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Hydrolytic Reaction by Zinc Finger Mutant Peptides: Successful Redesign of Structural Zinc Sites into Catalytic Zinc Sites

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To redesign a metal site originally required for the stabilization of a folded protein structure into a functional metal site, we constructed a series of zinc finger mutant peptides such as zf(CCHG) and zf(GCHH), in which one zinc-coordinating residue is substituted into a noncoordinating one. The mutant peptides having water bound to the zinc ion catalyzed the hydrolysis of 4-nitrophenyl acetate as well as the enantioselective hydrolysis of amino acid esters. All the zinc complexes of the mutant peptides showed hydrolytic activity, depending on their peptide sequences. In contrast, the zinc complex of the wild-type, zf(CCHH), and zinc ion alone exhibited no hydrolytic ability. These results clearly indicate that the catalytic abilities are predominantly attributed to the zinc center in the zinc complexes of the mutant peptides. Kinetic studies of the mutant peptides demonstrated that the catalytic hydrolysis is affected by the electron-donating ability of the protein ligands and the coordination environment. In addition, the pH dependence of the hydrolysis strongly suggests that the zinc-coordinated hydroxide ion participates the catalytic reaction. This report is the first successful study of catalytically active zinc finger peptides.

Introduction

The design and synthesis of new functional metalloproteins are some of the current topics in protein engineering. The successful design of metalloproteins significantly contributes to our understanding of the fundamental principles in chemistry and biology and also provides an economic alternative for biotechnological applications. This subject has been mainly approached through de novo protein design[1−4] as well as through the introduction of novel metal binding sites into naturally occurring proteins.[5,6]

The metal ions in proteins are roughly divided into two different classes: structural and catalytic metal ions. Although various metal ions are found as structural metals in many proteins, zinc is one of the most common metals. In zinc-containing metalloproteins, zinc can play both a structural role and a catalytic one.[7] Zinc ion in zinc finger motifs is a typical structural metal ion, stabilizing the compact structure required for specific DNA recognition.[8−11] On the other hand, a catalytic zinc ion directly participates in the catalytic mechanism, interacting with the substrate molecules undergoing reaction. In contrast to structural zinc sites, a unique feature of a catalytic zinc site is the existence of vacant site(s); that is, the zinc coordination geometry contains at least one water molecule in addition to three or four protein ligands.[12,13] Indeed, the zinc-bound water is a critical component for a catalytic zinc site. Several designs for three-histidine zinc sites have been reported, in which the three histidine sites were constructed in the four-helix bundle,[14,15] antibody,[16,17] and human retinal binding protein.[18] Regan et

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al. demonstrated the contribution of each ligand in the de novo designed protein to the geometry and affinity of metal binding,[19] and they created a de novo tetrahedral three-coordinate site with a vacant ligand position in a protein.[20] Lombardi, DeGrado, and their co-workers designed a series of dimeric four-helix bundles with metal binding sites.[3,4] One of these de novo metalloproteins has a carboxylate-bridged diiron center near the center of the structure, which is capable of binding azide, acetate, and small aromatic moelcules.[3] However, it still remains a significant challenge to effectively design metal binding sites with catalytic activities. As one interesting strategy to this objective, we now report a trial to redesign a metal site originally required for the stabilization of a folded protein structure into a functional metal site for catalytic hydrolysis.

The C_2H_2-type zinc finger motif comprises approximately 30 amino acids and folds into the $\beta\beta\alpha$ conformation,[21−24] stabilized by a structural zinc ion between a pair of cysteines from the β-sheet and a pair of histidines from the α-helix.[25,26] To clarify the role of the individual amino acids bound to the zinc ion, we constructed a series of zinc finger mutants, in which one zinc-coordinating residue is substituted into a noncoordinating one.[27] Our previous results confirmed that all of these mutants retain the ability of binding 1 equiv of zinc ion and folding into compact structures, like the wild-type zinc finger peptide, zf(CCHH), despite their tridentate coordinations. The study of Co(II)-substituted mutants also showed that the Zn(II) has a four-coordinated tetrahedral or five-coordinated geometry, depending on the peptide sequences. The unsaturated zinc sites seem to be occupied by one or two water molecules, and this information prompted us to examine the hydrolytic ability of these artificial zinc sites in these zinc finger mutants. Here we would like to report the first catalytically active zinc finger peptides and the relationship between the catalytic activity and the coordination environments of the zinc center of the zinc finger mutants.

Results and Discussion

Figure 1 summarizes the zinc finger peptides used in this study. The hydrolysis ability of the zinc finger mutants was investigated by using 4-nitrophenyl acetate (NA) as the substrate (Scheme 1 and Figure 2). Table 1 shows the NA hydrolysis rate constants of the Zn(II)−peptide complexes. Herein, the coordination numbers are deduced from the absorption spectral characteristics of the corresponding Co(II) complexes. All the zinc complexes of the mutant peptides

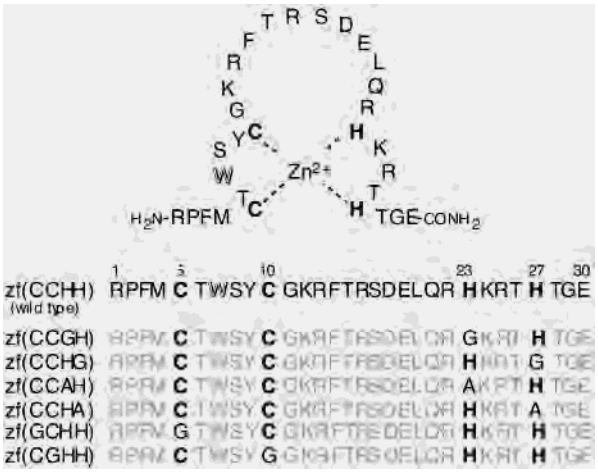


Figure 1. Amino acid sequences of zinc finger peptides. Ligands of Zn(II) and Trp residues are presented by bold and shadowed letters, respectively.

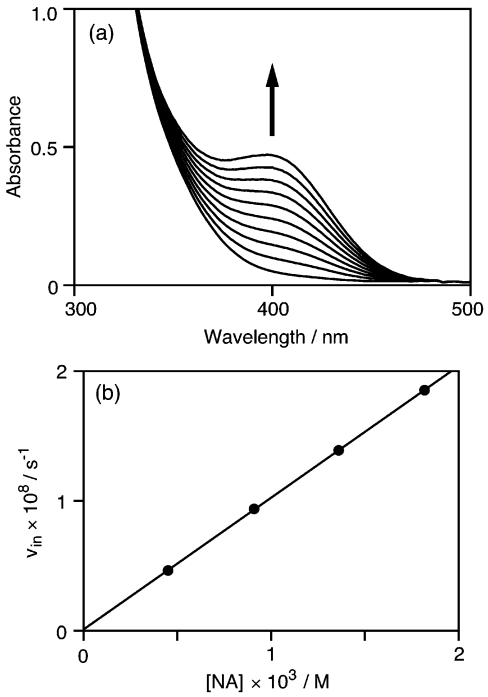
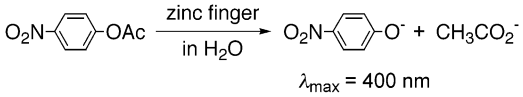


Figure 2. (a) Typical spectral change upon NA hydrolysis by Zn(II)−zf(CCHG). (b) Dependence of initial velocity on substrate concentration at pH 7.5.

Scheme 1



revealed appreciable hydrolytic activity, depending on their peptide sequences. These catalytic activities were evidently higher than that of the zinc−cyclen (1,4,7,10-tetraazacyclododecane) complex, which is one of the best modelcomplexes for hydrolytic zinc enzymes.[28,29] Although the free (uncomplexed with zinc ion) peptides also presented some hydrolytic activity due to the uncoordinated histidine residues[30] (entries h−l; vide infra), the zinc complex of the wild-

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Table 1. NA Hydrolysis Rate Constant k^a

entry	catal	α-helix induction	coord no.[b] (no. of vacant sites)	$10k$ (M^{-1} s^{-1})
a	Zn(II)−zf(CCHH)	yes	4 (0)	0
b	Zn(II)−zf(CCGH)	no	5 (2)	2.18 ± 0.085
c	Zn(II)−zf(CCAH)	no	5 (2)	2.32 ± 0.051
d	Zn(II)−zf(CCHG)	yes	5 (2)	3.51 ± 0.182
e	Zn(II)−zf(CCHA)	yes	4 (1)	5.68 ± 0.228
f	Zn(II)−zf(GCHH)	yes	5 (2)	3.99 ± 0.014
g	Zn(II)−zf(CGHH)	yes	5 (2)	4.58 ± 0.021
h	apo-zf(CCHH)			4.09 ± 0.102
i	apo-zf(CCGH)			3.45 ± 0.0761
j	apo-zf(CCHG)			3.49 ± 0.256
k	apo-zf(GCHH)			3.97 ± 0.0465
l	apo-zf(CGHH)			3.76 ± 0.235
m	Zn(II)−cyclen		5 (1)[c]	0.392 ± 0.0171
n	Zn(II)			0

[a] Data were acquired in 20 mM HEPES buffer (pH 7.5) containing 100 mM NaCl and 3.5% acetonitrile at 25 °C. [b] UV−visible absorption studies of Co(II)−peptide complexes, except for cyclen. [c] Reference 28.

type, zf(CCHH), exhibited no hydrolytic ability due to zinc coordination of the histidine residues. On the basis of these results, the histidine residues that are not involved in the zinc coordination and also directly bound to the zinc ion cannot hydrolyze NA. Zinc ion alone also exhibited no hydrolytic ability. Fluorescence studies confirmed that the mutant peptides were able to bind 1 equiv of Zn(II) under the present experimental condition; i.e., the fluorescence intensity of the zinc finger peptides was enhanced upon the addition of zinc ion and reached a plateau at the point of 1 equiv of Zn(II) in the same manner as previously reported.[27,31] Furthermore, the 1:1 complexes were verified by the electrospray ionization mass spectra of the Zn(II)−peptide complexes (data not shown). From these results, the contribution of apopeptides to the hydrolytic reaction rate observed for Zn(II)−peptide should be negligible due to their very low concentration. Therefore, the hydrolytic activities observed for the zinc complexes (entries b−g) are predominantly attributed to the zinc center in the zinc complexes of the mutant peptides.

Among these mutant zinc finger peptides, the zinc complex of zf(CCHA) showed the highest activity. This zinc complex folds into a structure similar to that of the wild-type peptide, zf(CCHH), and has a tetrahedral coordination geometry with one vacant ligand position presumably occupied by one water molecule.[27] Merkle et al. previously reported that a truncated C_2H zinc finger peptide does not show any hydrolytic activity, although the zinc−peptide complex possesses a coordination environment similar to zf(CCHA).[32] In our opinion, the zinc site in the truncated C_2H zinc finger is highly exposed to solvent, while the zinc site in zf(CCHA) is in a more hydrophobic environment due to the presence of its C-terminus. This difference may reflect the different hydrolytic activity between those two zinc finger peptides, because the hydrophobic environment can affect the substrate-binding event as well as the acidity of water bound to the zinc ion,[33] although we cannot exclude the possibility due to different reaction conditions.

Chart 1. Amino Acid Sequences of HHH- and HHHH-Type Zinc Finger Mutant Peptides[a]

	1 5 10 23 27 30
zf(AHHH)	RPFM A TWSY **H** GKRFTRSDELQR **H** KRT **H** TGE
zf(HAHH)	RPFM **H** TWSY A GKRFTRSDELQR **H** KRT **H** TGE
zf(HHAH)	RPFM **H** TWSY **H** GKRFTRSDELQR A KRT **H** TGE
zf(HHHA)	RPFM **H** TWSY **H** GKRFTRSDELQR **H** KRT A TGE
zf(HHHH)	RPFM **H** TWSY **H** GKRFTRSDELQR **H** KRT **H** TGE

[a] Coordinating amino acid ligands are presented in bold type.

Table 2. NA Hydrolysis Rate Constant k of HHH- and HHHH-Type Peptides[a]

catal	α-helix induction	coord no.[b] (no. of vacant sites)	$10k$ (M^{-1} s^{-1})
Zn(II)−zf(AHHH)	weak	6 (3)	4.78 ± 0.057
Zn(II)−zf(HAHH)	weak	6 (3)	4.97 ± 0.0058
Zn(II)−zf(HHAH)	no	6 (3)	3.70 ± 0.289
Zn(II)−zf(HHHA)	weak	6 (3)	4.43 ± 0.147
Zn(II)−zf(HHHH)	strong	6 (2)	9.66 ± 0.492

[a] Data were acquired in 20 mM HEPES buffer (pH 7.5) containing 100 mM NaCl and 3.5% acetonitrile at 25 °C. [b] UV−visible absorption studies of Co(II)−peptide complexes.

Except for zf(CCHA), all the other mutants have a five-coordinate geometry. Their hydrolytic activities decrease in the order of zf(CGHH) > zf(GCHH) > zf(CCHG) > zf(CCAH) > zf(CCGH). These zinc finger mutants can be categorized into two groups: the CHH-type and CCH-type zinc fingers. The latter two mutants in the CCH type do not form an α-helix, unlike zf(CCHG) in the same type, because His23 is a critical residue for the α-helix formation.[27] Zf(CCHG) shows the highest activity in the CCH type, probably because such a folded structure is preferred for the hydrolytic activity. The CHH-type peptides reveal higher activity than the CCH-type peptides. Presumably, this result can be rationalized by the Lewis acidity of the zinc center. In general, a zinc complex with a higher Lewis acidity exhibits a higher hydrolytic activity.[33] The zinc-coordinating cysteine residue decreases the Lewis acidity of the zinc ion through its electron-donating ability and, consequently, reduces the hydrolytic reactivity. To increase the Lewis acidity of the zinc center, we constructed four HHH-type peptides, zf(AHHH), zf(HAHH), zf(HHAH), and zf(HHHA) (Chart 1). The results of the metal binding study and their hydrolytic activities are listed in Table 2. As expected, the mutant peptides showed increased activity compared to the corresponding peptide, for example, zf(HHAH) > zf(CCAH). In this series of HHH-type peptides, we again observed that the compact folded structure provides a certain contribution to the high hydrolysis ability. We have demonstrated the HHHH-type peptide, which induces the α-helix structure upon zinc-binding.[34] The hydrolysis ability of zf(HHHH) should be higher than those of other mutants because of its compact structure and Lewis acidic zinc center. Therefore, we synthesized zf(HHHH), whose sequence is shown in

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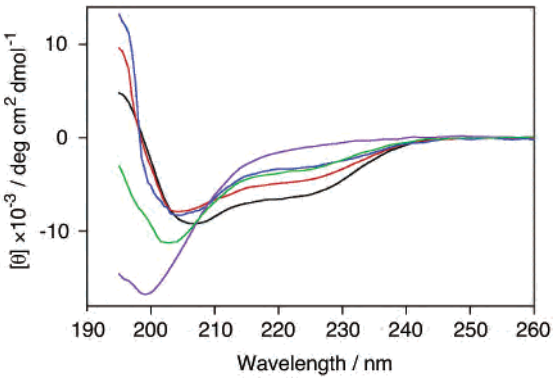

Figure 3. CD spectra of HHH- and HHHH-type zinc finger mutant peptides in 10 mM HEPES (pH 7.5) containing 50 mM NaCl in the presence of 1.5 equiv of $ZnCl_2$ at 20 °C. The peptide concentration was 25 μM. Red, blue, purple, green, and black lines show Zn(II)−zf(AHHH), Zn(II)−zf(HAHH), Zn(II)−zf(HHAH), Zn(II)−zf(HHHA), and Zn(II)−zf(HHHH), respectively.

Chart 1. The metal binding study demonstrated that the present mutant peptide induced an α-helix structure upon zinc binding (Figure 3), which is consistent with the previous report of the zinc-finger protein containing three tandem HHHH-type motifs.[34,35] The α-helix induction of Zn(II)−zf(HHHH) was stronger than those of other Zn(II) mutant peptide complexes. In addition, an electronic spectral study on the Co(II)−zf(HHHH) complex indicates the coordination geometry containing two vacant sites.[35−37] Since cobalt has a greater tendency to bind as an octahedral than zinc,[38] the possibility of other coordination geometries cannot be ruled out for the Zn(II) complex. However, the pH dependency of the hydrolytic activity by Zn(II)−zf(HHHH) strongly suggests that hydroxide ion(s) bound to the zinc ion play a central role in the hydrolysis of NA (see below and Figure 5). As expected, the hydrolytic activity of Zn(II)−zf(HHHH) was remarkably enhanced, compared with those of the tridentate-type zinc finger peptides. The catalytic reaction rate constant (k) is 1.7-fold greater than that observed for zf(CCHA).

(35) Although the NMR chemical shifts and NOEs of the histidine imidazole groups suggest three or four histidine imidazole residues in contact with Zn(II), the NMR data provided little information about the Zn(II) coordination geometry. The NMR structure[34] was calculated on the basis of the assumption that the zinc was tetrahedrally coordinated as can be seen in the wild type.[49] The visible spectra of the corresponding Co(II) complex and the high catalytic activity of the Zn(II) complex strongly suggest a 6-coordination rather than a 4-coordination for the mutant complex Zn(II)−zf(HHHH). In contrast, the wild complex Zn(II)−zf(CCHH) exactly adopts a tetrahedral geometry through two cysteine and two histidene residues, consistent with the spectral data of the Co(II) complex and no catalytic activity of the Zn(II) complex.

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(37) The d−d bands of Co(II)−zf(HHHH) and the pH dependence of the hydrolytic activity suggest that the zinc center of Zn(II)−zf(HHHH) should adopt a six-coordinate geometry rather than a tetrahedral geometry (Figures 5 and S1), although we have assumed in the previous report that each zinc finger motif of the related three tandem Zn(II)−zf(HHHH) protein has a tetrahedral geometry.[34] The three tandem Zn(II)−zf(HHHH) proteins have a high affinity for DNA, despite the structural change of the zinc site from a tetrahedral to a six-coordinate geometry. The three tandem Zn(II)−zf(HHHH) proteins exhibit an α-helix induction upon Zn(II) binding, whose helicity is comparable to the wild type. (The present Zn(II)−zf(HHHH) peptide also induces an α-helix to a similar extent as the wild type.) As well-known, the α-helix region of zinc finger motifs plays a central role in DNA recognition. We think that is the reason the Zn(II)−zf(HHHH) protein retains a high affinity for DNA. The structural change of the zinc center might not significantly decrease the DNA affinity for Zn(II)−zf(HHHH).

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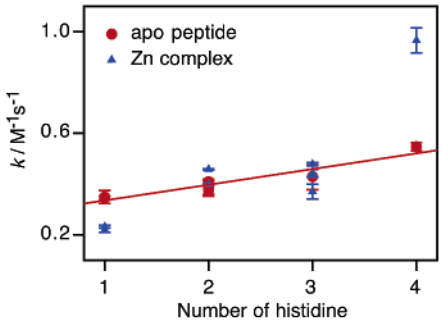

Figure 4. Plot of the hydrolysis rate constants vs the number of histidine residues at pH 7.5.

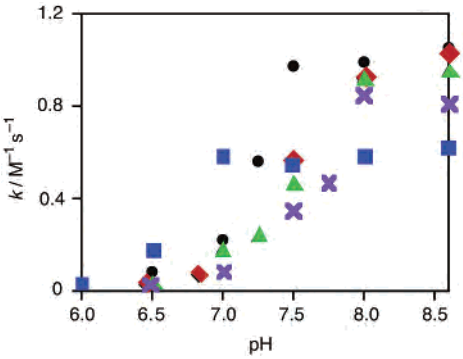

Figure 5. pH-dependence profile for the hydrolysis rate of NA by the zinc finger mutant peptides: Zn(II)−zf(CCHG) (purple), Zn(II)−zf(CCHA) (red), Zn(II)−zf(CGHH) (green), Zn(II)−zf(HHHH) (black), and apo−zf(HHHH) (blue) in neutral pH region.

Furthermore, it is noteworthy that the hydrolysis reactivity of apopeptides is approximately parallel with the number of histidine residues, which is contrast to those of the Zn(II)−peptide complexes (Figure 4). This result unequivocally supports that the hydrolysis by apopeptides is caused by histidine residues, while the hydrolysis by zinc−peptides is caused by the zinc center of zinc finger peptides. In the latter cases, the reaction is affected by the environmental factors of zinc coordination center such as electron-donating ability of ligands, coordination structure, and folding structure.

It is noteworthy that the Co(II)−peptide complexes also exhibited the hydrolytic ability with certain enhancements of reactivity compared with the corresponding Zn(II) complex (data not shown). This result strongly suggests the possibility that incorporation of other metals into the apopeptides can induce a larger hydrolytic activity or other new functions. The studies along this line are in progress.

To gain some insights into the hydrolytic reaction, the pH dependence was investigated. The inflection points of the pH dependence (Figure 5) correspond to the pK_a values of the reactive species for the catalytic hydrolysis of NA (Table 3). The pH dependence for the apo-zf(HHHH) is apparently different from those for the zinc−peptide complexes with respect to its inflection point and the maximum value of k (k_{Zn-OH}; see Experimental Section). Judging from the pH value of the inflection point, the active sites of apo-

Table 3. pH of the Inflection Point and the Maxima of *k* for the Zn(II)−Peptide Complexes[a]

catal	pH of the inflectn pt	k_{max} (M^{-1} s^{-1})
Zn(II)−zf(HHHH)	7.1	1.15
Zn(II)−zf(CGHH)	7.4	1.08
Zn(II)−zf(CCHG)	7.6	0.979
Zn(II)−zf(CCHA)	7.3	1.09
apo-zf(HHHH)	6.3	0.628

[a] Data were acquired in 20 mM buffer containing 100 mM NaCl and 3.5% acetonitrile at 25 °C.

Scheme 2

$$[Zn{-}(OH_2)_n] \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} [Zn{-}OH^-(OH_2)_{n-1}]$$

zf(HHHH) must be histidine residues.[39] For the zinc−peptide complexes, on the other hand, the pK_a values increase as the expected Lewis acidity of the zinc center decreases. This fact indicates that the monodeprotonated water bound to the zinc, Zn−OH, is the active species for the hydrolysis by the zinc−peptide complexes (Scheme 2). In addition, Zn(II)−zf(CCHA) showed only one inflection point, whereas the zinc complexes of zf(CGHH) and zf(HHHH) gave two inflection points (Figure S1). This result agrees with the number of coordinated water molecules of the mutant peptides previously discussed.[27]

Several anions, such as thiocyanate or azide, behave as competitive inhibitors of hydrolytic metalloenzymes.[40−43] X-ray crystallographic studies on carbonic anhydrase revealed that these anions coordinate directly the active zinc center and effectively prevent the hydrolytic reaction catalyzed by the enzymes.[40,41] In addition, such anions have been utilized as inhibitors for hydrolytic reaction by zinc complexes.[44−46] To confirm the hydrolytic role of zinc-bound water in the Zn(II)−peptide complexes, we employed thiocyanate anion as an inhibitor. The hydrolysis rate of the Zn(II)−zf(HHHH) peptide complex was remarkably decreased to about 20% in the presence of 1 equiv of sodium thiocyanate (Figure 6); on the other hand, apopeptides were not affected by thiocyanate anion. Interestingly, the activity of Zn(II)−zf(CCHG) was also decreased to the similar extent of that observed for the Zn(II)−zf(HHHH) peptide (to 34%), while Zn(II)−zf(CCHA) was nearly completely diminished (to 0.3%) with 1 equiv of thiocyanate anion (data not shown). These results are in agreement with d−d absorption studies on cobalt complexes and pH dependence of activity (Figures 5 and S1), suggesting that zinc-coordinated zf(HHHH) and zf(CCHG) contain two vacant sites and that zf(CCHA) possesses only one vacant site.[27] Moreover, excess thiocyanate anion caused no change for the CD spectra of Zn(II)−peptide complexes (data not shown). These studies unequivocally demonstrate that the zinc center of the Zn(II)−mutant peptide complexes is the active site of hydrolysis as hydrolytic zinc enzymes.

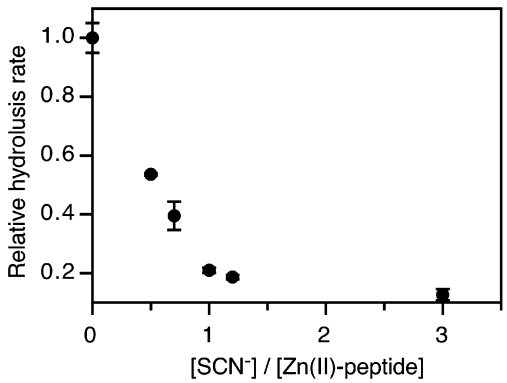

Figure 6. Relative hydrolysis rate constants catalyzed by Zn(II)−zf(HHHH) in the presence of sodium thiocyanate.

Table 4. Gln-ONp Hydrolysis Rate Constant *k*[a]

	k (M^{-1} s^{-1})		
catal	D-Gln-ONp	L-Gln-ONp	*k*L/*k*D
Zn(II)−zf(CCHH)	0	0	
Zn(II)−zf(HHHH)	0.70 ± 0.031	14.6 ± 0.145	20.8
Zn(II)−zf(CCHG)	1.05 ± 0.091	5.75 ± 0.239	5.48
Zn(II)−zf(CCHA)	0.53 ± 0.009	3.14 ± 0.110	5.92
apo-zf(HHHH)	3.13 ± 0.011	3.19 ± 0.165	1.02
Zn(II)−cyclen	1.43 ± 0.144	1.42 ± 0.113	0.993
Zn(II)	0	0	

[a] Data were acquired in 20 mM HEPES buffer (pH 7.5) containing 100 mM NaCl and 3.5% tetrahydrofuran at 25 °C.

Of special interest is the fact that the zinc complex of zf(HHHH) catalyzed the hydrolysis of amino acid esters (Boc-glutamine 4-nitrophenyl ester: D- or L-Gln-ONp) with considerable enantioselectivity (Table 4). Zn(II) complex with tridentate peptides, zf(CCHG) and zf(CCHA), also performed enantioselective hydrolysis of Gln-ONp; however, the hydrolytic activity of these two Zn(II)−peptide complexes for Gln-ONp was reversed compared with that for NA; i.e., *k*(zf(CCHG)) < *k*(zf(CCHA)) for NA and *k*(zf(CCHG)) > *k*(zf(CCHA)) for Gln-ONp. The results may be reflected with substrate accessibility to the zinc center, because Zn(II)−zf(CCHA) complex has only one accessible site in its a tetrahedral geometry and Zn(II)−zf(CCHA) has two vacant sites.[47] In contrast, the uncomplexed peptide, apo-zf(HHHH), showed virtually no enantioselectivity in this reaction. These results suggest that the active zinc centers in the mutant peptides are situated in asymmetric environments sufficient enough to perform enantioselective hydrolysis of Gln-ONp.

The study demonstrates that the redesign of a structural metal site in proteins is promising for the creation of novel catalytically active metalloproteins. Indeed, this report is the first example of the catalytically active zinc peptides complexes by redesign of the structural zinc ion of zinc finger

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peptides. Such a method is also utilized by "nature"; some matrix prometalloproteinases are activated by the conversion of a structural tetradentate-zinc site to a tridentate-zinc site.[48] Zinc finger peptides would provide a convenient scaffold for the design of novel functional metalloproteins, because the folded structures are well studied[25,26,49] and the relatively short sequence allows us to prepare a wide variety of derivatives by a peptide synthesizer. By redesign of the metal site in the zinc finger peptides, the exploration of new functions is currently underway.

Experimental Section

Materials. Tetrahydrofuran (dehydrated, no stabilizer) and 4-nitrophenyl acetate were purchased from Wako Pure Chemical Industries, Ltd., and Sigma, respectively. All other chemicals were of the highest commercial grade and were used without further purification.

Peptide Preparation. HHH- and HHHH-type peptides were synthesized by a Shimazu PSSM-8 peptide synthesizer using the Fmoc solid-phase method. The obtained peptides were identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS): zf(AHHH), calcd ($[M + H]^+$) *m*/*e* 3717.17, obsd ($[M + H]^+$) *m*/*e* 3717.11; zf(HAHH), calcd ($[M + H]^+$) *m*/*e* 3717.17, obsd ($[M + H]^+$) *m*/*e* 3716.99; zf(HHAH), calcd ($[M + H]^+$) *m*/*e* 3717.17, obsd ($[M + H]^+$) *m*/*e* 3717.11; zf(HHHA), calcd ($[M + H]^+$) *m*/*e* 3717.17, obsd ($[M + H]^+$) *m*/*e* 3717.89; zf(HHHH), calcd ($[M + H]^+$) *m*/*e* 3783.23, obsd ($[M + H]^+$) *m*/*e* 3783.56. Other mutant peptides were prepared, purified, and checked according to our previous report.[27]

Kinetics of Hydrolysis. The hydrolysis rate of 4-nitrophenyl acetate (NA) was measured by following the increase in the released 4-nitrophenolate (NP) in buffer solution[50−52] on a Beckman Coulter DU7400 diode array spectrophotometer. The buffers used (20 mM) were MES (pH 6.4−6.7), HEPES (pH 6.8−8.0), and CHES (pH 8.6−9.5) containing 100 mM NaCl and 3.5% acetonitrile. For the initial rate determination, the following typical procedure was employed: A catalyst and 1.2 equiv of $ZnCl_2$ were incubated in the buffer solution for 1 h. After NA was added to the solution of the Zn(II)−peptide complex, the increase in the UV absorption was immediately recorded and then generally followed until the formation (ca. 2%) of NP at 25 °C. The ϵ values for NP were 1700 (pH 6.0), 4200 (pH 6.5), 8700 (pH 7.0), 12 800 (pH 7.5), 16 200 (pH 8.0), 17 300 (pH 8.5), 17 800 (pH 9.0), and 18 200 M^{-1} cm^{-1} (pH 9.5) at 400 nm. Hydrolysis of the Boc-glutamine 4-nitrophenyl ester (Gln-ONp) was examined using tetrahydrofuran instead of acetonitrile. The other conditions for the hydrolysis of Gln-ONp followed those of the NA hydrolysis.

In our experimental conditions, the initial rate linearly increased with the concentration of NA (Figure 2b). The initial rate (v_{in}) is expressed by

$$v_{in} = k_{obs}[NA] \quad (1)$$

where k_{obs} (s^{-1}) is the pseudo-first-order rate constant. In the absence of the catalysts, hydrolysis was observed, indicating that OH^- and solvent can promote hydrolysis. The value k_{obs} includes all the catalytic species such as the zinc complex and base. Therefore, k_{obs} can be expressed using

$$k_{obs} = k[cat] + k_{OH}[OH^-] + k_0 \quad (2)$$

where k (M^{-1} s^{-1}) and k_{OH} (M^{-1} s^{-1}) are the second-order rate constants of the catalyst and OH^-, respectively, and k_0 (s^{-1}) is the first-order rate constant of solvolysis. In the absence of zinc complex, k_{OH} and k_0 were obtained, which were similar to the literature values.[52]

CD Spectroscopy. CD spectra were obtained using a Jasco J-720. Measurements were carried out in 10 mM HEPES buffer (pH 7.5) containing 50 mM NaCl in a capped 1-mm path length cell at 20 °C under nitrogen. All spectra represent the average of 8−16 scans. Spectra were baseline-corrected and noise-reduced using the Jasco software.

pH Dependence. The deprotonation constants of the coordinated water were determined by fitting to eq 3, where k_{Zn-OH_2} (M^{-1} s^{-1}) and k_{Zn-OH} (M^{-1} s^{-1}) are the second-order rate constants of the Zn(II)-bound water and the monodeprotonated water bound to Zn(II), respectively.

$$k = \{k_{Zn-OH_2} + k_{Zn-OH}10^{(pH-pK_a)}\}/\{1 + 10^{(pH-pK_a)}\} \quad (3)$$

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Supporting Information Available: pH dependence of the zinc finger peptides. This material is available free of charge via the Internet at http://pubs.acs.org.

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