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# MARKED TEMPERATURE EFFECT ON THE ENANTIOSELECTIVE HYDROLYSIS IN ARTIFICIAL MEMBRANES

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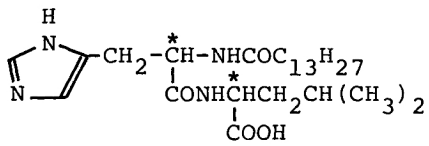
The enantioselectivity was markedly elevated at temperatures somewhat higher than the phase transitions for the hydrolysis of hydrophobic amino acid esters (p-nitrophenyl N-dodecanoyl-D(L)-phenylalaninate) by a hydrophobic dipeptide (tetradecanoyl-L-histidyl-L-leucine) in the artificial membrane (dialkyldimethylammonium bromides) systems.

It is widely known that most enzyme catalyses are highly stereospecific. For example, the α-chymotrypsin-catalyzed hydrolysis of p-nitrophenyl esters of D- and L-enantiomers of N-acetylamino acids demonstrates the interrelationship between substrate-specificity and stereoselectivity.[1] Enzyme-model studies[2-5] have been the subject of continued interest in such areas as the development of stereoselective reaction sites for the hydrolysis of enantiomeric esters and in aiding understanding the origins of stereoselectivity in the above-mentioned proteolytic enzymes.[1]

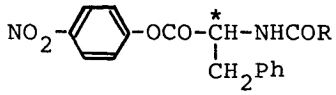
Relatively high stereoselectivity has recently been attained in the hydrolytic cleavage (hydrolysis) of diastereomeric dipeptide substrates with a thiol-functionalized surfactant,[3] in the hydrolysis of N-acylamino acid esters with dipeptide L-histidine derivatives in the presence of cationic surfactants,[4] or in that with L-histidine derivatives in bilayer systems.[5] Very recently, we have attempted to examine the kinetic origin of high enantioselectivity by measuring substrate-binding properties, activation parameters and kinetic salt and organic co-solvent effects in the co-micellar systems, and emphasized the importance of hydrophobicity of the enantiomer substrate for the elevation of enantioselectivity.[6] However, there have been only a few reports of the effect of membrane fluidity on stereoselective hydrolysis in artificial bilayer membranes.

In this paper, we wish to report the dramatic temperature effect on the enantioselective hydrolysis of p-nitrophenyl-D(L)-phenylalaninate (D(L)-$S_{12}$), which bears a long hydrophobic acyl chain, by the bilayer catalytic systems of tetradecanoyl-L-histidyl-L-leucine (MyrHisLeu) with didodecyldimethylammonium bromide ($2C_{12}$), ditetradecyldimethylammonium bromide ($2C_{14}$), and dihexadecyldimethylammonium bromide ($2C_{16}$) in connection with the fluidity of the membrane matrix.

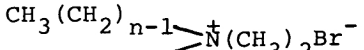
The kinetic studies were carried out at 10 - 45 °C (pH 7.6), 0.083 M Tris buffer (1 M=1 mol $dm^{-3}$) (0.083 M KCl) in (3:97 v/v) $CH_3CN$-$H_2O$. The bilayer stock



MyrHisLeu



D(L)-ZS (R=$PhCH_2O$)
D(L)-$S_2$ (R=$CH_3$)
D(L)-$S_{12}$ (R=$CH_3(CH_2)_{10}$)



$2C_n$ (n=12, 14, 16)

solutions were prepared by dissolving both nucleophile and surfactant in Tris-KCl buffer by sonication at 50 °C for 1 h. Each run was initiated by adding an acetonitrile solution of an ester substrate to a reaction medium which contained both nucleophile and surfactant. The reaction obeyed the usual pseudo-first-order rate law, and the rate constants, $k_\psi$, were obtained by the usual technique.[7]

The temperature dependence of enantioselectivity (reflected in $k_\psi^L/k_\psi^D$) for the hydrolysis of D(L)-ZS, D(L)-$S_2$, and D(L)-$S_{12}$ catalyzed by MyrHisLeu in the presence of $2C_{14}$ (or $2C_{12}$)[8] and $2C_{16}$ was examined as shown in Figs. 1 and 2. It is very noteworthy that the temperature dependence of enantioselectivity for the hydrolysis of $S_{12}$, which bears a long acyl chain, was bell-shaped with a maximum at 15 -25 °C, 25 °C, and 35 °C in the bilayer catalytic systems of MyrHisLeu + $2C_{12}$, MyrHisLeu + $2C_{14}$, and MyrHisLeu + $2C_{16}$, respectively. Especially, the highest enantiomer rate ratio ($k_\psi^L/k_\psi^D$ = 11) was attained at 25 °C in the system of MyrHisLeu + $2C_{14}$. The phase transition temperature ($T_c$) was determined to be 14.5 °C (ref. 9) 16 °C) for the Tris-KCl buffer solution of $2C_{14}$ by the DSC method. Interestingly, the optimum temperatures (15 - 25 °C, 25 °C, and 35 °C) with a maximum of enantioselectivity were in fair agreement with the inflection ranges ($2C_{12}$, 16 - 22 °C; $2C_{14}$, 25 - 30 °C; and $2C_{16}$, 20 - 33 °C)[10] in the Arrhenius plots, which were apparently related to the phase transition of the bilayers. However, it is desirable to note that the optimum temperatures with a maximum of enantioselectivity in the bilayer systems of MyrHisLeu + $2C_n$ (n = 12, 14, 16) were somewhat higher than the $T_c$ values[9] for the aqueous solutions of $2C_n$ (n = 12, 14, 16).

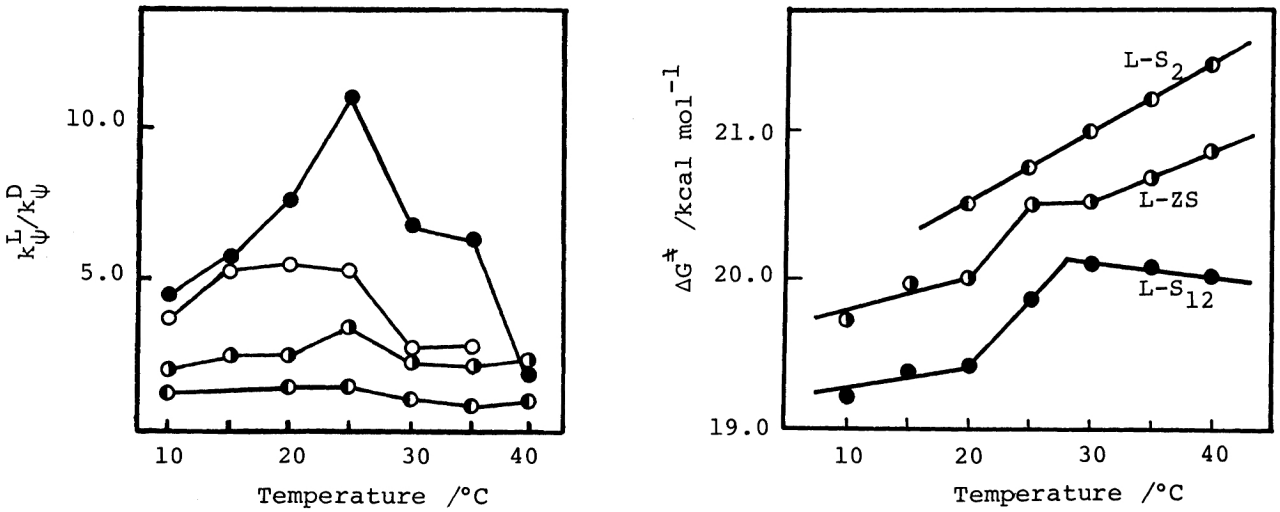



Fig. 1. Temperature dependence of enantioselectivity and free energy of activation ($\Delta G^{\neq}$) in the hydrolysis of enantiomers catalyzed by MyrHisLeu + $2C_{14}$ ( ●:$S_{12}$, ◐:$S_2$, ◑:ZS) and MyrHisLeu + $2C_{12}$ ( ○:$S_{12}$).

by the DSC method, respectively. On the other hand, the enantioselectivity in the hydrolysis of $S_2$ having a short chain is almost constant over the whole temperature range examined, though the hydrolysis of ZS having a bulky side part tends to be bell-shaped with a maximum at 25 °C in the bilayer catalytic system of $2C_{14}$. The magnitude of enantioselectivity for the long-chain substrate ($S_{12}$) is fairly larger than that for the short-chain substrates ($S_2$ and ZS) in the system of MyrHisLeu + $2C_n$ (n = 14 and 16). These results suggest that the hydrophobic interaction between substrate and nucleophile is of great importance to the enhancement of enantioselectivity. Interestingly, the enantioselectivity for the hydrolysis of all the substrates ($S_2$, $S_{12}$, and ZS) employed converged almost to a similar magnitude at 40 °C and 45 °C in the bilayer systems of $2C_{14}$ and $2C_{16}$, respectively. Probably this would be attributed to the weakening of the highly oriented structure of the membrane matrix at higher temperatures.

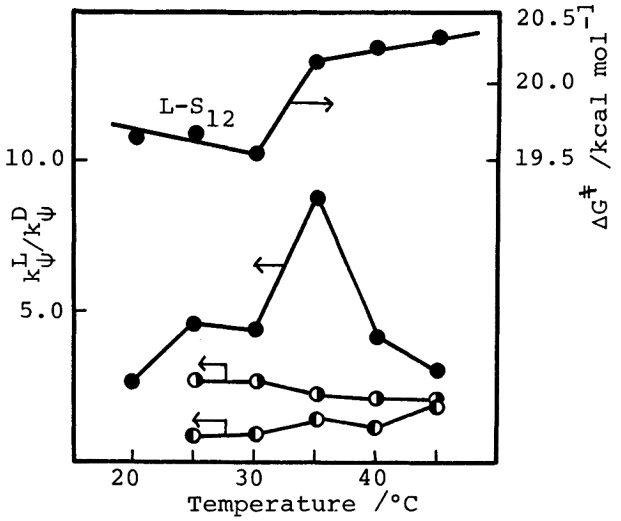



Fig. 2. Temperature dependence of enantioselectivity and $\Delta G^{\ddagger}$ in the hydrolysis of enantiomers catalyzed by MyrHisLeu + $2C_{16}$ (●:$S_{12}$, ◐:$S_2$, ◑:ZS).

Figs. 1 and 2 also show the correlations between the free energy of activation ($\Delta G^{\ddagger}$)[11] and temperature in the hydrolysis of $S_2$, $S_{12}$, and ZS. The plots are not linear in the hydrolysis of the long-chain substrate ($S_{12}$) and the bulky side-chain substrate (ZS), though the linear relationship is presented for the hydrolysis of the short-chain substrate ($S_2$). It is of interest that the inflection temperature range of 20 - 28 °C for the $S_{12}$ hydrolysis in the bilayer catalytic system of $2C_{14}$ was in the neighborhood of 25 °C with a maximum of enantioselectivity. A similar trend was observed in the bilayer system of $2C_{16}$ (Fig. 2). Furthermore, the isokinetic relationship appears to hold for the hydrolysis of $S_{12}$ and ZS over the temperature range of 10 - 20 °C and 30 - 40 °C, respectively, from the relation between the entropy and enthalpy of activation ($\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$) on the basis of the rate constant ($k_\psi$).[11] On the basis of the isokinetic temperature ($\beta$)[11] value in connection with $\bar{T}$ (the average of experimental temperature), the enantioselective hydrolysis in the bilayer system of MyrHisLeu + $2C_{14}$ may be governed by the entropy of activation above and below 25 °C, that is, $\bar{T}$ (288 K) exceeds $\beta$ (270 $\pm$ 6 K) below 25 °C and $\bar{T}$ (308 K) exceeds $\beta$ (287 $\pm$ 2 K) above 25 °C. It can be inferred from the $\beta$ value that the enantioselective hydrolysis would proceed through a strong hydrophobic interaction (entropy driven)[12] between the reactants above and below 25 °C (optimum temperature) in the bilayer system of $2C_{14}$.

It is concluded that the notable aspects of the present enantioselective

hydrolysis in the bilayer catalytic systems ( (a) the temperature dependence of enantioselectivity for the long-chain substrate ($S_{12}$) was bell-shaped with a maximum at the optimum temperature, and (b) the enantioselectivity for the hydrolysis of all the substrates ($S_2$, $S_{12}$, and ZS) having a various hydrophobic chain was converged almost to a similar magnitude at higher temperatures.) would be derived from the delicate change in the fluidity of the membrane matrix with temperature and are closely related to the hydrophobic microenvironment of $2C_n$ (n = 12, 14, and 16).

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