obtainable yield of the ester was only 32% for a K value of 0.56. In contrast, phenylvaleric acid was a more efficient acyl donor, because menthyl phenylvalerate is more soluble in the organic phase. The position of equilibrium is shifted to a K value of 0.19, and a 42% recovery of the ester was obtained. When the water content of the medium is increased, the rate of the reverse hydrolytic process is not only enhanced but also the value of K is raised as illustrated by the two examples of menthyl laurate synthesis (Figure 2A–B).

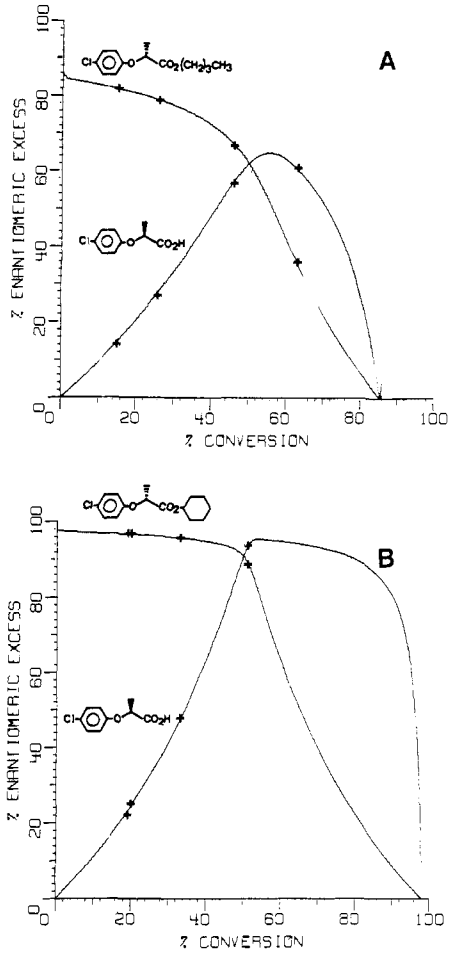
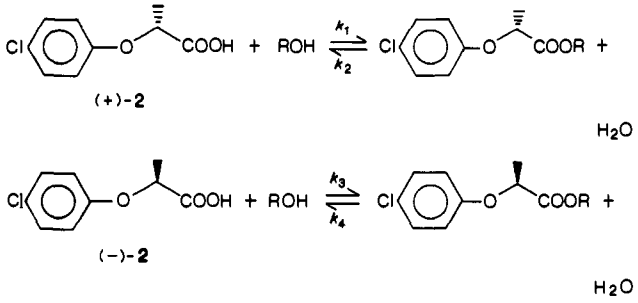


Figure 3. Enantiospecific synthesis of 2-(*p*-chlorophenoxy)propionic esters in isooctane catalyzed by *C. cylindracea* lipase. # = experimentally determined values. The curves depicting the relationship between ee and *c* were computer generated from eq 7 and 8 with use of the apparent constants (*E* and *K*) listed in Table II. (A) Formation of butyl 2-(chlorophenoxy)propionate; (B) formation of cyclohexyl 2-(chlorophenoxy)propionate.

(b) Enzymatic Resolution of (±)-2. The enzymatic kinetic resolution of (±)-**2** by *C. cylindracea* lipase was carried out with



butanol or cyclohexanol as the acyl acceptor. The reaction media again were isooctane saturated with phosphate buffer, pH 7.0, and phosphate buffer, pH 7.0. The enzyme preferentially attacked the *R* enantiomer [(+)-**2**], and the values of *E* and *K* calculated from the experimental results under various conditions are listed in Table II. It is apparent that the relationships of ee_S and ee_P to *c* are consistent with the prediction of eq 8 and 9 (Figure 3). Cyclohexanol is a better acyl acceptor than butanol in terms of enantiospecificity. In contrast to the results obtained for the menthyl ester formation, the reactions carried out in aqueous buffer gave higher enantiospecificity than those conducted in aqueous–organic solvent mixture (Table II). However, because of the unfavorable equilibrium in water, low product yield was obtained (10 and 18% for butanol and cyclohexanol, respectively).

Discussion

Hydrolytic enzymes (esterases, lipases) are usually used in classic hydrolytic reactions for the kinetic resolution of enantiomers. When the reaction is irreversible, the enantiomeric ratio, *E*, may be calculated from the values of ee_S and ee_P with use of the homocompetitive eq 7. This relationship also holds for lipases, which catalyze reactions at the lipid–water interface because the surface active properties of enantiomers are identical. Hence, the net penetration rate of the enzyme into the interface is not affected by the changes in the relative concentrations of the enantiomers.[11] The *E* value is a quantitative index of enantiospecificity, independent of the substrate and enzyme concentrations and the extent of conversion (*c*).

When hydrolases are used for kinetic resolution via enantiospecific esterifications in aqueous–organic biphasic media, the reactions are reversible. Hence a new expression, which incorporates the important thermodynamic parameter *K*, is required for the calculation of *E* (eq 6). The equilibrium constant, *K*, is directly related to the water content of the medium and determines the maximal obtainable chemical yield [$c = 1/(1 + K)$]. Although there is a general tendency to assume that if no exogenous water is added to the organic phase, reverse hydrolysis becomes negligible; in reality, the small quantity of residual water normally present in the enzymic protein and the water generated as the product of esterification are sufficient to serve as substrate for reverse hydrolysis.[7a]

Several approaches may be used to minimize the reverse biocatalyzed hydrolysis. In principle, the addition of an excess amount of achiral acyl donor or acceptor to the medium could drive the reaction to completion, provided that the enzyme is not denatured under these conditions. However, it is more advantageous to select a suitable acyl donor or acceptor, so that the product ester cannot be hydrolyzed efficiently by the enzyme. In such a case, there is a large difference in the specificity constants (k_{cat}/K_m) for the forward and reverse reactions, resulting in an equilibrium constant, $K = (k_{cat}/K_m)_{reverse}/(k_{cat}/K_m)_{forward}$, that favors esterification. For example, in the enzyme-catalyzed esterification of (±)-**2** with either butanol or cyclohexanol as the acyl acceptor, a much lower *K* value was observed for the cyclohexyl ester system (0.02 vs. 0.15). Apparently the ester of a secondary alcohol is more resistant to enzymic hydrolysis than that of a primary alcohol.

Either the acid or its corresponding ester may be used as the acyl donor in enzyme-catalyzed esterification or transesterification reactions. Mechanistically, these two types of reactions proceed via different transition states for all steps leading to and including the formation of the acyl–enzyme intermediate. Hence, despite the apparent similarity, the *E*[12] and *K* values for esterification and transesterification will be different. Further, the physical state of the substrate has a pronounced effect on the enzymatic catalytic rate. For example, in a nonpolar organic medium, the ester substrate is fully dispersed and is less susceptible to enzymic lipolysis due to the lack of a lipid–water interface. On the other hand, the acid substrate forms micelles or emulsions, which are readily attacked by the lipase. However, if the acid is insoluble in the organic medium, the reaction rate is markedly reduced due to the sluggish enzymic action on a solid substrate.

As shown in Tables I and II, esterification takes place even in aqueous media. Therefore during the lipase-catalyzed hydrolysis of esters containing long-chain acids such as menthyl laurate, reverse enzyme-catalyzed equilibration becomes prominent, and low optical and chemical yields are obtained.[1] A useful diagnostic indicator of a reversible biocatalytic kinetic resolution system is that the optical purity of the remaining substrate (ee_S) decreases when the conversion is extended beyond 50% in contradistinction to the irreversible case where ee_S increases as the conversion is extended.

(11) Verger, R.; Mieras, M. C. E.; DeHaas, G. H. *J. Biol. Chem.* **1973**, *248*, 4023.

(12) The *E* values are different for the kinetic resolution of a racemic acid and its corresponding ester, whereas the value for the kinetic resolution of acyl acceptor is independent of the acyl donors used.

All available experimental data appear to support the contention that the sense of chirality for the forward and reverse reactions is usually maintained. For example, the ester of the (−)-1*R*,2*S*,5*R* enantiomer of **1** was preferentially formed and hydrolyzed by the lipase; likewise the (+)-*R*-**2** and its ester were preferentially esterified and hydrolyzed, respectively.

As far as the enantiospecificity for the forward and reverse reactions is concerned, the theory predicts that if the reaction conditions are identical, the *E* value for the forward and the reverse reactions should be the same. However, in practice the *E* values are always different because the hydrolytic (water) and the esterification (water-organic) reactions proceed through different transition states under different reaction conditions.

There are several inherent disadvantages associated with this procedure. The enzyme has lower catalytic efficiency in biphasic media and a larger quantity of enzyme is required for the reaction. Also, the enzyme is less stable in biphasic media and only nonpolar solvents (such as isooctane, hexane, cyclohexane) are used. In addition, it is seldom possible to obtain the remaining substrate fraction of high optical purity (ee ≥ 0.98) because of the problem of reversible biocatalysis. On the other hand, by selecting a suitable acyl donor or acceptor, enzyme-catalyzed esterification in biphasic media can be highly enantiospecific. In many instances, the *E* values of the enantiospecific esterification are higher than those of the corresponding enzymic hydrolysis of racemic esters. For example, in the *C. cylindracea* lipase-catalyzed hydrolysis of the butyl and cyclohexyl esters of (±)-**2**, the *E* values are 6 and 22,[13] respectively, whereas the *E* values for the corresponding esterification are 12 and 80. Moreover, the experimental protocol is simple to execute because the acid and alcohol are added directly to the reaction mixture without prior chemical manipulation(s). Finally, substrate and product inhibition can often be alleviated in biphasic media. As such, this method provides a useful alternative to the classical hydrolytic resolution procedure for the laboratory preparation of optically active compounds.

Experimental Section

^{1}H NMR spectra were recorded on a Varian EM-390 spectrometer in deuteriochloroform solution with tetramethylsilane as the internal standard. Gas chromatography (GC) was performed with a Varian Aerograph Model 2400 instrument. A Model M-6000 pump equipped with a U6K injector and a Model 77 double-beam UV (254 nm) detector (Waters Associates) were used for high-pressure liquid chromatography (HPLC). Optical rotations were measured with a Perkin-Elmer Model 241C instrument in chloroform unless otherwise stated. Column chromatography was performed with MN-Kieselgel 60 (0.05–0.2 mm; 70–270 mesh, Brinkmann). All solvents were glass distilled prior to use.

dl-Menthol, *dl*-2-(*p*-chlorophenoxy)propionic acid, hexanoic acid, lauric acid, phenylvaleric acid, cyclohexanol, *n*-butanol, *R*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid [(+)-MTPA], and tris(3-(heptafluoropropylhydroxymethylene)-(+)-camphorato)europium(III) derivative, 99% [Eu(hfc)$_3$], were products of Aldrich. *Candida cylindracea* lipase powder (L1754 type VII) was purchased from Sigma.

Determination of *E* and *K*. The equilibrium constant *K* was determined by allowing the reaction to proceed until the fast-reacting species attained equilibrium. The value of *K* could then be estimated from the concentrations of the remaining substrate and product of the fast-reacting species. From the experimentally determined data of ee_S, ee_P, *c*, and *K*, the *E* value was calculated with use of eq 8 or 9.

Enantiospecific Esterification of (±)-1. Three acyl donors (10 mmol), hexanoic, lauric, and phenylvaleric acids, were each incubated separately with 1 g of *C. cylindracea* lipase and (±)-menthol (780 mg, 5 mmol) in 20 mL of isooctane, saturated with 0.2 M phosphate buffer, pH 7.0, or in 20 mL of 0.2 M phosphate buffer, pH 7.0, at 25 °C with shaking. A separate incubation experiment was used for each time interval. The reaction was terminated by filtering the organic medium to remove the enzyme or by extracting the aqueous medium with ethyl acetate at the following time intervals: hexanoic acid (isooctane–H_2O) 115, 163, 240, and 360 h and (phosphate buffer) 40, 64, 120, 138, and 200 h; lauric acid (isooctane–H_2O) 24, 54, 78, 103, 127, and 151 h and (phosphate buffer) 1.5, 4, 7.5, 11, and 23 h; phenylvaleric acid (isooctane–H_2O) 45, 90, 200, 317, and 360 h and (phosphate buffer) 17, 40, 64, 112, and 120 h. The organic extract was dried over Na_2SO_4 and was then concentrated to dryness under reduced pressure. The residue was dissolved in hexane–ethyl acetate (30:1) and chromatographed over 60 g of silica gel. Elution of the column with hexane–ethyl acetate (30:1) gave pure menthyl ester; residual menthol was eluted from the column with a hexane–ethyl acetate (7:1) mixture.

Preparation and Analyses of (+)-MTPA Esters of 1. To 5 mg of purified menthol was added 15 mg of (+)-MTPA chloride.[14] After the mixture was stirred for 1 min, one drop of anhydrous pyridine was added and the reaction mixture was stirred for 16 h at 25 °C. The reaction mixture was then diluted with water and extracted with 10 mL of diethyl ether. The ethereal layer was washed with 1% HCl and water until the pH of the solution was neutral. The organic layer was dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The resulting (+)-MTPA ester was analyzed by HPLC on a 50 cm Alltech μporasil (10 μm) column (i.d. 4.6 mm). The column was eluted with a solvent system consisting of hexane–ether (150:1) at a flow rate of 1 mL/min. The absorbance at 254 nm was monitored, and the retention times for the menthol–MTPA diastereomers were 8.2 min (1*S*,2*R*,5*S*) and 9.7 min (1*R*,2*S*,5*R*). The menthyl esters, obtained in the enzymatic reaction, were hydrolyzed by treatment with 2 N NaOH in ethanol for 1 h. The ee of the resulting menthol was determined as described above.

Enantiospecific Esterification of (±)-2. Two acyl acceptors (10 mmol), *n*-butanol and cyclohexanol, were each incubated separately with 1 g of *C. cylindracea* lipase in the presence of (±)-**2** (1 g, 5 mmol) in either 20 mL of isooctane saturated with 0.2 M phosphate buffer, pH 7.0, or 20 mL of 0.2 M phosphate buffer, pH 7.0. The reaction was terminated by filtering the organic medium to remove the enzyme. The aqueous phosphate buffer solution was adjusted to pH 2.0 with 6 N HCl prior to extraction with ethyl acetate. Samples were taken at the following intervals: butanol (isooctane) 27, 49, 79, and 120 h and (phosphate buffer) 4, 9, 24, and 34 h; cyclohexanol (isooctane) 105, 115, 163, and 240 h and (phosphate buffer) 7, 45, 68, and 240 h. The organic extract was dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was dissolved in hexane–ethyl acetate (7:1) and chromatographed over 60 g of silica gel. Elution of the column with hexane–ethyl acetate (7:1 and 3:1) gave the pure ester and the residual acid, respectively. The ester was converted to the acid by alkaline hydrolysis (2 N NaOH in ethanol for 1 h). The acid from both fractions was treated with diazomethane, and the resulting methyl ester was used for optical purity determination by ^{1}H NMR spectroscopy with CCl_4 as the solvent in the presence of 0.3 equiv of Eu(hfc)$_3$.

Acknowledgment. This investigation was supported in part by Grant GM-33149 of the National Institutes of Health.

(13) Sih, C. J., unpublished data.

(14) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543.