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A FACILE HYDROLYSIS OF HYDROPHOBIC ESTERS BY CYCLO(D-LEU-L-HIS)

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In the hydrolysis of p-nitrophenyl laurate, cyclo(D-Leu-L-His) was much more effective catalyst than imidazole, the corresponding linear peptide and cyclo(L-Leu-L-His). The importance of binding the ester by the catalyst, and of keeping the isobutyl group of the D-leucyl residue and the imidazole group of the L-histidyl residue into a juxtaposition with the aid of the rigid cyclic backbone was suggested.

The use of simple model compounds has been proved to be very helpful to investigate the mechanism of enzyme reaction.[1] Since the interactions of enzyme protein with other molecules and the cooperation of functional groups in the protein molecule (macromolecule) are essential to the enzyme activity, an enormous number of polymer model compounds carrying functional groups have been constructed, most of which were linear polymers.[2]

In the course of our study on the efficiency and the specificity of enzyme reactions, we used cyclic oligopeptides as an enzyme model compound.[3] Cyclic peptides are advantageous for the ease of synthesis, the detailed analysis of conformation by NMR spectroscopy, and the establishment of the clear-cut relationship between the structure and the activity.

The imidazole group is often found at a catalytic site of enzyme, and a hydrophobic binding of a substrate to enzyme is also important for the enzyme activity.[1] It seemed to be useful to construct a rigid enzyme model carrying an imidazole group and a hydrophobic group. For the simplest cyclic peptide containing histidine (catalytic site) and leucine (hydrophobic site), cyclo(D-Leu-L-His) was synthesized and used as a catalyst for the hydrolysis of a series of carbonic acid esters. As will be seen below in the hydrolysis of p-nitrophenyl laurate cyclo-(D-Leu-L-His) was found to be about 200 times as reactive as cyclo(L-Leu-L-His).

Cyclo(D-Leu-L-His) and cyclo(L-Leu-L-His) were synthesized by the cyclization of D- and L-Leu-L-His-OMe, respectively. The linear dipeptide esters were synthesized by a usual method. For comparison cyclo(Gly-L-His), which lacks a hydrophobic group, was also synthesized in a similar way. By titration pK_1 values of various imidazole functions were determined and are shown in

Table 1. pK_1 values for the cyclic peptide catalysts are lower than imidazole, but no marked difference of pK_1 values is observed among the cyclic peptides.

p-Nitrophenyl esters of acetic acid, β-phenylpropionic acid, and lauric acid were synthesized from the corresponding carbonic acids and p-nitrophenol. These esters will be represented subsequently as PNPA, PNβP, and PNPL, respectively. The hydrophobicity of these esters may increase as the elongation of acyl chain length,[4,5] and thus in the above order.

The hydrolysis was carried out in water containing 20 vol% dioxane at 25 °C and pH 7.9(phosphate buffer, μ=0.1). The substrate concentration was 3.0×10^{-5} M, and the concentration of the total imidazole function was $1.6—9.0 \times 10^{-4}$ M. The reaction was followed by UV absorption at 400nm due to p-nitrophenolate ion liberated in the rate-determining acylation of the imidazole function by a substrate. The first-order plot of the optical densities at 400 nm gave a pseudo first-order rate constant, $k_1(min^{-1})$. k_1 was corrected for the spontaneous hydrolysis with water(k_0). The concentration of the effective (unprotonated) imidazole functions, [E](M), was determined on the basis of the titration curve. $(k_1-k_0)/[E]$ gave the second-order rate constant, $k_2(M^{-1}min^{-1})$, and the catalytic activities were compared in this term. PNPL often associates in aqueous solution,[5,6] but it may not associate in 20 % aqueous dioxane solution at the concentration of 3×10^{-5} M.[6] Addition of urea (4.2 M) did not affect the k_2 value, which confirms the above consideration. In the present study, the effect of association was ignored.

In Table 1 the k_2 values determined for various combinations of the substrate and the catalyst

Table 1 pK_1 Values of cyclic peptides containing L-histidine and the second-order rate constants for the hydrolysis

	pK_1	PNPA	PNβP	PNPL
Imidazole	7.05	17.4	14.0	1.9
Cyclo(Gly-L-His)	6.20	1.5	1.7	1.1
Cyclo(L-Leu-L-His)	6.25	1.6	1.5	0.2∿0.3
Cyclo(D-Leu-L-His)	6.10	3.3	7.0	34∿ 68

are shown. The most interesting is the hydrolysis of PNPL by cyclo(D-Leu-L-His). With PNPL, because of a large steric hindrance, k_2 for imidazole is quite low. Linear dipeptides containing L-His residue, which are not shown here, were almost inactive for PNPL, presumably because the steric situation around the imidazole functions got worse as a result of connecting the imidazole group with a peptide backbone. However, the cyclization of the peptide catalysts restored the activity, and thus cyclo(Gly-L-His) showed the activity comparable to imidazole. This might have been caused by weak hydrophobic interactions between the dikeropiperazine ring and PNPL. To increase the hydrophonic interaction, the leucyl residue was replaced for the glycyl residue.

The catalytic activity of the resultant cyclic peptide was surprisingly affected by the configuration of C^{α} of leucyl residue; that is, cyclo(L-Leu-L-His) was only slightly active for the hydrolysis, whereas cyclo(D-Leu-L-His) was very active. k_2 for the latter was about 200 times as large as k_2 for the former. This enhanced catalytic activity cannot be explained in terms of a high nucleophilicity of the imidazole function in cyclo(D-Leu-L-His) as is evident in Table 1.

The efficiency of cyclo(D-Leu-L-His) became more emphasized as the hydrophobicity of the substrate increased (Table 1); that is, the k_2 values for the hydrolysis increased in the following order.

PNPA < PNβP < PNPL

This implies that the high reactivity of cyclo(D-Leu-L-His) towards PNPL arises from binding of the substrate by the catalyst. Further evidence for this view came from the study of the solvent effect. When the hydrolysis was carried out in 50 % aqueous dioxane, k_2 for the imidazole catalysis was 1.4 while that for cyclo(D-Leu-L-His) catalysis was only 0.59. For the increasing hydrophobicity of the medium, in which the hydrophobic binding of the substrate by the catalyst is no longer important, the cyclic peptide was almost unreactive.

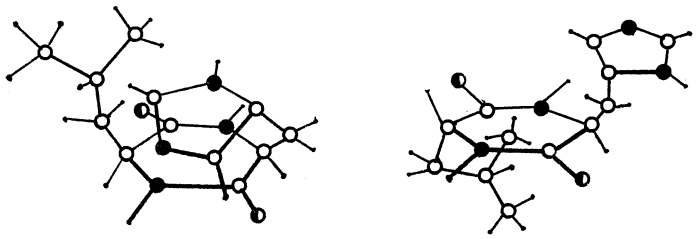
The preliminary analysis of the solution conformation of the cyclic peptides was made by NMR spectroscopy. The NH-C^{α}H dihedral angles of the cyclic peptide backbone and the conformation of the side chains were determined on the basis of the coupling constant,[7] and are shown in Table 2. From these values it follows that the cyclic peptide backbone is nearly planar (NMR measurement

Table 2 Conformation of the cyclic peptide backbone and the side chain [a]

	NH-C^{α}H Dihedral angle		Side chain conformation, %					
			Leu			His		
	Leu	His	F	U_I	U_{II}	F	U_I	U_{II}
Cyclo(L-Leu-L-His)	68°	63°	25	26	49	58	21	21
Cyclo(D-Leu-L-His)	63°	63°	38	31	31	30	49	21

a) For F, U_I, and U_{II} conformation, see ref 7).

in DMSO-d_6). It also follows that in cyclo(L-Leu-L-His) the isobutyl group of the L-leucyl residue directs outside the diketopiperazine ring (U_{II} form) and the imidazole group of the L-histidyl residue faces to the diketopiperazine ring (F form), whereas in cyclo(D-Leu-L-His) the isobutyl group of the D-leucyl residue faces to the diketopiperazine ring (F form) and the imidazole group of the L-histidyl residue directs outside the diketopiperazine ring (U_I form). The most plausible conformation in solution can be depicted as in Figure 1. In cyclo(L-Leu-L-His) the steric hindrance around the imidazole group is very large and the isobutyl (binding) and the imidazole (catalytic) groups are



Cyclo(L-Leu-L-His) Cyclo(D-Leu-L-His)

Figure 1 The skeletal arrangement of the cyclic peptides.

○ C, ● N, ◐ O, • H.

too closely situated to cooperate. On the other hand, in cyclo(D-Leu-L-His) the two functional groups are situated so favourably as the cooperation between them is exhibited in the reaction of PNPL carrying a long acyl chain.

The experimental findings showed unambiguously that a specific arrangement of the binding group and the catalytic group fixed on a rigid backbone such as cyclic peptide is very important for the catalytic activity.

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