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Co-operative Effects in the Catalytic Action of *N*-Decanoyl-L-histidine in Micelles

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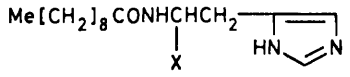
Summary A comparison of catalytic effects in the hydrolysis of the enantiomeric esters (**2**) by *N*-decanoyl-L-histidine (**1a**) and its methyl ester (**1b**) in the presence of cetyltrimethylammonium bromide micelle strongly suggests that the carboxylate ion of (**1a**) enhances the reactivity of the imidazole group through intramolecular hydrogen-bonding.

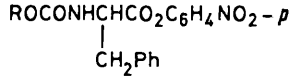
Studies on the catalytic action of α-chymotrypsin and related enzymes suggested a charge-relay mechanism involving interaction between the carboxylate and imidazole groups.[1] In previous papers,[2,3] we have established that the functionalized mixed micelles of *N*-acyl-L-histidines and cetyltrimethylammonium bromide (CTABr) were effective stereoselective catalysts for the hydrolysis of enantiomeric substrates, and a mechanism was suggested for the stereoselective catalysis involving acylation of optically active histidine residues.

This communication describes a comparative analysis of the catalysis of enantiomeric ester hydrolysis by *N*-decanoyl-L-histidine (**1a**) and its methyl ester (**1b**) in the presence of CTABr micelles. Both the imidazole and carboxylate functions are involved in the hydrolysis by (**1a**) and we sought to verify possible co-operative effects between them in aqueous micellar systems.



(1)

a; X = CO_2H
b; X = CO_2Me



(2)

a; R = $PhCH_2$
b; R = Me

Pseudo-first-order rate constants (k_ψ) for hydrolysis of the enantiomeric substrates (**2**) were determined by monitoring the release of *p*-nitrophenol spectrophotometrically at 400 nm (pH>6), and 317 nm (pH<6), with [CTABr]>[catalyst]>[substrate], at 25 °C. The apparent catalytic second-order rate constants (k_c) were obtained from the slopes of linear plots of k_ψ against catalyst concentration at fixed [CTABr]. The Table summarizes the results for several micellar systems at pH 7·30, 0·02 M phosphate buffer.

The catalytic effects of both (**1a**) and (**1b**) lead to a decrease in rates with increasing CTABr concentration (Table, conditions A and B), but the ratios of the decrease are almost the same for (**1a**) and (**1b**), suggesting that there is no significant structural difference between the mixed micelles of (**1a**) and (**1b**). Additions of salt (C) or organic solvent (D) to the reaction media also markedly decrease the rates.† However, the influence of these additives is smaller for (**1a**) than for (**1b**) in mixed micellar systems.

† Micellar catalysis is usually inhibited by additions of salts or organic solvents. These additives affect the catalytic rates by changing the dissociation behaviour of the catalyst and/or the structure of the micelles. For a discussion of salt effects see T. Kunitake, Y. Okahata, and T. Sakamoto, *J. Am. Chem. Soc.*, 1976, **98**, 7799.

TABLE. Apparent catalytic rate constants k_c in micellar systems.[a]

		k_c/l mol^{-1} s^{-1}					
		(2a)			(2b)		
Conditions[b]	Catalyst	L	D	L/D	L	D	L/D
A	(1a)[c]	1730	690	2·51	877	403	2·16
	(1b)	851	496	1·72	495	327	1·51
B	(1a)[c]	572	231	2·51	314	145	2·17
	(1b)	283	154	1·84	184	114	1·61
C	(1a)	130	50·5	2·57	82·0	37·5	2·19
	(1b)	29·0	13·9	2·09	18·4	10·9	1·69
D	(1a)	36·0	18·8	1·91	10·6	6·28	1·69
	(1b)	10·1	6·38	1·58	3·38	2·32	1·46

[a] pH 7·30, 0·02 M phosphate buffer, 25 °C, 0·83% v/v MeCN. The k_c values were calculated by least-squares and generally have correlation coefficients >0·98. [b] (A): In 2·00 × 10^{-3} M CTABr; (B): in 6·00 × 10^{-3} M CTABr; (C): in 6·00 × 10^{-3} M CTABr and 0·40 M KCl; (D): in 6·00 × 10^{-3} M CTABr and 30% v/v EtOH. [c] From ref. 3.

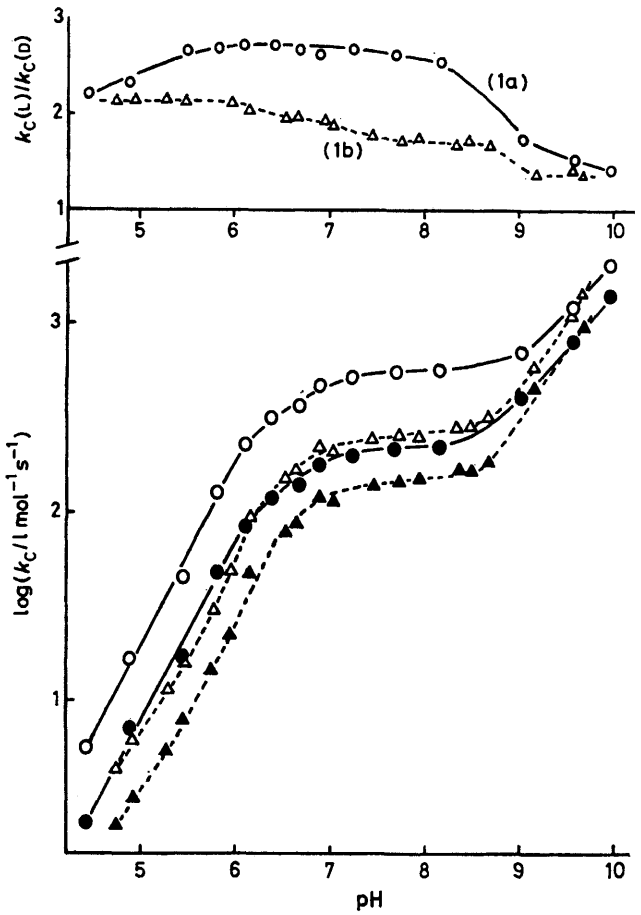


FIGURE. pH–rate profiles for the hydrolysis of (**2a**) by (**1a**) and (**1b**) in CTABr at 25 °C, μ = 0·05 M (KCl), 0·04 M acetate (pH < 6), 0·02 M phosphate (9 > pH > 6), and 0·02 M carbonate (pH > 9); [CTABr] = 6·00 × 10^{-3} M; [(**1a**) or (**1b**)] = (1·00—2·00) × 10^{-4}M; [(**2a**)] = (0·5—1·0) × 10^{-5} M. (——) (**1a**): (●) with D-(**2a**), (○) with L-(**2a**); (- - - -) (**1b**): (▲) with D-(**2a**), (△) with L-(**2a**).

Furthermore, the kinetic results for (**1a**) and (**1b**) show that (**1a**) is more reactive than (**1b**) for all conditions studied. Greater stereoselectivity is also observed for (**1a**) than (**1b**). In general, ionization of the carboxy-group of (**1a**) suppresses ionization of the imidazole group, and also partially neutralizes the positively charged CTABr head groups. Both factors reduce the catalytic potential in micellar systems. Thus, addition of *N*-decanoyl-L-phenylalanine to the reaction medium decreases the rates under comparable conditions. Therefore, the enhanced reactivity and stereoselectivity of (**1a**) suggest that some of the co-operative effects are due to the active site of the catalyst.

In order to gain further insight into co-operative effects in the catalytic action of (**1a**), pH–rate profiles of the reaction were examined and are shown in the Figure. The log-k_c–pH profiles indicate that (**1a**) is more reactive than (**1b**) over the pH range studied. This result shows that appreciable co-operative interaction exists between the imidazole and carboxylate groups in these micellar systems.

For compound (**1a**) at pH 4—7, stereoselectivity increases with increasing pH (increasing concentrations of both carboxylate anion and free imidazole base). This behaviour is quite different from that of (**1b**) [the stereoselectivity remains unchanged (pH 4—6) and then decreases with increasing pH]. Therefore, we suggest that the carboxylate ion of (**1a**) enhances the reactivity of the imidazole group and results in an increase in stereoselectivity.‡ These results can reasonably be explained in terms of intramolecular hydrogen-bonding between the carboxylate and imidazole groups in the micellar phase. It appears that proton transfer accompanies nucleophilic attack by the imidazole which is expected to be much more nucleophilic than a normal imidazole group. This micellar co-operative system should be of considerable interest in connection with studies on enzyme mechanisms.

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‡ The kinetic pK_a values were determined from the plots of [H$^+$] *vs.* 1/k_c by using the data at pH 5·3—7·0 in the Figure; the pK_a values were 6·35 ± 0·08 and 6·24 ± 0·10 for (**1a**) and (**1b**), respectively. This result also suggests that the enhanced reactivity of (**1a**) compared with that of (**1b**) is due to a co-operative interaction between the carboxy- and imidazole groups rather than to a pK_a difference between the imidazole groups.

[1] D. M. Blow and T. R. Steitz, *Annu. Rev. Biochem.*, 1970, **39**, 63.
[2] Y. Ihara, *J. Chem. Soc., Chem. Commun.*, 1978, 984.
[3] Y. Ihara, *J. Chem. Soc., Perkin Trans.* 2, 1980, 1483.