Hydrolysis of *p*-Nitrophenyl Esters by Histidine-containing Linear and Cyclic Peptides

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Synopsis. Hydrolysis of *p*-nitrophenyl acetate (PNPA) and *p*-nitrophenyl hexanoate (PNPH) catalyzed by several linear and cyclic peptides containing histidine and other amino acid residues was studied. The catalytic activity of the peptides was less than that of imidazole for PNPA and PNPH. On the other hand, in the presence of a cationic surfactant and a hydrophobic peptide the hydrolysis of PNPH was significantly enhanced.

Sheehan *et al.*[1] and Kopple *et al.*[2,3] investigated cyclic hexapeptides containing histidyl and seryl residues or histidyl and tyrosyl residues as hydrolytic enzyme models. In these cases, however, detail study about the conformation of the cyclic peptides was not undertaken. On the other hand, Imanishi *et al.* studied the catalytic behavoir and the conformation of *cyclo*-D-leucyl-L-histidyl and found out that D-leucyl residue was an effective binding site for a hydrophobic substrate.[4,5] Recently they also synthesized the cyclic hexapeptide having L-histidyl-D-leucyl sequences and studied its catalytic activity and conformation in detail, where a substrate seems to be bound to D-leucyl residue by hydrophobic interaction.[6,7] In the previous studies, the author synthesized and investigated the cyclic hexapeptide having D-leucyl-L-histidyl sequences and found out that D-leucyl-L-histidyl sequence brings about β-turn.[8,9] In the present paper, therefore, the author would like to describe the catalytic behavior of the analogous peptides as well as the peptides investigated previously.[8,9] The effect of a cationic micelle on the catalytic activity was studied, too.

Experimental

Linear and cyclic peptides were synthesized in a similar manner as described previously.[8] Other reagents were commercially available. The reaction rate was determined by using the absorbance of *p*-nitrophenolate at 400 nm with a JASCO UVIDEC-1 spectrophotometer. Second-order rate constant (k_2) was estimated from the following equations,

$$\ln\{(OD_\infty - OD_0)/(OD_\infty - OD_t)\} = k_1 t,$$

$$k_2 = (k_1 - k_{spont})/[E],$$

where OD_0, OD_t, and OD_∞ mean the optical density at the time, 0, t, and ∞; k_1 and k_{spont} sinify the pseudo-first-order rate constant in the presence and absence of the catalyst, respectively; [E] designates the catalyst concentration per histidine residue.

Results and Discussion

In Table 1 is shown the second-order rate constant (k_2) of the hydrolytic reaction of *p*-nitrophenyl acetate (PNPA) and *p*-nitrophenyl hexanoate (PNPH) at pH 7.9 (0.1 mol dm^{-3} phosphate buffer) at 25 °C. All the peptides show less activity than imidazole. For PNPA the cyclic peptide **5** has lower activity than the linear peptide **4**. However, **9** has higher activity than **5**, while **8** has similar activity to **4**. There could be seen no significant difference in the behavior of the optical density at 245 nm (*N*-acylimidazole)[10] between **7** and **9**. Therefore it could not be confirmed that acyl group was transferred from the *N*-acylimidazole to the seryl hydroxyl group in **7**.[10] As a whole, the activity for

Table 1. Second-order rate constant (k_2) for the hydrolysis of *p*-nitrophenyl acetate (PNPA) and *p*-nitrophenyl hexanoate (PNPH)[a]

Catalyst	k_2 / mol^{-1} dm^3 min^{-1}	
	PNPA	PNPH
Imidazole	23	18
Boc-His-OCH_3 (**1**)	7.0	6.4
Boc-D-Leu-His-OCH_3 (**2**)	4.0	3.0
Boc-Cys(*S*-Acm)-D-Leu-His-$NHNH_2$ (**3**)	4.0	3.0
Boc-Cys(*S*-Acm)-D-Leu-His-Cys(*S*-Acm)-D-Leu-His-OCH_3 (**4**)	4.3	5.2
cyclo(-Cys(*S*-Acm)-D-Leu-His-Cys(*S*-Acm)-D-Leu-His-) (**5**)	2.6	—
cyclo(-Ser(*O*-Bzl)-D-Leu-His-Ser(*O*-Bzl)-D-Leu-His) (**6**)	3.8	—
cyclo(-Ser-D-Leu-His-Ser-D-Leu-His-)(**7**)	5.4	—
Boc-Ala-D-Leu-His-Ala-D-Leu-His-OCH_3 (**8**)	4.5	3.5
cyclo(-Ala-D-Leu-His-Ala-D-Leu-His-) (**9**)	6.0	4.3

a) 25 °C, pH 7.9 (0.1 mol dm^{-3} phosphate buffer), [PNPA]=[PNPH]=5.0×10^{-5} mol dm^{-3}, [catalyst]=1.0×10^{-3} mol dm^{-3} per histidine residue. 5 v/v % DMF was contained.

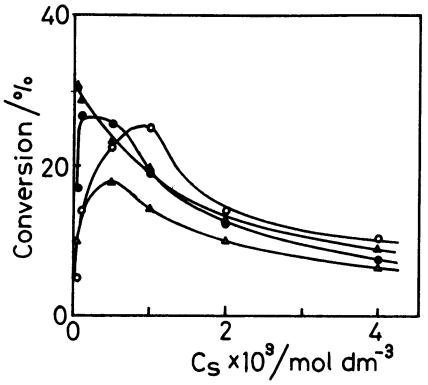

Fig. 1. Relation between the conversion at 20 min and the concentration of the cationic surfactant (C_s): HTAC-**10** (—○—), HTAC-**11** (—●—), BHDAC-**10** (—△—), BHDAC-**11** (—▲—). **10**; Boc-Ser(*O*-Bzl)-D-Leu-His-Ser(*O*-Bzl)-D-Leu-His-OCH_3, **11**; Boc-Ser(*O*-Bzl)-Leu-His-Ser(*O*-Bzl)-Leu-His-OCH_3.

PNPH is lower than that for PNPA. However, in the case of **4** the activity for PNPH is a little higher than that for PNPA. The activation energy of the hydrolysis of PNPA was almost equal to each other, which suggests that the hydrolytic rate is dominated by the frequency factor.

In Fig. 1 is plotted the conversion at 20 min against the concentration of a cationic surfactant. The surfactants used are hexadecyltrimethylammonium chloride (HTAC) and benzylhexadecyldimethylammonium chloride (BHDAC). Maximum of conversion can be seen in Fig. 1. It is considered that the first increasing stage in the lower concentration region may be caused by the formation of the micelle and the subsequent decreasing stage in the higher concentration region may be caused by the dilution of the catalytic sites and the substrate in the micelle. There is some difference in the catalytic property between **10** and **11**. The configurational difference between them is only that of leucyl residue (D and L). Therefore the conformational difference must be responsible for the different catalytic behavior. The rate enhancement by the combination of the peptide and the cationic micelle must be partly due to the formation of the hydrophobic ion pair between the ammonium group of the surfactant and the imidazolate anion which is a potent nucleophile and partly due to the activation of the neutral imidazole itself placed in the hydrophobic environment.[11,12]

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