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THE HYDROLYSIS OF p-NITROPHENYL ESTERS OF α-AMINO ACIDS BY N-LAUROYL L OR D-HISTIDINE IN CATIONIC MICELLES

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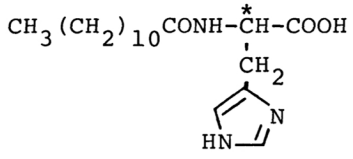
In order to investigate the enantioselectivity of enzyme-catalyzed reactions the hydrolysis of L and D-S_{EP} were carried out using optically active catalyst, L and D-LauHis, in the presence of the mixed micelles with an optically active surfactant, L-TALAI, or inactive surfactant, CTABr. The enantiometric rate ratios of 1.5-1.6 were obtained in these systems with both surfactants.

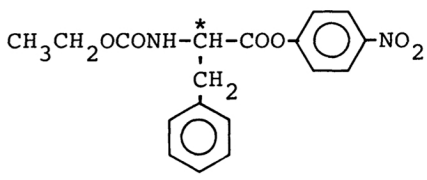
α-Chymotrypsin, which has been studied extensively, exhibits a characteristic enantioselectivity[1] as well as a high reactivity[2] in its catalytic hydrolysis. Many catalytic functions, such as synthetic polymers,[3] macrocyclic compounds[4] and functional surfactants[5] have been investigated. In micellar systems few studies have been reported in regard to the enantioselectivity catalyzed hydrolysis.[6] No significant selectivity was provided by the catalyst in these studies but by one of a cationic surfactant type containing a L-Histidine function, by which the hydrolysis of p-nitrophenyl ester of N-acetyl-L-phenylalanine proceeded three times as fast as that of its D-enantiomer.[7]

In this work, we designed much simpler enzyme models which fill the following requirements:

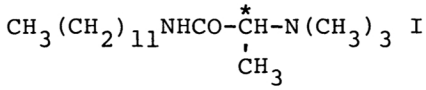
1) Asymmetric center and active site must exist closely in the reaction system.
2) Strong interactions must exist among reagents.
3) The catalyzed hydrolysis must occur in hydrophobic field in order to avoid the reaction with non-selective hydroxide ion.



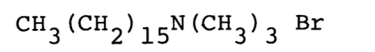
L or D-LauHis



L or D-S_{EP}



L-TALAI



CTABr

These considerations led us to employ mixed micelles of cationic surfactants and catalysts. This mixed micellar system facilitated the study on the asymmetric circumstances to induce enantioselectivities by using a chiral surfactant or chiral catalysts. It was also expected that this system reduces the concentration of the catalyst in contrast to the compound like Bunton's in which a catalyst is chemically combined to a surfactant innevitably requiring a higher concentration than the critical micelle concentration(cmc).

In this communication is described the hydrolysis of L-S_{EP} and D-S_{EP} catalyzed by trimethyl-L-alanyllaurylamide iodide(L-TALAI) and cetyltrimethylammonium bromide(CTABr) micelles[8] containing N-lauroyl L or D-histidine(L or D-LauHis). The reagents possess hydrophobic groups and acyl functions which might provide hydrophobic interactions and inter-amide hydrogen bondings among them.

L or D-LauHis were prepared by standard method,L(+);162-163°C, D(-);161-162°C (ref[9] 161-162°C). L or D-S_{EP} were prepared by the reaction of N-ethoxycarbonyl-L or D-phenylalanine and p-nitrophenol with dicyclohexylcarbodiimide,[10] L(-);120.5-121.0°C, D(+);120.0-120.5°C. L-TALAI was prepared by quarternization of L-alanyl laurylamide obtained by the debenzyloxycarbonylation of N-benzyloxycarbonyl-L-alanyllaurylamide in the presence of Pd/c. N-benzyloxycarbonyl-L-alanyllaurylamide was prepared by the reaction of N-benzyloxycarbonyl-L-alanine[11] and laurylamine with dicyclohexylcarbodiimide, L(-);191°C.

Hydrolysis was followed spectrophotometrically at 25°C, pH 7.17, in 0.05 M. Tris. buffer, 0.2 M. KCl. From the kinetic analysis are observed the apparent differences of pseudo-first-order rate constants in the hydrolysis of L-S_{EP} and D-S_{EP} as shown in Fig.I. The concentration of the catalyst is 1.0×10^{-4} M. It should be noted that there have been so far no clear-cut example of enantioselectivity in such low concentration. Further Fig.II shows that the enantioselectivity was independent on the concentration of the catalyst in the range of

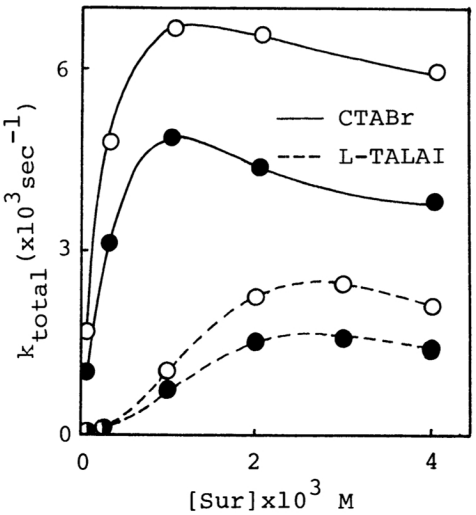
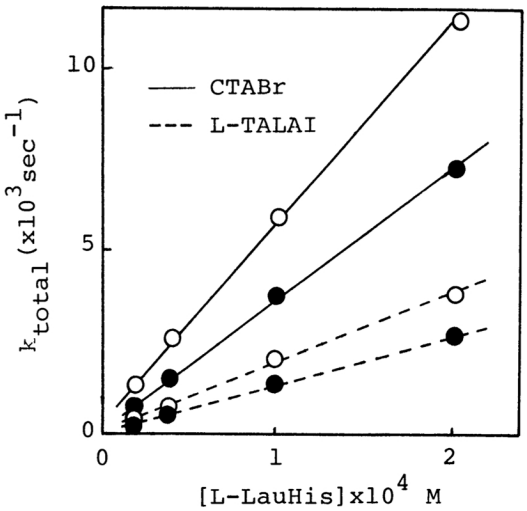


Fig.I. k_{total} vs. [sur]
[L-LauHis]=1×10^{-4} M.
-O- L-S_{EP} -●- D-S_{EP}



FigII. k_{total} vs. [L-LauHis]
[sur]=4×10^{-3} M.
-O- L-S_{EP} -●- D-S_{EP}

Table I. Hydrolysis of L or D-S_{EP}.

Sur	Cat	$k_{a,obs.}$ ($M^{-1}sec^{-1}$) L-S_{EP}	D-S_{EP}	L/D
L-TALAI	L-LauHis	20.5 ± 0.1	13.5 ± 0.1	1.52 ± 0.01
L-TALAI	D-LauHis	16.5 ± 0.1	23.5 ± 0.2	0.70 ± 0.02
L-TALAI	LauHX	65.7 ± 0.5	70.0 ± 2.7	0.94 ± 0.04
CTABr	L-LauHis	56.3 ± 3.6	36.2 ± 1.8	1.56 ± 0.05
CTABr	D-LauHis	36.2 ± 0.7	55.8 ± 1.4	0.65 ± 0.05
CTABr	LauHX	110.7 ± 2.3	111.0 ± 2.7	1.00 ± 0.03

pH 7.17, 25°C, 0.05 M Tris. buffer, 0.2 M KCl, 10.0-6.67 v/v% CH_3OH-CH_3CN-H_2O. [Sur]=4 x 10^{-3} M, [Cat]=1 x 10^{-4} M, [Sub]= 4 x 10^{-5} M.

4 x 10^{-5}-20 x 10^{-5} M. Unexpectedly the system with the chiral surfactant(L-TALAI) and the achiral one(CTABr) gave the selectivity similar to each other. We suppose as hereunder: first the surfactants afforded only appropriate hydrophobic fields (there was observed less clear selectivity in the case of an anionic micellar system[12]), second a substituent group(-CH_3) adjacent to the asymmetric carbon of L-TALAI is sterically so small that its effect is not clear.

Table I summarizes the result in each mixed micellar system. In each case it was found that L-Catalyst reacted more selectively with L-Substrate, and D-Catalyst with D-Substrate, vice versa. A mixed micellar system with L-TALAI gave rise to no significant selectivity when L or D-LauHis was replaced by an optically inactive catalyst, lauroylhydroxamic acid(LauHX).[13] We believe that the enantioselectivity can be obtained in simple models such as mixed micellar system in this work when considering above-mentioned regards. An asymmetric effect of surfactant such as L-TALAI probably is negligible unless a strong interaction is formed between surfactant and catalyst or substrate.

More effective selectivity may be realized by modifying the steric structure of catalyst or substrate.

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8) cmc of L-TALAI and CTABr at the condition of hydrolysis were 5×10^{-4} M. and 6×10^{-5} M.,respectively.

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12) $k_{a,obs}$(L-S_{EP})= 1.55 $M^{-1}sec^{-1}$, (D-S_{EP})= 1.21 in the mixed micelles with sodium dodecyl sulfate, pH 7.7, 25°C, 0.05M.Tris. buffer.

13) LauHX : $CH_3(CH_2)_{10}CONHOH$, mp. 94.5-96.0°C (ref 94°C ; H. Takahashi, H. Kashiwase, T. Kuwamura, Yukagaku, 16, 633 (1966).)

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