Rate enhancement and enantioselectivity in ester hydrolysis catalysed by cyclodextrin–peptide hybrids

Hiroshi Tsutsumi, Keita Hamasaki, Hisakazu Mihara and Akihiko Ueno *

Department of Bioengineering, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama 226-8501, Japan. Fax: 81-45-924-5833; E-mail: aueno@bio.titech.ac.jp

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A pair of cyclodextrin–peptide hybrids (CD–peptides) having three functional groups, β-cyclodextrin (β-CD), imidazole and carboxylate, in this order and in the reverse order were designed and synthesized as hydrolytic catalysts. These CD–peptides were designed so as to make three functional groups placed on the same side of the α-helix peptide work together. Another pair of CD–peptide hybrids which lack the carboxylate were also designed and synthesized in order to examine the effect of the carboxylate in the novel catalysts. Circular dichroism studies revealed that these CD–peptides have stable α-helix structures and their α-helix contents were high enough (around 70%) to place the functional groups at appropriate positions in the CD–peptides. Boc-D-alanine *p*-nitrophenyl ester and Boc-L-alanine *p*-nitrophenyl ester were chosen as substrates and the enantioselectivity of the catalysts in the hydrolysis was examined. Kinetic studies suggested that the presence of carboxylate in the CD–peptides enhances the ester hydrolysis with substrate selectivity.

Introduction

Many enzymes have high activity and substrate specificity or selectivity because they arrange multiple functional groups in a masterly way. But the usage of enzymes in industrial fields is limited due to their fragilility with regard to pH, temperature, and so on. Therefore, artificial enzymes which overcome this difficulty have been sought. One of the essential factors for constructing useful catalysts is arranging the multiple functional groups at appropriate positions. Among many candidates for artificial enzymes, enzyme mimics using modified cyclodextrins (CDs)[1] or peptides[2] have been studied extensively. In the modified CDs used as catalysts, CD acts as a substrate binding site because of its remarkable ability to bind a guest molecule in its central cavity. On this basis, CD derivatives having a benzoate conjugated imidazole,[3,4] and a tripeptide (serine-histidine-aspartate)[5] were proposed as serine protease mimics. However, it is not clear whether those functional groups work cooperatively or not. On the other hand, peptides, which can form well-defined secondary structures,[6] are useful as a scaffold on which to place multiple functional moieties at their appropriate positions. For example, a 4α-helix bundle was synthesized as a hydrolytic catalyst, in which multiple functional groups are arranged.[2] In this catalyst, it was confirmed that multiple functional groups act together. But it is difficult to construct a substrate binding site only from small peptide molecules. The use of CD as the binding site might solve this problem.

Recently, we have prepared cyclodextrin–peptide hybrids (CD–peptides) with multiple functional groups. It was proved that the CD unit acts as the substrate binding site and the carboxylate as one of the functional groups plays a key role in ester hydrolysis.[7] In this study, Boc-D-alanine *p*-nitrophenyl ester and Boc-L-alanine *p*-nitrophenyl ester were chosen as substrates and the enantioselectivity of the CD–peptide hybrids was studied.

Results and discussion

Design and syntheses of CD–peptides

In the design of these CD–peptides, to avoid any undesirable influence of the amino acid side chains, alanine was chosen as a main component because it favors an α-helix structure.[8] Additionally, in order to stabilize an α-helical structure, two pairs of intramolecular salt bridges (Glu4-Arg8 and Glu12-Arg16)[9] were introduced into the opposite face of the catalytic side. Schematic illustrations of the CD–peptides and their amino acid sequences are shown in Fig. 1. EHβ19 has a carboxylate, an imidazole moiety and β-CD in this order from the N-terminus side and Hβ19 has Ala6 instead of Glu6, and consequently it lacks the carboxylate. βHE19 has three functional groups in the reverse order of EHβ19. βH19 lacks the carboxylate with Ala14 instead of the Glu14 of βHE19.

Scheme 1 shows the synthetic route to EHβ19. The α-helix peptide was synthesized by the stepwise elongation of Fmoc protected amino acids. The side chains of His, Arg and Glu except for Glu14 were protected with trityl (Trt), mesitylene-2-sulfonyl (Mts) and benzyl (Bzl) groups, respectively. The side chain of Glu14 was protected with the *tert*-butyl (tBu) group. Synthesized peptide was cleaved from the resin by trifluoroacetic acid, and at this stage, tBu of Glu14 and Trt of His10 were removed. 6-Mono-deoxy-6-amino-β-CD was prepared as previously described[10] and selectively introduced into the side chain of Glu14 *via* an amide bond linkage. Finally, all protection groups were removed by trimethylsilyl trifluoromethanesulfonate.[11] The other CD–peptides were synthesized in the same manner as EHβ19. All the products were purified using an HPLC instrument equipped with a C4 reversed phase column and identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS) and amino acid analysis.

Structural analysis of peptide backbones in the CD–peptides

Circular dichroism studies revealed that all the CD–peptides showed a typical α-helical pattern with double negative maxima at 208 and 222 nm at various pH's in phosphate buffer solutions[12] (Fig. 2). The secondary structure of the peptides did not depend on pH (Fig. 3) in the pH region examined. The α-helix contents[13] of EHβ19, Hβ19, βHE19 and βH19 were 73%, 67%, 72% and 68%, respectively. All the CD–peptides maintain a

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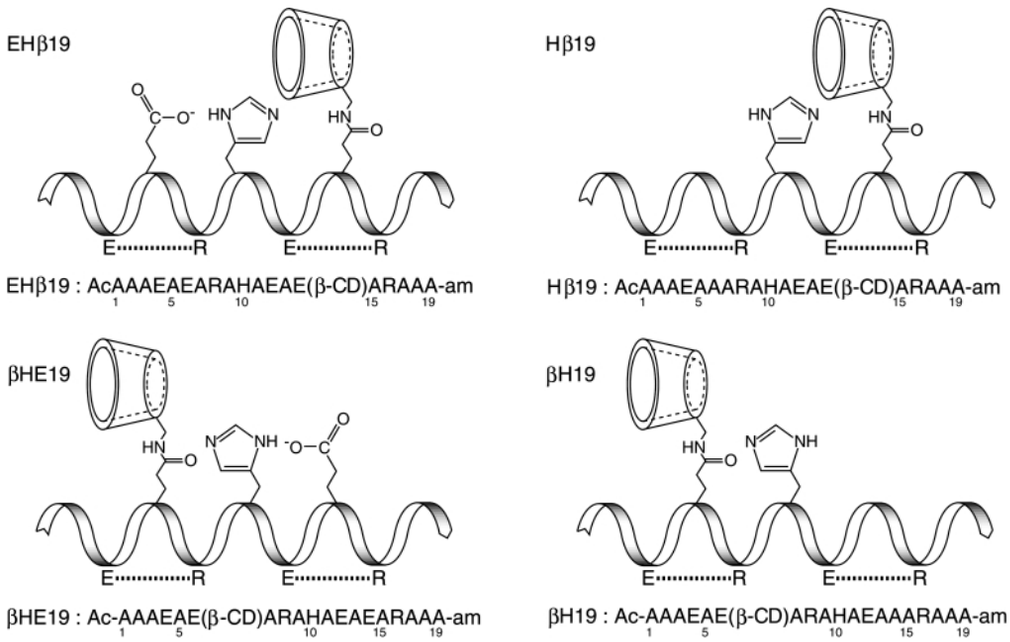
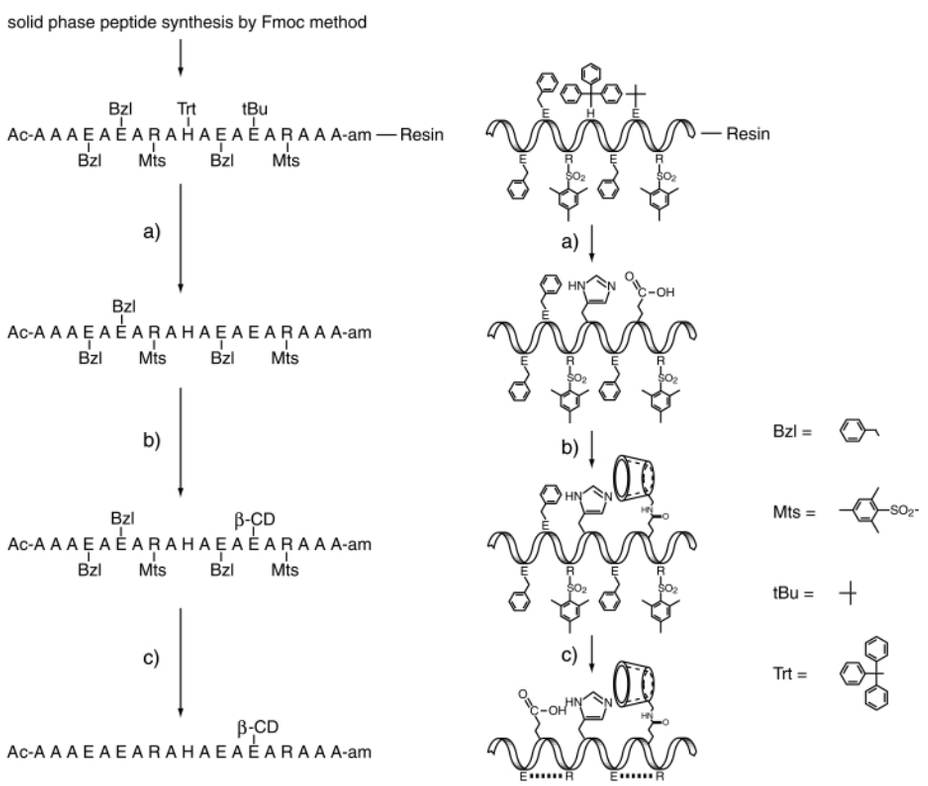


Fig. 1 Illustrations and amino acid sequences of CD–peptides.



Scheme 1 Synthetic route to EHβ19. a) Cleavage of the peptide from the resin. TFA, *m*-cresol, 25 °C, 1.0 h. b) Coupling of the peptide with NH_2-β-CD. HBTU, HOBt, DIEA, NMP, 25 °C, 24 h. c) Deprotection of all protecting groups on the peptide. TFA, *m*-cresol, thioanisole, TMSOTf, 0 °C, 2.0 h. TFA = trifluoroacetic acid, HBTU = *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, HOBt = 1-hydroxybenzotriazole, DIEA = diisopropylethylamine, NMP = *N*-methylpyrrolidone, TMSOTf = trimethylsilyl trifluoromethanesulfonate.

stable α-helix structure at various pH in the buffer solutions with helix contents high enough for them to maintain an appropriate arrangement of the functional groups. Though the orders of amino acids and functional groups in the CD–peptides are different from each other, all the CD–peptides have similar α-helix contents. On the other hand, the α-helix content of another CD–peptide that lacks two Glu-Arg salt bridges was 22%.[14] These results suggest that the α-helix structures of the CD–peptides are governed not only by alanines as the main component but also by two pairs of Glu-Arg intramolecular salt bridges.

Ester hydrolysis and its kinetic study

Boc-D-alanine *p*-nitrophenyl ester (D-AlaONp) and Boc-L-alanine *p*-nitrophenyl ester (L-AlaONp) were chosen as enantiomeric substrates. The hydrolysis was performed at various pH in phosphate buffer solutions and monitored by increasing

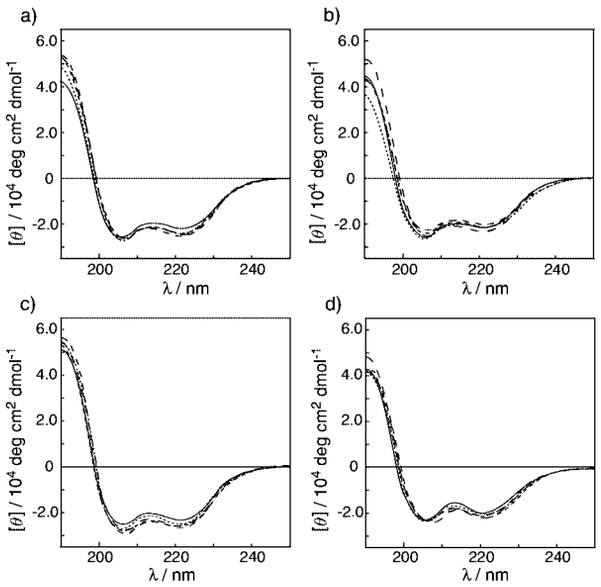


Fig. 2 Circular dichroism spectra of CD–peptides in 50×10^{-2} mol dm^{-3} phosphate buffer solutions containing 1.25 vol% trifluoroethanol at 25 °C. a) EHβ19; b) Hβ19; c) βHE19; d) βH19, pH 5.0 (——), 6.0 (------), 6.5 (– – – –), 7.0 (— — —), 8.0 (— —).

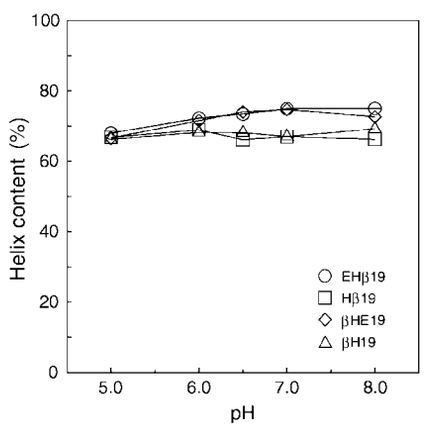


Fig. 3 pH profiles of α-helix content for EHβ19 (○), Hβ19 (□), βHE19 (◇) and βH19 (△).

absorbance of the product, *p*-nitrophenolate.[15] In all measurements, the concentration of each CD–peptide was fixed at 2.5×10^{-5} mol dm^{-3}. The substrate concentration was varied from 1.0×10^{-4} to 5.0×10^{-4} mol dm^{-3}. Conditions of excess substrate were kept in each measurement.

Initial rates of hydrolysis were measured in buffer solutions[16] with different pH's under the conditions of a fixed substrate concentration of 3.0×10^{-4} mol dm^{-3}. Both EHβ19 and βHE19 had a maximum point around pH 6.0 in the hydrolysis of D-AlaONp and L-AlaONp. On the other hand, Hβ19 and βH19 did not have any maximum point (Fig. 4). Since the secondary structures of the CD–peptides do not depend on pH, the difference in the catalytic activities around pH 6.0 must arise from the presence of the carboxylate in EHβ19 or βHE19. In the pH region from 6.0 to 7.0, the activities decreased in EHβ19 and βHE19. One possible explanation is that the hydrogen bond between carboxylate and imidazole is inhibited by water molecules in the pH region, resulting in the inactivation of the imidazole group. A pH around 6.0 might be optimal for forming a hydrogen bond between carboxylate and imidazole. The catalytic activities of all the CD–peptides increased beyond pH 7.0 because the nucleophilicity of the imidazole group simply increases in the pH region.

In the absence of any catalyst, the rate constants of the hydrolysis of D-AlaONp and L-AlaONp (k_{un}) were 1.84×10^{-5}

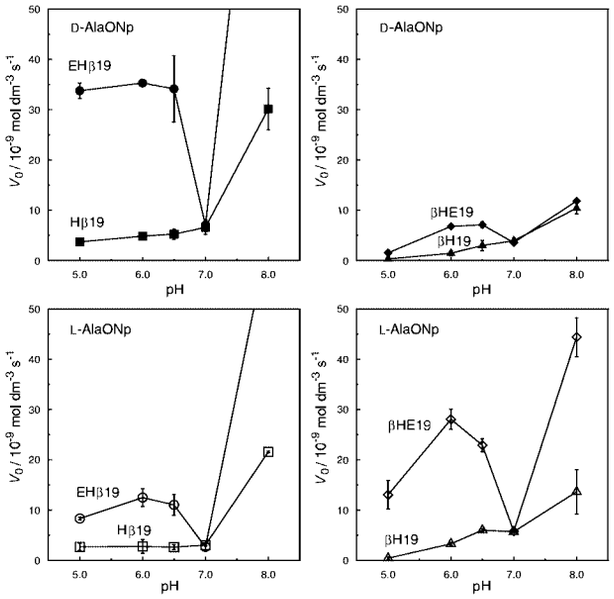


Fig. 4 pH dependence of hydrolysis of Boc-D-alanine *p*-nitrophenyl ester (closed symbol) and Boc-L-alanine *p*-nitrophenyl ester (open symbol) for EHβ19 (●, ○), Hβ19 (■, □), βHE19 (◆, ◇) and βH19 (▲, △). The concentration of all catalysts was 2.5×10^{-5} mol dm^{-3}. The concentration of the substrates was fixed at 3.0×10^{-4} mol dm^{-3}.

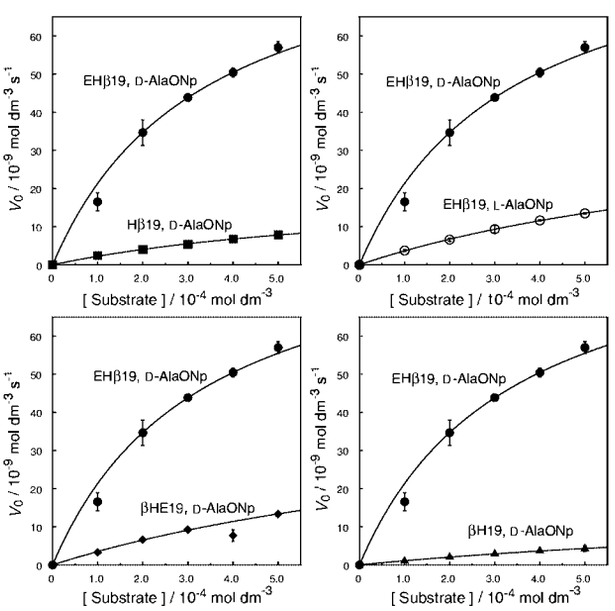


Fig. 5 Hydrolysis of Boc-D-alanine *p*-nitrophenyl ester (closed symbol) and Boc-L-alanine *p*-nitrophenyl ester (open symbol) for EHβ19 (●, ○), Hβ19 (■), βHE19 (◆), βH19 (▲). The concentration of all catalysts was 2.5×10^{-5} mol dm^{-3}. The concentration of the substrates was varied from 1.0×10^{-4} to 5.0×10^{-4} mol dm^{-3}. The solid lines were obtained by least squares curve fitting based on the Michaelis–Menten equation.

and 1.78×10^{-5} s^{-1}, respectively. Because EHβ19 and βHE19 showed maximum catalytic activity at pH 6.0, the kinetic parameters of all the CD–peptides were compared at this pH (Fig. 5) and are summarized in Table 1. The k_{cat}[17] values of EHβ19 and βHE19 having carboxylate, imidazole and β-CD were higher than the CD–peptides Hβ19 and βH19, which lack a carboxy group, for the hydrolysis of the substrates. When EHβ19 and Hβ19 were compared, EHβ19 showed 4.5-fold and 1.7-fold higher k_{cat} values (3.70×10^{-3} and 1.67×10^{-3} s^{-1}) than Hβ19 (0.82×10^{-3} and 0.96×10^{-3} s^{-1}) for D-AlaONp and L-AlaONp, respectively. The superior k_{cat} values of EHβ19 over those of Hβ19 must derive from the presence of the carboxylate. The carboxylate in EHβ19 might enhance the

Table 1 Kinetic parameters for the hydrolysis of Boc-D-alanine *p*-nitrophenyl ester and Boc-L-alanine *p*-nitrophenyl ester catalysed by CD–peptide hybrids at 25 °C in 5.0×10^{-2} dm^{-3} phosphate buffer solution (pH 6.0)

Catalyst	Substrate	k_{cat}/ 10^{-3} s^{-1}	K_m/ 10^{-4} mol dm^{-3}	(k_{cat}/K_m)/ mol^{-1} dm^3 s^{-1}	K_{TS}/ 10^{-5} mol dm^{-3}
EHβ19	D-AlaONp	3.70	3.33	11.1	0.16
	L-AlaONp	1.67	10.5	1.59	1.11
Hβ19	D-AlaONp	0.82	8.18	1.00	1.90
	L-AlaONp	0.96	12.5	0.77	2.29
βHE19	D-AlaONp	1.60	11.9	1.34	1.34
	L-AlaONp	2.53	6.89	3.67	0.49
βH19	D-AlaONp	0.66	14.1	0.47	4.08
	L-AlaONp	1.16	6.75	1.72	1.04

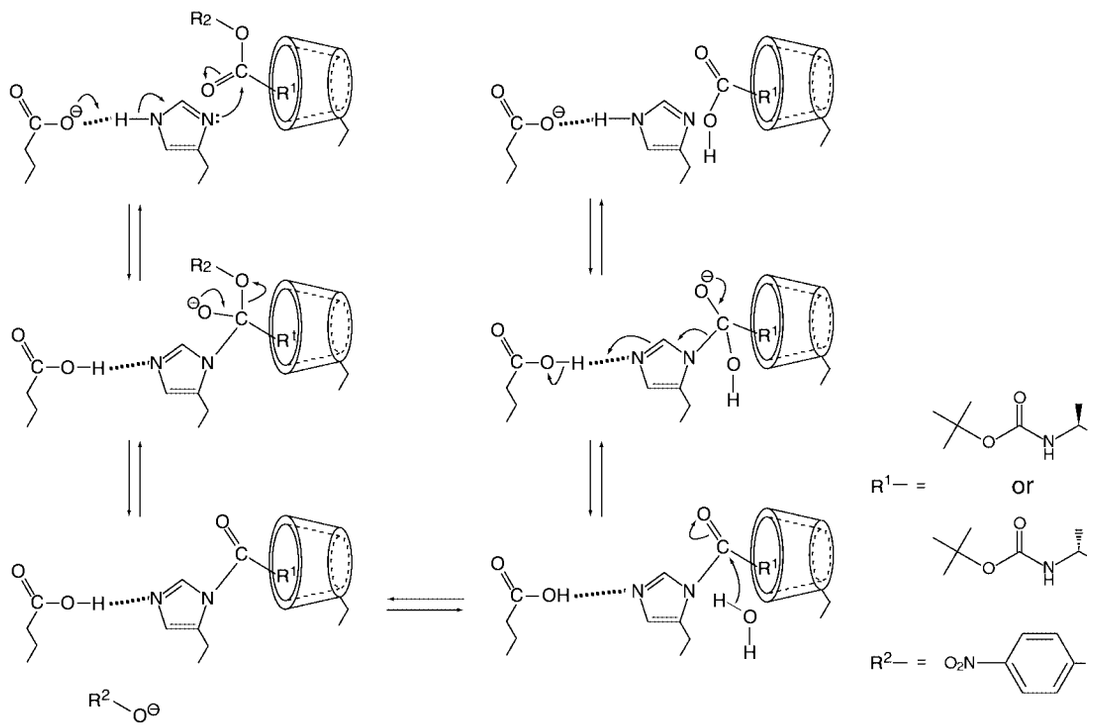


Fig. 6 Schematic illustration of ester hydrolysis performed by the cooperation of imidazole, carboxylate and β-CD units.

nucleophilicity of the imidazole group by forming a hydrogen bond with the imidazole. A plausible mechanism,[2,3] for the ester hydrolysis is shown in Fig. 6. On the other hand, EHβ19 showed a 2.5-fold superiority in K_m value over Hβ19 for the hydrolysis of D-AlaONp. K_m is considered as an approximation of the dissociation constant of the catalyst–substrate complex. Namely, the lower K_m value is more favorable to the hydrolytic reaction. This result suggests that the presence of the carboxylate in EHβ19 is favorable for the binding of D-AlaONp. In the case of L-AlaONp as the substrate, the superiority in the binding is not large. Tee *et al*. evaluated the dissociation constant of the catalyst–substrate complex in the transition state as K_{TS} ($=k_{un}/(k_{cat}/K_m)$).[18] The K_{TS} values of EHβ19 for D-AlaONp and L-AlaONp are smaller than those of Hβ19, suggesting that the complexes in the transition state are stabilized by the presence of the carboxylate group. In the K_m values of the CD–peptides, there is no significant difference between βHE19 and βH19 for D-AlaONp and L-AlaONp although βHE19 showed 2.4 and 2.2-fold higher values of k_{cat} than βH19 for D-AlaONp and L-AlaONp, respectively. These results suggest that the presence of the carboxylate in βHE19 did not improve the binding for these substrates. Also, βHE19 showed 3.0- and 2.1-fold superiority in the value of K_{TS} over βH19 for D-AlaONp and L-AlaONp, respectively, and these results indicate that the presence of the carboxylate in βHE19 stabilizes the transition state of the substrate–catalyst complexes.

When the K_m values for the substrates of the enantiomers are compared for CD–peptides with different arrangements of the functional units, EHβ19 and Hβ19, which have a β-CD unit on the C-terminus side, showed the superior value for D-AlaONp. On the other hand, βHE19 and βH19, which have a β-CD unit on the N-terminus side, showed an advantage for L-AlaONp. This result suggests that the order of the β-CD unit and the imidazole group on the α-helix peptide determines the selectivity for the substrate binding. The selectivity difference may arise from the topological difference, which reflects the arrangement of the functional units on the α-helix. With respect to the k_{cat} values of the CD–peptides, EHβ19 showed higher values for D-AlaONp while βHE19 and βH19 showed higher values for L-AlaONp. On the other hand, EHβ19 had a 7-fold higher k_{cat}/K_m value for D-AlaONp than for L-AlaONp. In the case of Hβ19, in spite of the fact that the k_{cat} value for D-AlaONp was a little inferior to that for L-AlaONp, the k_{cat}/K_m value for D-AlaONp was superior to that for L-AlaONp. This superiority is probably due to the advantage in K_m for D-AlaONp. In contrast to the D-substrate preference of EHβ19 and Hβ19, βHE19 and βH19 had about 3-fold higher k_{cat}/K_m values for L-AlaONp than for D-AlaONp. This result indicates that the substrate selectivity is mostly determined by the order of the functional groups in the CD–peptides. Namely, the topological aspect of the functional groups is important in these catalytic systems.

All these results demonstrate that this new approach using CD–peptides is useful for finding appropriate systems with high catalytic activity and high substrate selectivity by arranging the multiple functional groups on the α-helix peptide.

Experimental

Materials and methods

All chemicals and solvents were of reagent or HPLC grade. Amino acid derivatives and reagents for peptide synthesis were purchased from Watanabe Chemical Co. (Hiroshima, Japan). β-Cyclodextrin (β-CD) was kindly donated by Nihon Shokuhin Kako Co., Ltd., and was used as received. Mono-6-deoxy-6-amino-β-CD (NH_2-β-CD) was prepared as previously reported.[10] Boc-L-alanine *p*-nitrophenyl ester was purchased from Sigma. It was used without further purification. Boc-D-alanine *p*-nitrophenyl ester was synthesized. All peptides were synthesized manually by the solid-phase method using the Fmoc-strategy. The CD–peptides were purified by reversed-phase HPLC. HPLC was carried out on a C4 reversed phase column (YMC-Pack A-823 column, YMC Co.) (10φ × 250 mm) by employing a Hitachi L-7100 HPLC system. The CD–peptides were identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS). MALDI-TOFMS was measured on a Shimadzu KOMPACT MALDI III mass spectrometer by using 3,5-dimethoxy-4-hydroxycinnamic acid or 2,5-dihydroxybenzoic acid as a matrix. Amino acid analysis was carried out with a Shimadzu LC-10AD*vp* system equipped with a Wakopak WS-PTC column (4.0φ × 200 mm, Wako Co.) after hydrolysis of each CD–peptide in 6.0 mol dm^{-3} HCl at 110 °C for 24 h in a sealed tube and labeling amino acids with phenyl isothiocyanate. The CD–peptide concentrations were determined by quantitative amino acid analysis using valine as an internal standard.

Syntheses of CD–peptides

Synthesis of EHβ19. 4-(2′,4′-Dimethoxyphenyl-Fmoc-aminomethyl)phenoxy resin (Rink amide resin) was employed and the peptide was synthesized manually by stepwise elongation of Fmoc-protected amino acids on the resin according to the reported procedure.[19] The amino group on the resin (156 μmol of 260 mg resin) was coupled with Fmoc amino acid (3 equiv.) in the presence of *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU, 3 equiv.), 1-hydroxybenzotriazole hydrate ($HOBt·H_2O$, 3 equiv.) and diisopropylethylamine (DIEA, 6 equiv.) in 2 ml of *N*-methylpyrrolidone (NMP) for 20 min. The Fmoc group was removed by treatment with 2 ml of 20% piperidine in NMP solution for 15 min. The terminal amino group of the peptide was acetylated with acetic anhydride (10 equiv.) in NMP for 20 min. Coupling efficiency on the resin was checked by the Kaiser test.[20] Then 487 mg of the acetylated peptide–resin (Ac-peptide–resin) was obtained. The Ac-peptide was cleaved from the resin and partially deprotected with 10 ml of trifluoroacetic acid (TFA) in the presence of 0.25 ml of *m*-cresol as scavenger, by stirring for 1.0 h at 25 °C, then peptide was sedimented by diethyl ether. The crude peptide was confirmed as the desired product from the molecular ion peak $[M + H]^+$ in MALDI-TOFMS. Ac-peptide (60 mg, *ca.* 24 μmol) and NH_2-β-CD (3 equiv.) were dissolved in 3 ml of NMP in the presence of HBTU (3 equiv.), $HOBt·H_2O$ (3 equiv.) and DIEA (6 equiv.) and stirred at 25 °C for 24 h. The crude product was obtained as a precipitate (140 mg, *ca.* 24 μmol) in ether and analyzed by reversed phase HPLC and identified by MALDI-TOFMS (*m*/*z* 3628.6 $[(M+H)^+]$, calc. 3628.0). The crude peptide was treated with 2.5 ml of trimethylsilyl trifluoromethanesulfonate[10] in the presence of 1.5 ml of thioanisole and 0.25 ml of *m*-cresol in 8 ml of TFA solution at 0 °C for 2.0 h. The final product, EHβ19, was purified by HPLC using a reversed C4 column and identified by MALDI-TOFMS (*m*/*z* 2993.7 $[(M + H)^+]$, calc. 2993.1). Total yield from the resin was 10%.

Synthesis of βHE19. This compound was synthesized and purified in an identical way to EHβ19, except for the introduction of Glu4(tBu) and Glu14(Bzl) instead of Glu4(Bzl) and Glu14(tBu), respectively. MALDI-TOFMS (*m*/*z* 2993.3 $[(M+H)^+]$, calc. 2993.1). Total yield from the resin was 23%.

Synthesis of βH19. This compound was synthesized and purified in an identical way to EHβ19, except for the introduction of Glu4(tBu) and Ala14 instead of Glu4(Bzl) and Glu14-(tBu), respectively. MALDI-TOFMS (*m*/*z* 2934.4 $[(M + H)^+]$, calc. 2935.1). Total yield from the resin was 20%.

Synthesis of Hβ19. This compound was synthesized and purified in an identical way to EHβ19, except for the introduction of Ala4 instead of Glu4(Bzl). MALDI-TOFMS (*m*/*z* 2935.4 $[(M + H)^+]$, calc. 2935.1). Total yield from the resin was 6%.

Preparation of substrate

Boc-D-alanine *p*-nitrophenyl ester was synthesized by coupling Boc-D-alanine with *p*-nitrophenolate in the presence of dicyclohexylcarbodiimide in DMF. Product was purified by silica gel chromatography and recrystallization from hexane and identified by ^1H NMR in $CDCl_3$.

Circular dichroism measurements

Circular dichroism spectra were recorded on a J-720WI spectropolarimeter equipped with a thermoregulator using a quartz cell with 0.1 cm pathlength in the amide region (190–250 nm) at 25 °C. Stock solutions of all CD–peptides were prepared as a trifluoroethanol solution (2.0×10^{-3} mol dm^{-3}) and diluted in 5.0×10^{-2} mol dm^{-3} phosphate buffer solution (pH 5.0, 6.0, 6.5, 7.0 and 8.0). The final concentration of CD–peptides was 2.5×10^{-5} mol dm^{-3} in each measurement.

Hydrolysis measurement

Initial rates in the hydrolysis were measured in 5.0×10^{-2} mol dm^{-3} phosphate buffer solution (pH 5.0, 6.0, 6.5, 7.0 and 8.0) and determined by the increase of the absorbance at 320 nm (pH 5.0, 6.0 and 6.5) or 400 nm (pH 7.0 and 8.0) for released *p*-nitrophenolate as a product. All the absorbance was monitored using a UV/Vis spectrophotometer (Shimadzu) 3100 using a quartz cell with 1.0 cm pathlength at 25 °C. Stock solutions of all CD–peptides were prepared as an NMP solution (2.0×10^{-3} mol dm^{-3}) and diluted in phosphate buffer solution (the final concentration in each measurement was 2.5×10^{-5} mol dm^{-3}). Stock solutions of substrates were prepared with acetonitrile (1.0×10^{-3} mol dm^{-3}) and diluted in phosphate buffer solution (3.0×10^{-4} mol dm^{-3}) in each measurement.

Kinetics study

Substrate concentrations were varied from 1.0×10^{-4} to 5.0×10^{-4} mol dm^{-3} at 25 °C and pH 6.0. Initial rates of substrate hydrolysis were plotted against substrate concentrations. Kinetic parameters were obtained by least squares curve fitting analysis with the Michaelis–Menten equation [eqn. (1)] using Kaleida Graph (Synergy Software).

$$V_0 = k_{cat}[C]_0[S]/([S] + K_m) \quad (1)$$

V_0 represents the initial rate of hydrolysis, k_{cat} and K_m represent the first-order rate constant from the catalyst–substrate complex and the Michaelis–Menten constant, respectively, and $[C]_0$ and [S] represent the initial concentration of CD–peptide and the concentration of substrate, respectively.

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References

1 For a review of cyclodextrins as enzyme mimics, (*a*) R. Breslow and S. Dong, *Chem. Rev.*, 1998, **98**, 1997; (*b*) M. Komiyama and H. Shigekawa, *Comprehensive Supramolecular Chemistry*, *Vol. 3*, *Cyclodextrins*; J. Szejtli and T. Osa, eds.; Pergamon: Oxford, New York, Tokyo, 1996, p. 401.
2 L. Baltzer, K. S. Broo, H. Nilsson, J. Nilsson and A. Flodberg, *Bioorg. Med. Chem. Soc.*, 1999, **7**, 83.
3 V. T. D'Souza and M. L. Bender, *Acc. Chem. Res.*, 1987, **20**, 146.
4 V. T. D'Souza, K. Hanabusa, T. O'Leary, R. C. Gadwood and M. L. Bender, *Biochem. Biophys. Res. Commun.*, 1985, **129**, 727.
5 B. F. Ekberg, L. I. Anderson and K. Mosbach, *Carbohydr. Res.*, 1989, **192**, 111.
6 For a review of the *de novo* design of peptides, (*a*) W. F. DeGrado, C. M. Summa, V. Pavone, F. Nastri and A. Lombardi, *Annu. Rev. Biochem.*, 1999, **68**, 779; (*b*) J. P. Schneider and J. W. Kelly, *Chem. Rev.*, 1995, **95**, 2164.
7 H. Tsutsumi, K. Hamasaki, H. Mihara and A. Ueno, *Bioorg. Med. Chem. Lett.*, 2000, **8**, 741.
8 P. Y. Chou and G. D. Fasman, *Annu. Rev. Biochem.*, 1978, **47**, 258.
9 (*a*) L. Serrano, A. Horovitz, B. Avron, M. Bycroft and A. R. Fersht, *Biochemistry*, 1990, **29**, 9343; (*b*) S. Dao-pin, U. Sauer, H. Nicholson and B. W. Matthews, *Biochemistry*, 1991, **30**, 7142.
10 K. Hamasaki, H. Ikeda, A. Nakamura, A. Ueno, F. Toda, I. Suzuki and T. Osa, *J. Am. Chem. Soc.*, 1993, **115**, 5035.
11 N. Fujii, A. Otaka, O. Ikemura, K. Akaji, S. Funakoshi, Y. Hayashi, Y. Kuroda and H. Yajima, *J. Chem. Soc.*, *Chem. Commun.*, 1987, 274.
12 Due to the poor solubility of the cyclodextrin–peptide hybrids in water, trifluoroethanol (TFE) stock solutions of CD–peptides (2.0×10^{-3} mol dm^{-3}) were prepared and diluted in phosphate buffer (5.0×10^{-2} mol dm^{-3}). The final concentration of the catalysts was 2.5×10^{-5} mol dm^{-3}. The pH was varied from 5.0 to 8.0.
13 J. M. Scholtz, H. Qian, E. J. York, J. M. Stewart and R. L. Baldwin, *Biopolymers*, 1991, **31**, 1463.
14 H. Tsutsumi, K. Hamasaki, H. Mihara and A. Ueno, unpublished data.
15 The UV absorption wavelengths of the *p*-nitrophenolate were varied depending on the pH (320 or 400 nm).
16 For the study of ester hydrolysis, the stock solutions of CD–peptides (2.0×10^{-3} mol dm^{-3}) were prepared with *N*-methyl-2-pyrrolidone (NMP) and diluted in phosphate buffer (5.0×10^{-2} mol dm^{-3}). The final concentration of both catalysts was 2.5×10^{-5} mol dm^{-3}. The pH was varied from 5.0 to 8.0.
17 k_{cat} is the first order rate constant for the reaction from the catalyst-substrate complex.
18 A. J. Kirby, J. K. Kochi, H. A. Kurtz, O. S. Tee and R. V. Williams, *Adv. Phys. Org. Chem.*, 1994, 29.
19 E. Atherton and R. C. Sheppard, *Solid Phase Synthesis*: *A Practical Approach*, IRL Press, Oxford, 1989.
20 Kaiser test reagents were ninhydrin (0.5 g in 10 ml of ethanol), phenol (8 g in 2 ml of ethanol) and potassium cyanide (0.2 ml of 10 mmol dm^{-3} in 10 ml of pyridine). The coupling efficiency was checked by utilizing the ninhydrin reaction of the amino group. The resin was heated for 2 min in the presence of Kaiser test reagents (50 μl, respectively).