Cyclodextrin-Mediated Deacylation of Peptide Esters with Marked Stereoselectivity

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Functional molecular assemblies composed of surfactants and reactive species have attracted much attention in recent years as efficient enzyme models for clarification of the catalytic specificities of native enzymes from the biomimetic viewpoint.[1] In the course of our study on the stereoselective hydrolysis of amino acid esters in coaggregate systems, we emphasized that the stereochemical control is attained by regulating temperature[2] and ionic strength[3] and by changing the composition of the coaggregates.[4] In particular, the authors observed almost complete L-enantiomer-selective catalysis, which was attained by controlling the reaction microenvironment.[5] The observation can be attributed to the optimization of the enzyme model conformation in the coaggregate systems.[6]

On the other hand, considerable efforts have been devoted in the past decade to the investigation of various properties of cyclodextrins (CyD) because of their usefulness as serine protease models.[7] However, little is known about the relation between the cavity size of cyclodextrins and the stereospecificity in the hydrolysis of enantiomeric esters.[8] The enantioselectivity reported so far for the enzyme mimetic catalysis does not seem attractive. In the present study, we report the first successful experiment resulting in marked stereoselectivity in the hydrolysis of diastereomeric peptide esters (Z-(D or L)-Phe-L-Phe-PNP (**1**)) as mediated by CyD.

$PhCH_2OCONHC^*H(CH_2Ph)CONHC^*H(CH_2Ph)OCO\text{-}C_6H_4\text{-}p\text{-}NO_2$

1: Z-(D or L)-Phe-L-Phe-PNP

$PhCH_2OCONHC^*H(CH_2CHMe_2)CONHC^*H(CH_2Ph)\text{-}OCO\text{-}C_6H_4\text{-}p\text{-}NO_2$

2: Z-(D or L)-Leu-L-Phe-PNP

Exploring the stereochemical component of the "micelle enzyme analog",[9] Moss and others examined the kinetic diastereoselectivity exercised in the cleavage of dipeptide esters in nucleophile-functionalized micellar,[10] vesicular,[11] and coaggregate systems.[12] Particularly, these authors emphasized that the diastereoselectivity must originate from supramolecular interactions of the substrates with the micellar assembly.

In this study, the kinetic data were treated on the basis of the Michaelis–Menten principle.[13] The binding constants (K_b) and the rate constants (k_2) for the deacylation (hydrolysis) of Z-(D or L)-Phe-L-Phe-PNP and Z-(D or L)-L-Leu-L-Phe-PNP[14] catalyzed by α-, β-, and γ-CyD are summarized in Table I.

The rate constants (k_2) for the DL diastereomeric substrate are much superior to those for the corresponding LL isomer in the hydrolysis of Z-Phe-Phe-PNP, while both the k_2 and K_b values for the LL isomer are slightly larger than those for the DL isomer in the hydrolysis of Z-Leu-Phe-PNP. It should be noted that the markedly large stereoselectivity (k_2DL/k_2LL = 42 for β-CyD and 46 for γ-CyD) and the preferential binding property (K_bDL/K_bLL = 2.4 for γ-CyD) are evident. Furthermore, the rate enhancement is also remarkable in the hydrolysis of Z-D-Phe-L-Phe-PNP as mediated by γ-CyD.[15] The large stereospecificity must originate from a favorable accommodation of the substrate (Z-D-Phe-L-Phe-PNP) in the hydrophobic cavity of γ-CyD, which provides a specific spatial environment for the substrate.

In order to examine the conjecture as regards the origin of the markedly enhanced stereoselectivity in the hydrolysis of depeptide esters as mediated by CyD, we assembled space-filling molecular

Table I. Kinetic Parameters for the Hydrolysis of Dipeptide Esters by Cyclodextrins (CyD) at pH 9.5, 25 °C[a]

CyD	kinetic parameter	Z-Phe-Phe-PNP LL	DL	DL/LL	Z-Leu-Phe-PNP LL	DL	LL/DL
α-CyD	k_2 (s^{-1})	0.00104	0.0230	22	0.0150	0.00261	5.7
	K_b (M^{-1})	28.7	22.2	0.8	188	103	1.8
β-CyD	k_2 (s^{-1})	0.0180	0.758	42	0.140	0.0428	3.3
	K_b (M^{-1})	23.0	26.4	1.1	28.5	26.2	1.1
γ-CyD	k_2 (s^{-1})	0.0170	0.778	46	0.0916	0.0543	1.7
	K_b (M^{-1})	32.7	79.9	2.4	50.4	39.7	1.3

[a] 0.02 M carbonate buffer, 0.05 M KCl, 3% (v/v) CH_3CN–H_2O, [sub] = 5×10^{-6} M, [CyD] = (0.4–1.5) × 10^{-2} M. Under the conditions [CyD] ≫ [substrate], pseudo-first-order rate constants (k_t in the presence of CyD and k_s in the absence of CyD) were evaluated from monitoring *p*-nitrophenol liberation from *p*-nitrophenyl esters of dipeptides at 400 nm. The reaction proceeds via the scheme of eq 1, and the K_b ($= k_1/k_{-1}$) and k_2 values were determined by the least-squares method from Lineweaver–Burk plots between $1/(k_t - k_s)$ and 1/[CyD] in eq 2.[13]

$$\mathrm{CyD} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{CyD{\cdot}S} \xrightarrow{k_2} \text{acyl-CD} + \text{product}, \quad \mathrm{S} \xrightarrow{k_s} \text{products} \qquad (1)$$

$$1/(k_t - k_s) = 1/(k_2 - k_s) + 1/K_b(k_2 - k_s)[\mathrm{CyD}] \qquad (2)$$

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(14) These diastereomers were synthesized by mixed anhydride coupling of Z-(D or L)-Phe(or Leu) to L-Phe-PNP as described in Moss, R. A.; Hendrickson, T. F.; Ueoka, R.; Kim, K. Y.; Weiner, P. K. *J. Am. Chem. Soc.* **1987**, *109*, 4363. These isomers were fully characterized.
(15) Interestingly, on the basis of k_t in the presence of CyD (1×10^{-2} M), the diastereoselectivity (k_tDL/k_tLL = 73) was large: k_tDL = 0.339 s^{-1} and k_tLL = 0.004 65 s^{-1}. On the other hand, the diastereoselectivity (k_sDL/k_sLL = 9.2) without CyD was not so attractive: k_sDL = 0.005 47 s^{-1} and k_sLL = 0.000 595 s^{-1}.

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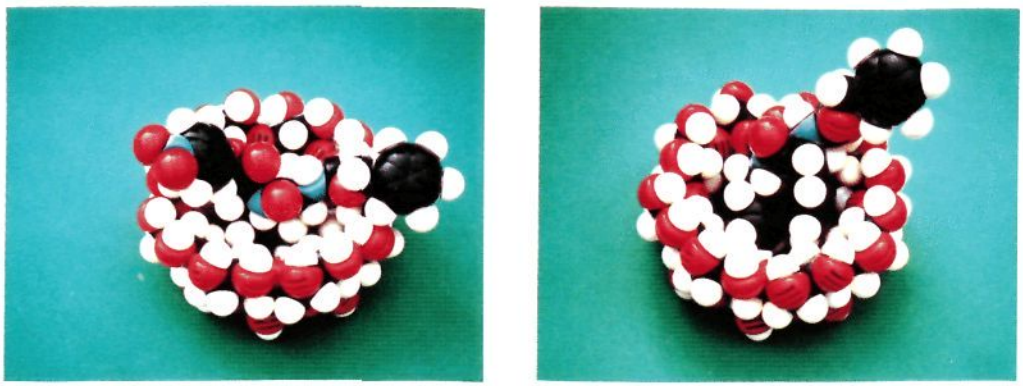

Figure 1. A conceivable fitting model of γ-CyD and Z-D-Phe-L-Phe-PNP (left) and the acylated γ-CyD (right).

models (CPK model).[16] Figure 1 shows an enzyme-like fitting of the specific substrate (Z-D-Phe-L-Phe-PNP) into the cavity of γ-CyD. The hydrophobic interaction between the D-Phe-L-Phe portion of the substrate and the cavity of γ-CyD, as well as the hydrogen-bonding interaction between the C=O group of the Z moiety and/or the *p*-nitrophenyl ester group of the substrate and the OH group of γ-CyD, seems to be crucial for the demonstration of such an effect in light of the molecular model study. In addition, the acylated γ-CyD seems to retain a similar state of affairs when the D-Phe-L-Phe portion of the substrate is incorporated into the cavity of γ-CyD, though partial distortion of γ-CyD might occur in this acylation process.

In conclusion, it needs to be pointed out as the most significant feature that even the unmodified cyclodextrins exhibit markedly high diastereoselectivity in the hydrolysis of specific dipeptide substrates (Z-D(L)-Phe-L-Phe-PNP). The CyD–substrate complex can be referred to one of the typical examples of the so-called "supramolecular assemblies" which demonstrate novel functions only after association of individual molecular components.

(16) Preliminary molecular modeling calculations have been performed with the extended MM2(91) program on a SUN workstation. The energy is minimized around the structure which was obtained from the CPK models for two complexes, DL-CyD and LL-CyD. The tentative result indicates that the DL-CyD complex has lower energy than LL-CyD by a few kilocalories.

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