

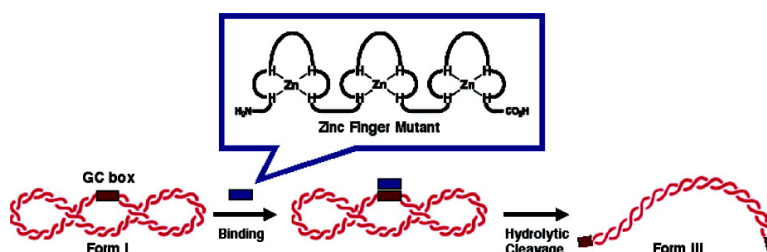
Communication

**Sequence-Selective and Hydrolytic Cleavage of DNA by Zinc Finger Mutants**

Akiko Nomura, and Yukio Sugiura

*J. Am. Chem. Soc.*, **2004**, 126 (47), 15374-15375 • DOI: 10.1021/ja045663l • Publication Date (Web): 03 November 2004

Downloaded from <http://pubs.acs.org> on April 5, 2009



**More About This Article**

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 1 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

[View the Full Text HTML](#)

## Sequence-Selective and Hydrolytic Cleavage of DNA by Zinc Finger Mutants

Akiko Nomura and Yukio Sugiura\*

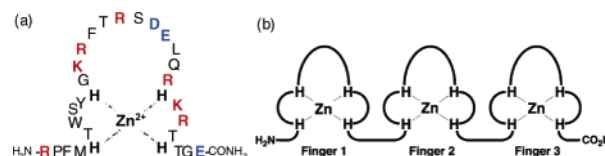
*Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan*

Received July 19, 2004; E-mail: sugiura@scl.kyoto-u.ac.jp

The molecules that can hydrolyze the DNA phosphoester at specific positions are valuable tools in biotechnology, thus facilitating DNA manipulation in a variety of applications. Although many naturally occurring nucleases have been utilized in the laboratory for this purpose, designing artificial nucleases is one of the most interesting current topics in the field of protein engineering because artificial nucleases could expand a set of recognizable DNA sequences beyond the limited sequences of currently available nucleases. Most of the artificial nucleases so far reported have been constructed by linking DNA binding moieties with DNA cleavage moieties.<sup>1,2</sup> For example, Ni(II)–peptide complexes<sup>1a,3</sup> have been attached as a DNA cutter at the terminus of DNA recognition proteins or molecules.<sup>4</sup> The other fascinating strategy for artificial nucleases is to construct a hydrolytic active site in the DNA binding peptides; for example, novel types of artificial nucleases have been developed based on HTH and EF-hand DNA binding peptides by introducing lanthanide ions in the original calcium binding site.<sup>5</sup>

We recently succeeded in constructing zinc finger peptides with hydrolytic ability by mutating amino acid residues coordinated to the zinc ion.<sup>6</sup> Since zinc finger motifs have been known to bind to the major groove of the DNA duplex in a sequence-specific fashion,<sup>7</sup> we expected that our zinc finger mutants would function as a novel type of artificial nuclease with high sequence specificity for DNA duplexes. In addition, DNA recognition by the zinc finger motifs is expected to cover a wide range of DNA sequences, compared to restriction enzymes, because zinc finger motifs bind to asymmetric sequences while the DNA sequences recognized by the restriction enzymes must be palindromic. In this context, we have studied the expansion of recognizable DNA sequences by zinc finger motifs (e.g., nine-tandem zinc finger proteins that can bind unusually longer DNA sequences<sup>8</sup> or  $\alpha$ -helix-exchanged zinc finger peptides that can bind to A/T-rich sequences instead of the original G/C-rich sequences).<sup>9</sup> Furthermore, the efforts to realize the DNA recognition of a wide variety of sequences by zinc finger motifs have been also enthusiastically pursued by other groups.<sup>10</sup> Aiming to create peptide-based biochemical tools exhibiting a site-specific DNA cleavage ability, in this study, we examined the ability of the zinc finger mutant peptides for sequence-selective DNA hydrolysis.

First of all, we examined the ability of a zinc finger mutant zf(HHHH) (**Zn•1**),<sup>6</sup> whose sequence is based on the second finger in the three-tandem zinc finger protein Sp1 (Figure 1a), to hydrolyze bis(4-nitrophenyl) phosphate (BNP) as a model compound for DNA.<sup>11</sup> It was revealed that **Zn•1** catalyzed the hydrolysis of BNP to afford 4-nitrophenolate and 4-nitrophenyl phosphate.<sup>12</sup> The estimated second-order rate constant, *k*, was notably higher than that observed for the zinc–cyclen complex, known to be an excellent functional model for hydrolytic zinc enzymes,<sup>11a–c,13</sup> by 2 orders of magnitude under identical conditions (Table 1). This result encouraged us to examine the DNA hydrolytic ability of **Zn•1**. We used a supercoiled plasmid DNA, pUC19GC, as a substrate since pUC19GC contains a GC box to which Sp1 specifically

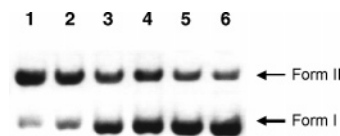


**Figure 1.** Zinc finger mutant peptide and protein. (a) The second zinc finger mutant zf(HHHH) (**Zn•1**). Ligands of the zinc ion, cationic amino acids, and anionic amino acids are denoted by bold black, red, and blue letters, respectively. (b) The three-tandem zinc finger protein (**Zn•2**).

**Table 1.** BNP Hydrolysis Rate Constant, *k*, by the Zinc Complexes<sup>a</sup>

catalyst	<i>k</i> × 10 <sup>-5</sup> (M <sup>-1</sup> s <sup>-1</sup> )
<b>Zn•1</b>	502 ± 24.5
apo-form of <b>Zn•1</b>	65.0 ± 5.28
zinc–cyclen	7.58 ± 0.696

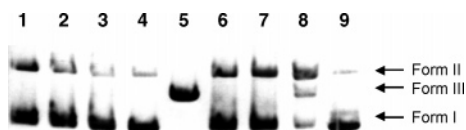
<sup>a</sup> The [catalyst] = 10–20 mM, [BNP]<sub>0</sub> = 2–6 mM in 20 mM HEPES buffer (pH 7.5) containing 0.1 M NaCl and 3.5% THF at 37 °C.



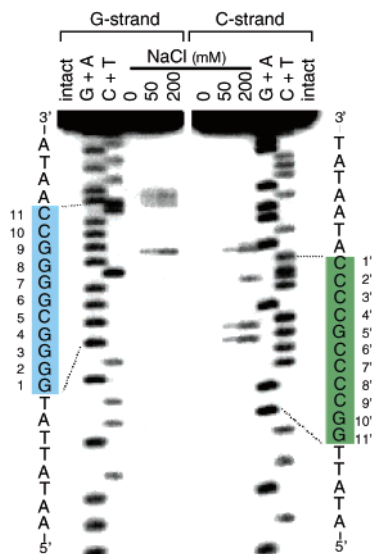
**Figure 2.** Ionic strength dependence of the plasmid DNA cleavage by **Zn•1**. The reactions were carried out at 37 °C for 96 h. [**Zn•1**] = 2 μM, [pUC19GC] = 57.4 nM (0.154 mM bp). Lane 1, 0 mM NaCl; lane 2, 3 mM NaCl; lane 3, 8 mM NaCl; lane 4, 18 mM NaCl; lane 5, 48 mM NaCl; lane 6, 98 mM NaCl.

binds.<sup>14</sup> DNA cleavage converts the supercoiled plasmid DNA (form I) to the nicked circular form (form II) and then to the linear form (form III). Despite the inactivated phosphoester, **Zn•1** converted pUC19GC to form II but not to form III (Figure S1 of the Supporting Information). The cleavage efficiency linearly increased as the zinc concentration increased and reached a plateau at the point of 1 equiv of zinc ion (Figure S2 of the Supporting Information). These results emphasize that the zinc ion plays a critical role in DNA hydrolysis.<sup>15</sup> However, the hydrolytic reactivity decreased along with an increase in the ionic strength (Figure 2), which suggests that the positively charged zinc finger peptide binds with the phosphate of DNA, rather than with the DNA bases, via electrostatic interactions. The same hydrolytic efficiency of **Zn•1** for pUC19, which contains no GC box, as for pUC19GC indicates nonselective binding of **Zn•1** with DNA (Figure S3 of the Supporting Information).

Tandem alignment of zinc finger mutants, like the native Sp1, should be a strategy to achieve selective binding at the GC box by the zinc finger mutants because repeated zinc finger motifs show a drastic enhancement in DNA affinity compared with that of a single zinc finger motif.<sup>16</sup> We already reported that a three-tandem zinc finger protein (**Zn•2**, Figure 1b), where two of the cysteine ligands in each finger motif were replaced by histidine ligands, retains a high affinity for the DNA duplex containing a GC box,



**Figure 3.** Cleavage of the plasmid DNA by **Zn<sup>2</sup>**. The reactions were carried out with 57.4 nM (0.154 mM bp) DNA and 6  $\mu$ M **Zn<sup>2</sup>** in 5 mM HEPES buffer (pH 7.5) at 37 °C for 48 h. Lanes 1–4, pUC19, and lanes 5–9, pUC19GC: lanes 1 and 6, 0 mM NaCl; lanes 2 and 7, 50 mM NaCl; lanes 3 and 8, 200 mM NaCl; lanes 4 and 9, DNA only; lane 5, HindIII-treated DNA.



**Figure 4.** Cleavage of 37 bp DNA by **Zn<sup>2</sup>**. The reactions were carried out with 10  $\mu$ M **Zn<sup>2</sup>** in 5 mM HEPES buffer (pH 7.5) containing 75 ng/ $\mu$ L poly(dI-dC) at 37 °C for 60 h. G + A and C + T are Maxam–Gilbert sequencing lanes. The GC box sequences are highlighted by light blue (G-strand) and yellowish green (C-strand). The numbers describe the positions of the base pairs.

with  $K_d = 85$  nM.<sup>17</sup> As expected, **Zn<sup>2</sup>** showed an enhanced cleavage activity for pUC19GC (Figure 3). Moreover, in sharp contrast to that of **Zn<sup>1</sup>**, the reactivity of **Zn<sup>2</sup>** for pUC19GC increased as the ionic strength increases. At [NaCl] = 200 mM, **Zn<sup>2</sup>** converted pUC19GC even to form III. On the other hand, **Zn<sup>2</sup>** showed a small hydrolytic activity for pUC19 at a high ionic strength. These results indicate that nonselective binding to DNA may be prevented at a higher ionic strength, and as a consequence, the hydrolytic cleavage of the GC box by **Zn<sup>2</sup>** was achieved through specific interactions, such as hydrogen bonding with DNA bases of the GC box, like the wild-type Sp1.

To specify in detail the cleaved sites within the GC box, a 37 bp DNA duplex containing a GC box was hydrolyzed by **Zn<sup>2</sup>**. Figure 4 shows the cleavage sites in the G- and C-strands of the DNA oligomer, in which the hydrolyzed sites appear as dark bands. Similar to that in pUC19GC, a higher ionic strength afforded a higher cleavage activity for the DNA oligomer. In addition, the results revealed that **Zn<sup>2</sup>** hydrolyzes the DNA phosphates around G(7) on the G-strand and around C(3'), C(6'), and C(7') on the C-strand. Cleavage of the two strands at the same positions is consistent with the transformation of the supercoiled pUC19GC to form III. Guanine methylation interference studies showed that the guanine at the 7-position weakly interacts with **Zn<sup>2</sup>**.<sup>17</sup> Such a weak interaction may cause an effective hydrolytic reaction at around the G(7) site by **Zn<sup>2</sup>**.

In conclusion, we successfully demonstrated selective hydrolysis of the DNA duplex at the GC box by the three-tandem zinc finger

mutant **Zn<sup>2</sup>**. Interestingly, at a high ionic strength, **Zn<sup>2</sup>** converted the plasmid DNA to the form III. The combination of the present strategy and the expansion of recognizable DNA sequences by zinc finger motifs would provide a wide range of artificial nucleases. Additionally, this hydrolytic activity may be the reason an H<sub>4</sub>-type zinc finger protein does not exist in nature. Detailed mechanistic studies about the site-specific hydrolysis of DNA and the construction of a family of artificial nucleases based on the zinc finger motif are currently ongoing in our laboratory.

**Acknowledgment.** This study was supported in part by Grants-in-Aid for the COE Projects “Element Science” and “Kyoto University Alliance for Chemistry”, and Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology.

**Supporting Information Available:** Experimental details and results of DNA hydrolysis. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References

- (1) For reviews, see: (a) Long, E. C. *Acc. Chem. Res.* **1999**, *32*, 827–836. (b) Sreedhara, A.; Cowan, J. A. *J. Biol. Inorg. Chem.* **2001**, *6*, 337–347. (c) Franklin, S. J. *Curr. Opin. Chem. Biol.* **2001**, *5*, 201–208.
- (2) For recent examples, see: (a) Sissi, C.; Rossi, P.; Felluga, F.; Formaggio, F.; Palumbo, M.; Tecilla, P.; Tonioli, C.; Scrimin, P. *J. Am. Chem. Soc.* **2001**, *123*, 3169–3170. (b) Bibikova, M.; Carroll, D.; Segal, D. J.; Trautman, J. K.; Smith, J.; Kim, Y. G.; Chandrasegaran, S. *Mol. Cell. Biol.* **2001**, *21*, 289–297. (c) Copeland, K. D.; Fitzsimons, M. P.; Houser, R. P.; Barton, J. K. *Biochemistry* **2002**, *41*, 343–356. (d) Boseggia, E.; Gatos, M.; Lucatello, L.; Mancini, F.; Moro, S.; Palumbo, M.; Sissi, C.; Tecilla, P.; Tonellato, U.; Zagotto, G. *J. Am. Chem. Soc.* **2004**, *126*, 4543–4549.
- (3) (a) Huang, X.; Pieczko, M. E.; Long, E. C. *Biochemistry* **1999**, *38*, 2160–2166. (b) Ananias, D. C.; Long, E. C. *Inorg. Chem.* **1997**, *36*, 2469–2471.
- (4) (a) Mack, D. P.; Iverson, B. L.; Dervan, P. B. *J. Am. Chem. Soc.* **1988**, *110*, 7572–7574. (b) Nagaoka, M.; Hagihara, M.; Kuwahara, J.; Sugiura, Y. *J. Am. Chem. Soc.* **1994**, *116*, 4085–4086. (c) Harford, C.; Narindrasorasak, S.; Sarkar, B. *Biochemistry* **1996**, *35*, 4271–4278.
- (5) (a) Welch, J. T.; Sirish, M.; Lindstrom, K. M.; Franklin, S. J. *Inorg. Chem.* **2001**, *40*, 1982–1984. (b) Kovacic, R. T.; Welch, J. T.; Franklin, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 6656–6662.
- (6) Nomura, A.; Sugiura, Y. *Inorg. Chem.* **2004**, *43*, 1708–1713.
- (7) (a) Laity, J. H.; Lee, B. M.; Wright, P. E. *Curr. Opin. Struct. Biol.* **2001**, *11*, 39–46. (b) Wolfe, S. A.; Nekudova, L.; Pabo, C. O. *Annu. Rev. Biophys. Biomol. Struct.* **2000**, *29*, 183–212.
- (8) Kamiuchi, T.; Abe, E.; Imanishi, M.; Kajii, T.; Nagaoka, M.; Sugiura, Y. *Biochemistry* **1998**, *37*, 13827–13834.
- (9) Nagaoka, M.; Doi, Y.; Kuwahara, J.; Sugiura, Y. *J. Am. Chem. Soc.* **2002**, *124*, 6526–6527.
- (10) (a) Segal, D. J.; Beerli, R. R.; Blancfort, P.; Dreier, B.; Effertz, K.; Huber, A.; Koksche, B.; Lund, C. V.; Magnenat, L.; Valente, D.; Barbas, C. F., III. *Biochemistry* **2003**, *42*, 2137–2148. (b) Beerli, R. R.; Barbas, C. F., III. *Nat. Biotechnol.* **2002**, *20*, 135–141. (c) Segal, D. J.; Barbas, C. F., III. *Curr. Opin. Biotechnol.* **2001**, *12*, 632–637. (d) Sera, T.; Uraga, C. *Biochemistry* **2002**, *41*, 7074–7081.
- (11) (a) Koike, T.; Kimura, E. *J. Am. Chem. Soc.* **1991**, *113*, 8935–8941. (b) Kimura, E.; Kodama, Y.; Koike, T.; Shiro, M. *J. Am. Chem. Soc.* **1995**, *117*, 8304–8311. (c) Hettich, R.; Schneider, H.-J. *J. Am. Chem. Soc.* **1997**, *119*, 5638–5647. (d) Deck, K. M.; Tseng, T. A.; Burstyn, J. N. *Inorg. Chem.* **2002**, *41*, 669–677.
- (12) The product of BNP hydrolysis, 4-nitrophenyl phosphate, was quite slowly hydrolyzed;<sup>18</sup> therefore, only the degradation of BNP proceeded under our experimental conditions (ref 11a).
- (13) (a) Koike, T.; Takamura, M.; Kimura, E. *J. Am. Chem. Soc.* **1994**, *116*, 8443–8449. (b) Kimura, E. *Acc. Chem. Res.* **2001**, *34*, 171–179. (c) Jang, B.-B.; Lee, K. P.; Min, D. H.; Suh, J. J. *J. Am. Chem. Soc.* **1998**, *120*, 12008–12016.
- (14) (a) Kadonaga, J. T.; Jones, K. A.; Tjian, R. *Trends Biochem. Sci.* **1986**, *11*, 20–23. (b) Kriwacki, R. W.; Schultz, S. C.; Steitz, T. A.; Caradonna, J. P. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 9759–9763. (c) Kuwahara, J.; Yonezawa, A.; Futamura, M.; Sugiura, Y. *Biochemistry* **1993**, *32*, 5994–6001.
- (15) The present DNA cleavage did not change in anaerobic conditions, indicating that the possibility of a metal ion-driven oxidative cleavage process can be ruled out.
- (16) Yokono, M.; Saegusa, N.; Matsushita, K.; Sugiura, Y. *Biochemistry* **1998**, *37*, 6824–6832.
- (17) Hori, Y.; Suzuki, K.; Okuno, Y.; Nagaoka, M.; Futaki, S.; Sugiura, Y. *J. Am. Chem. Soc.* **2000**, *122*, 7648–7653.
- (18) Kirby, A. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1965**, *87*, 3209–3216.

JA045663L