Hydrolysis of *p*-Nitrophenyl Esters by Hydrophobic Linear Molecule Containing Two Histidine Residues

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Synopsis. A new type of linear nucleophile, CH_3O–His–CO–$(CH_2)_8$–CO–His–OCH_3(HisC$_8$His), was designed, and its catalytic behavior for the hydrolysis of *p*-nitrophenyl acetate, hexanoate and laurate was investigated. It was shown that HisC$_8$His enhanced the hydrolysis of *p*-nitrophenyl laurate and hexanoate as compared with imidazole both in the absence and presence of methyltrioctylammonium chloride.

Hydrophobic interaction has proved to be very significant for the enzyme activity by the use of simple model compounds, such as functional polymers,[1,2] peptides,[3–5] cyclodextrins,[6,7] micelles[8–13] or other hydrophobic aggregates.[14,15] In the present paper, the author wishes to describe the catalytic behavior of a new type of simple-structure nucleophile which is composed of a long alkyl chain and two histidine residues placed on both sides of the long alkyl chain. The linear molecule with such a structure will have enough hydrophobicity but simultaneously have enough solubility in water. Furthermore, in the present study the effect of the hydrophobic quaternary ammonium salt, methyltrioctylammonium chloride (MTAC), was investigated, since there is a possibility that the hydrolysis might be considerably activated due to the formation of hydrophobic ion pairs between imidazolate anion and ammonium cation.[14,15] In relation to this, the author tried to combine MTAC with histidine residue covalently.

Experimental

CH_3O–His–CO–$(CH_2)_8$–CO–His–OCH_3(HisC$_8$His) or $CH_3(CH_2)_4$CO–His–OCH_3(C$_5$His) was synthesized from L-histidine methyl ester and sebacoyl chloride or hexanoyl chloride. $Br(CH_2)_5$CO–His–OCH_3 was prepared from L-histidine methyl ester and 6-bromohexanoyl chloride, and then it was converted to $Br^-[CH_3(CH_2)_7]_3N^+(CH_2)_5$CO–His–$OCH_3$($N^+C_5$His) by the reaction with trioctylamine. Imidazole, *N*-*t*-butoxycarbonyl-L-histidine methyl ester (Boc–His–OCH_3), methyltrioctylammonium chloride (MTAC), and *p*-nitrophenyl esters were commercially available.

The hydrolysis was carried out in 10 v/v% ethanol–water at 25 °C, μ=0.5(KCl), buffered with 1/10 mol dm^{-3} potassium dihydrogenphosphate–1/20 mol dm^{-3} sodium borate for pH 7—9, 1/20 mol dm^{-3} sodium borate–1/20 mol dm^{-3} sodium carbonate for above pH 9. The reaction rate was determined by using the absorbance of *p*-nitrophenolate at 400 nm with a Hitachi Model 100-10 spectrophotometer. Pseudo-first-order rate constant (k_1) was estimated by correcting the total rate constant (k_{total}) for the spontaneous hydrolysis as follows:

$$k_1 = k_{total} - k_{spont}.$$

In the presence of MTAC, k_{spont} means the rate constant for the hydrolysis only by MTAC. Second-order rate constant (k_2) was obtained by dividing k_1 by the total catalyst concentration per histidine residue.

Results and Discussion

Second-order rate constants (k_2) for the hydrolysis of *p*-nitrophenyl esters are summarized in Table 1. It should be noted that HisC$_8$His showed much higher activity than imidazole, Boc–His–OCH_3 and C$_5$His for the hydrolysis of *p*-nitrophenyl laurate (PNPL). For *p*-nitrophenyl hexanoate (PNPH), HisC$_8$His showed a little higher activity than imidazole and 2—4 times higher activity than Boc–His–OCH_3 and C$_5$His. HisC$_8$His gave a little greater activity for *p*-nitrophenyl acetate (PNPA) than Boc–His–OCH_3 and C$_5$His but smaller activity than imidazole. These results suggest that the rate enhancement for PNPL and PNPH was primarily ascribed to the hydrophobic interaction between the long alkyl chain of HisC$_8$His and those of the substrates. The significance of the hydrophobic interaction is also indicated by the following results. When 3 mol dm^{-3} urea was added the hydrolytic rate of PNPL by HisC$_8$His was decreased by *ca.* 80%, while that of PNPA only by *ca.* 20%. It is generally known that urea denaturates enzymes partly owing to the inhibition of hydrophobic interaction.[16] The analogous linear molecules which have a long alkyl chain but only one histidine residue, $CH_3(CH_2)_6$CO–His–OCH_3(C$_7$His) and $CH_3(CH_2)_{14}$CO–His–OCH_3(C$_{15}$His), also seem to be hydrophobic but they are poorly soluble in water, and therefore these catalysts could not be investigated in the present study. In this respect, the bi-histidine catalyst like HisC$_8$His designed in the present study is superior to the mono-histidine catalysts like C$_7$His and C$_{15}$His.

As reported by Kunitake *et al.*, hydrophobic nucleophiles having imidazoles gave enormously large rate enhancement in the hydrolysis of *p*-nitrophenyl ester in the presence of MTAC, due to the increase in the fraction of imidazolate anion facilitated by the formation of hydrophobic ion pairs between imidazolate anion and quaternary ammonium cation.[14,15] In the present study, therefore, the author has attempted the hydrolysis in the presence of both HisC$_8$His and MTAC in order to increase the catalytic ability of HisC$_8$His. The kinetic data are summarized in Table 2. Also in this case, HisC$_8$His gave considerably high catalytic activity for PNPL and PNPH as compared with the other nucleophiles, while it gave virtually no enhancement for PNPA. The hydrolysis of PNPL by HisC$_8$His was enhanced by *ca.* 20 times in the presence of MTAC. Figure 1 shows the relation between the pseudo-first-order rate constant (k_1) and the concentration of HisC$_8$His in the presence of MTAC. The k_1 values increase almost linearly with the HisC$_8$His concentration for both PNPH and PNPL. In Fig. 2 are shown the pH profiles of k_2 for PNPH and PNPL in the presence of HisC$_8$His and MTAC, where k_2 begins to increase exceedingly above pH 8.5—9.0. This suggests that the imidazolate anion is the catalytic nucleophile mainly contributing to the hydrolysis.[11,14,15] Since HisC$_8$His showed considerably higher catalytic activity on addition of MTAC, the author attempted then to design the catalyst, N^+C$_5$His, which has both MTAC

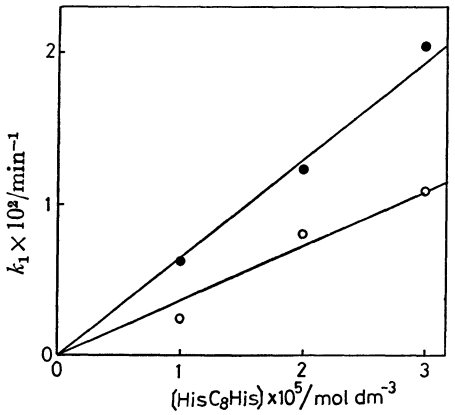

Fig. 1. Dependence of the pseudo-first-order rate constant (k_1) on the HisC$_8$His concentration in the presence of MTAC: (●) PNPL, (○) PNPH; 25 °C, pH 8.0, μ=0.5(KCl), 1/10 mol dm^{-3} KH_2PO_4-1/20 mol dm^{-3} $Na_2B_4O_7$ buffer, [PNPL]=[PNPH]=2.0×10^{-5} mol dm^{-3}, [MTAC] = 2.0 × 10^{-5} mol dm^{-3}. 10 v/v% EtOH was contained.

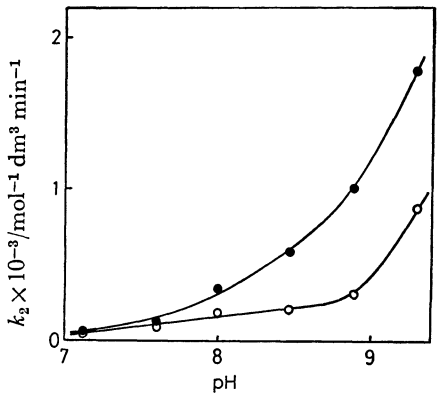

Fig. 2. pH-rate profiles for the hydrolysis of *p*-nitrophenyl esters in the presence of HisC$_8$His and MTAC: (●) PNPL, (○) PNPH; 25 °C, μ=0.5(KCl), 1/10 mol dm^{-3} KH_2PO_4-1/20 mol dm^{-3} $Na_2B_4O_7$ buffer(pH 7—9), 1/20 mol dm^{-3} $Na_2B_4O_7$-1/20 mol dm^{-3} Na_2CO_3 buffer(pH>9), [PNPL] = [PNPH] = 2.0 × 10^{-5} mol dm^{-3}, [HisC$_8$His]=3.0×10^{-5} mol dm^{-3}, [MTAC]=2.0×10^{-5} mol dm^{-3}. 10 v/v% EtOH was contained.

Table 1. Second-order rate constants (k_2) for the hydrolysis of *p*-nitrophenyl esters[a]

Catalyst	k_2 / mol^{-1} dm^3 min^{-1}		
	PNPA	PNPH	PNPL
Imidazole	20	15	0.3
Boc-His-OCH$_3$	6.7	5.7	0.5
C$_5$His	7.0	7.9	1.7
HisC$_8$His[b]	9.0	20	15
N$^+$C$_5$His	9.7	8.1	2.2

a) 25 °C, pH 8.0, μ=0.5(KCl), 1/10 mol dm^{-3} KH_2PO_4-1/20 mol dm^{-3} $Na_2B_4O_7$ buffer, [PNPA]=[PNPH]=[PNPL]=2.0×10^{-5} mol dm^{-3}, [catalyst]=1.0×10^{-3} mol dm^{-3} except for HisC$_8$His, [HisC$_8$His]=5.0×10^{-4} mol dm^{-3}. 10 v/v% EtOH was contained. b) The values of k_2 were estimated per histidine residue.

Table 2. Second-order rate constants (k_2) for the hydrolysis of *p*-nitrophenyl esters in the presence of methyltrioctylammonium chloride(MTAC)[a]

Catalyst	$k_2 \times 10^{-1}$ / mol^{-1} dm^3 min^{-1}		
	PNPA	PNPH	PNPL
Imidazole	<0.05	2.1	0.4
Boc-His-OCH$_3$	<0.05	0.8	2.0
C$_5$His	<0.05	0.3	1.9
HisC$_8$His[b]	<0.05	18	34
N$^+$C$_5$His	<0.05	0.8	1.8

a) 25 °C, pH 8.0, μ=0.5(KCl), 1/10 mol dm^{-3} KH_2PO_4-1/20 mol dm^{-3} $Na_2B_4O_7$ buffer, [PNPA]=[PNPH]=[PNPL]=2.0×10^{-5} mol dm^{-3}, [catalyst]=3.0×10^{-5} mol dm^{-3}, [MTAC] = 2.0 × 10^{-5} mol dm^{-3}. 10 v/v% EtOH was contained. b) The values of k_2 were estimated per histidine residue.

and histidine parts intramolecularly. The kinetic data for N$^+$C$_5$His are also shown in Table 1. As a whole, N$^+$C$_5$His does not have so large activity, although it gives a little enhancement for PNPA. As shown in Table 2, N$^+$C$_5$His does not give so large enhancement even in the presence of MTAC, too. N$^+$C$_5$His studied in the present report might not be able to form hydrophobic aggregates like MTAC in itself and not be able to be incorporated into MTAC aggregates, although there is still great room for improvement in this catalyst.

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