Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 333 (2005) 167–173
BBRC
www.elsevier.com/locate/ybbrc

An artificial six-zinc finger peptide with polyarginine linker: Selective binding to the discontinuous DNA sequences ☆

Miki Imanishi *, Wei Yan, Tatsuya Morisaki, Yukio Sugiura *,[1]

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

Received 16 May 2005
Available online 31 May 2005

Abstract

Artificial DNA binding peptides recognizing separated sequences would expand varieties of the target genes for desirable transcriptional control. Here we demonstrated that polyarginine linker between two 3-zinc finger domains gives DNA binding selectivity to the separated target sequences. We created a six-zinc finger peptide, Sp1ZF6(Arg)8, by connecting two DNA binding domains of transcription factor Sp1 with a bulky and cationic polyarginine linker. The DNA binding properties to continuous and discontinuous target sequences were examined and compared to those of Sp1ZF6(Gly)10 containing a flexible and neutral polyglycine linker. The dissociation constants indicate that Sp1ZF6(Arg)8 has an obvious DNA binding preference to discontinuous target sequences but not Sp1ZF6(Gly)10. Footprinting analyses also showed that Sp1ZF6(Arg)8 binds properly only to the discontinuous target sites, while Sp1ZF6(Gly)10 does not distinguish them. The results provide helpful information for linker design of future zinc finger peptides to various states of DNA as gene expression regulators.

Keywords: Artificial zinc finger protein; DNA binding; Sp1; Linker

Artificial DNA binding proteins with desired sequence specificities are useful tools to control gene expressions. A zinc finger motif of the C_2H_2 type, one of the most common DNA binding motifs in eukaryotes, presents an attractive framework for the design of such peptides [1–4] because of its characteristic DNA binding manner as follows [5]: (i) recognition of three bases per one zinc finger motif, (ii) the structure of tandemly connected zinc finger motifs, and (iii) binding to non-palindrome sequences. So far, artificial zinc finger peptides have been created by engineering amino acid residues important for base recognition [6–12] and/or by connecting multiple zinc finger domains to extend the length of the target DNA sequence [12–15]. Six-zinc finger peptides, in which two 3-zinc finger peptides are connected with the consensus linker, have succeeded in regulating the gene expression by binding to continuous 18 bp of DNA even in plants [16,17].

In order to expand the targetable DNA sequences, artificial peptides that could bridge discontinuous binding sequences are required. We have previously connected two 3-zinc finger motifs of transcription factor Sp1 with different numbers of glycine residues and have shown that the six-zinc finger peptides with long linkers could bind to discontinuous targets by 10 bp [18,19]. Other groups have also demonstrated binding to discontinuous targets using artificial dimeric zinc finger peptides [20,21] or six-zinc finger peptides with long structured linkers [22]. However, no peptide has

☆ This study was supported in part by Grants-in-Aid for COE Project "Element Science" (12CE2005) and Scientific Research (14370755, 16659028) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.
* Corresponding authors. Fax: +81 774 32 3038.
E-mail addresses: imiki@scl.kyoto-u.ac.jp (M. Imanishi), ysugiura@dwc.doshisha.ac.jp (Y. Sugiura).
[1] Present address: Department of Pharmaceutical Sciences, Doshisha Women's College of Liberal Arts, Kyotanabe, Kyoto 610-0395, Japan.

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.05.090

succeeded in strictly distinguishing the discontinuous targets from the continuous ones. In this study, we aim to create a six-zinc finger peptide which has preference to separated target sequences rather than continuous ones. Arginine is known to be the most basic amino acid. A previous report suggested that the linker region of a six-zinc finger peptide, which consists of alternate residues of arginine and glycine, non-specifically interacts with the phosphate backbone of DNA [19]. We thought that bulky and cationic side chains in a linker could change the DNA binding mode of a six-zinc finger peptide dependent on the distribution of two target DNA sequences. Therefore, an artificial six-zinc finger peptide with polyarginine residues as a linker, Sp1ZF6(Arg)8, was created. The DNA binding properties for the discontinuous and continuous targets were examined and compared to those of a six-zinc finger protein with a flexible and neutral polyglycine linker, Sp1ZF6(Gly)10.

Materials and methods

Chemicals. The T4 polynucleotide kinase and restriction enzymes were purchased from New England Biolabs (Beverly). The labeled [γ-^{32}P]ATP compound was supplied from DuPont, and dimethyl sulfate was obtained from Aldrich (Milwaukee). The plasmid pBS-Sp1-fl was kindly provided by Dr. R. Tjian. All other chemicals were of commercial reagent grade.

Preparations of zinc finger peptides and substrate DNA fragments. Sp1ZF6(Arg)8 is coded on the plasmids pEV/Sp1ZF6(Arg)8. pEV/Sp1ZF6(Arg)8 was constructed by exchanging the region coding the TGEKP linker of pEV/Sp1ZF6 [15] with the oligonucleotide coding GRRRRRRRRQ. Sp1ZF6(Arg)8 and Sp1ZF6(Gly)10 were overexpressed as a soluble form in *Escherichia coli* BL21(DE3)pLysS at 20 °C and purified as following procedure at 4 °C. *E. coli* cells were resuspended and lysed in PBS buffer. The soluble form was purified by cation-exchange chromatography using a High S Cartridge (Bio Rad) followed by a Mono S 5/50 column (Amersham). Final purification was achieved by a gel filtration technique (Superdex 75; Amersham) using TN buffer (10 mM Tris–HCl (pH 8.0), 50 mM NaCl, and 1 mM dithiothreitol). For the substrate DNA, complementary oligonucleotides containing 1GC (5′-GGGGCGGGGC-3′), 2GC(0) (5′-GGGGC GGGGGGGGCGGGGC-3′), and 2GC(10) (5′-GGGGCGGGG CTATA ATTATAGGGGCGGGGC-3′) sequences (Fig. 1) were purchased from Amersham Pharmacia Biotech.

Gel mobility shift assays. The 37 and 48 bp oligonucleotides containing the 2GC(0) and 2GC(10) sequences, respectively, were ^{32}P-labeled and annealed with the complementary oligonucleotide. For the substrate 1GC, the *Hin*dIII– *Xba*I fragment (41 bp) of pBS-GC [23] was ^{32}P-labeled. Gel mobility shift assays were carried out under the following conditions. Each reaction mixture contained 10 mM Tris–HCl (pH 8.0), 50 mM NaCl, 1 mM dithiothreitol, 0.05% Nonidet P-40, 5% glycerol, 5 mM $MgCl_2$, 25 ng/μL poly(dI–dC) (Amersham Pharmacia Biotech), the 5′-end-labeled DNA fragment (<20 pM), and different concentrations of the zinc finger peptides. After incubation at 20 °C for 1 h, the sample solutions were electrophoresed on an 8% nondenaturing polyacrylamide gel with Tris–borate buffer (88 mM Tris–HCl (pH 8.0) and 88 mM boric acid) at room temperature. The bands were visualized using a STORM instrument (Amersham Pharmacia Biotech) and analyzed by ImageQuant software (Molecular Dynamics). The equilibrium dissociation constants (K_d) of each peptide–DNA fragment complex were evaluated by fitting the experimentally obtained values of θ_b (θ_b; the fraction of labeled DNA bound to the peptide) to the binding isotherm equation (1) using the Kaleida Graph program (Abelbeck software)

$$\theta_b = [\text{peptide}]/([\text{peptide}] + K_d). \quad (1)$$

DNase I footprinting analyses. DNase I footprinting experiments were performed according to the method of Brenowitz et al. [24]. The substrate DNA fragment was prepared by labeling the 5′-end of the *Xba*I fragment of 2GC(0)/pBS [15] or *Hin*dIII fragment of 2GC(10)/pBEND [18] and digesting with *Hin*dIII or *Eco*RI, respectively. The binding mixture contained 10 mM Tris–HCl (pH 8.0), 50 mM NaCl,

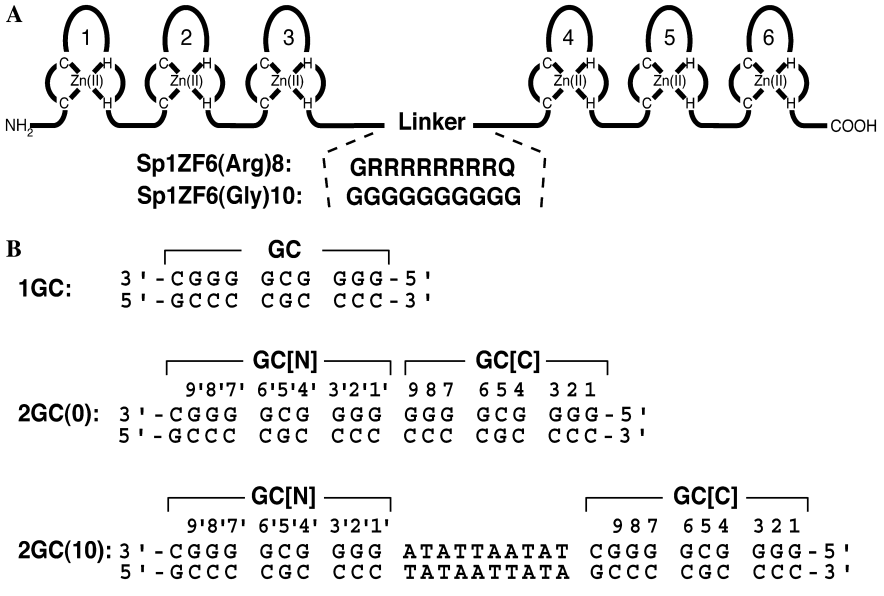

Fig. 1. Schematic representation of Sp1ZF6(Gly)10 and Sp1ZF6(Arg)8 (A), and their target DNA sequences, 1GC, 2GC(0), and 2GC(10) (B). The 1GC, 2GC(0), and 2GC(10) sequences contain single GC-box (5′-GGGGCGGGGC-3′), two continuous GC-box sequences, and two discontinuous GC-box sequences with 10 bp intervening, respectively.

1 mM dithiothreitol, 0.05% Nonidet P-40, 5% glycerol, 25 ng/μL poly(dI–dC), 5 mM $CaCl_2$, 10 mM $MgCl_2$, the 5′-end-labeled substrate DNA fragment, and 0–1 μM peptide. After incubation at 20 °C for 1 h, the following steps were accomplished according to the previous procedure [15]. The bands were visualized using a STORM instrument (Amersham Pharmacia Biotech).

Methylation interference assays. Methylation interference assays were performed as previously described [25]. The binding reaction was performed under the same conditions as the gel mobility shift assay. After incubation at 20 °C for 1 h, the peptide-bound and free DNAs were separated on a 10% non-denaturing polyacrylamide gel and eluted from the gel with a standard elution buffer. In order to examine both the strong and weak base contacts in the methylation interference experiment, we selected the experimental condition such that the fraction of the labeled DNA bound to the peptide is about 10%. The recovered methylated DNA was reacted with 100 μL of 1 M piperidine at 90 °C for 30 min. The cleavage products were analyzed on a 15% polyacrylamide/7 M urea sequencing gel. The bands were visualized using a STORM instrument (Amersham Pharmacia Biotech).

Results

Creation of a six-zinc finger peptide with a polyarginine linker and substrates

A novel six-zinc finger peptide, Sp1ZF6(Arg)8, was created by linking two 3-zinc fingers of the transcription factor Sp1, Sp1ZF3, with polyarginine (GRR RRRRRRQ) and the DNA binding properties were compared with those of Sp1ZF6(Gly)10, which has a polyglycine (GGGGGGGGGG) linker with the same number of residues as the Sp1ZF6(Arg)8 linker (Fig. 1). Sp1ZF3 strongly binds to the dsDNA containing the GC (5′-GGGGCGGGGC-3′) sequence called the GC-box [23,26] and Sp1ZF6(Gly)10 has a sufficient linker length to bind to two discontinuous GC-box regions with one helical turn (10 bp) intervening (2GC(10)) [18]. As the targets of the artificial six-zinc finger peptides, the DNA sequences containing two continuous GC-box sequences (2GC(0)) or two discontinuous GC-box sequences (2GC(10)) were used (Fig. 1). We name the 5′- and 3′-portion GC-boxes as GC[C] and GC[N], respectively, as shown in Fig. 1.

DNA binding affinities of artificial six-zinc finger peptides

Gel mobility shift assays were performed using Sp1ZF6(Arg)8 and Sp1ZF6(Gly)10 for the duplex DNA fragments containing the 2GC(0) or 2GC(10) sequences (Fig. 2, Table 1), and also for the 1GC substrate. Sp1ZF6(Gly)10 showed a single shifted band against both the 2GC(0) and 2GC(10) substrates dependent on the concentration (Figs. 2A and C), suggesting the formation of a single 1:1 complex with the two

Table 1
Apparent dissociation constants (K_d) for Sp1ZF6(Gly)10 and Sp1ZF6(Arg)8 bindings to 2GC(0), 2GC(10), and 1GC

Binding site[b]	K_d (nM)[a]	
	Sp1ZF6(Gly)10	Sp1ZF6(Arg)8
2GC(0)	0.96 ± 0.36	71 ± 7.7
2GC(10)	1.5 ± 0.14	3.1 ± 0.94
1GC	75 ± 0.12	68 ± 4.5

[a] Apparent dissociation constants were determined by titration using the gel mobility shift assay as described in Materials and methods. Values are averages of three or more independent determinations with standard deviations.

[b] The nomenclature is described in the text (see Fig. 1).

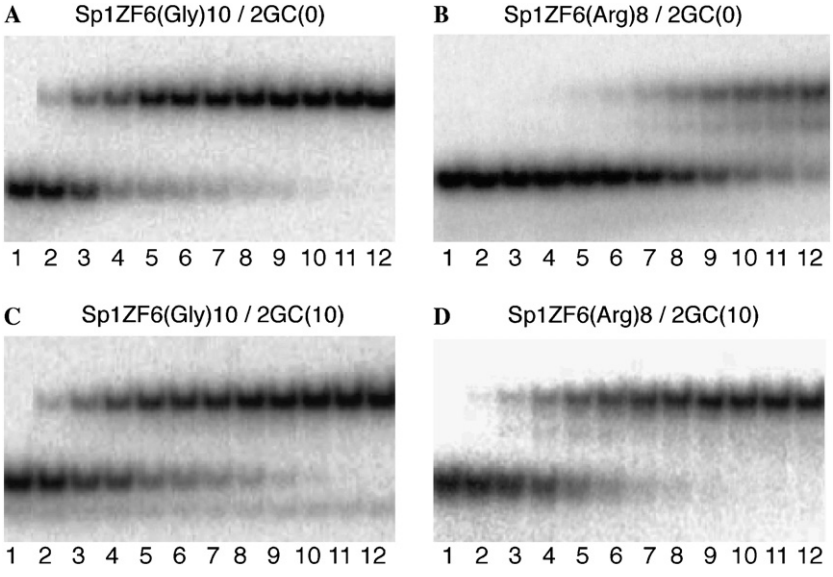

Fig. 2. Gel mobility shift assays for Sp1ZF6(Gly)10 binding to the 2GC(0) substrate (A), Sp1ZF6(Arg)8 binding to the 2GC(0) substrate (B), Sp1ZF6(Gly)10 binding to the 2GC(10) substrate (C), and Sp1ZF6(Arg)8 binding to the 2GC(10) substrate (D). Lanes 1–12 contain 0, 0.3, 0.6, 1.3, 2.5, 5.0, 10, 20, 40, 80, 160, and 320 nM peptides, respectively.

GC-box sequences. On the other hand, Sp1ZF6(Arg)8 showed an ambiguous shifted band for the 2GC(0) substrate (Fig. 2B), and a clear single shifted band against the 2GC(10) substrate (Fig. 2D). The obtained dissociation constants (K_d) to the 1GC substrate were 75 ± 0.12 and 68 ± 4.5 nM for Sp1ZF6(Gly)10 and Sp1ZF6(Arg)8, respectively (Table 1). Those of Sp1ZF6(Gly)10 to the 2GC(0) and 2GC(10) substrates were 0.96 ± 0.36 and 1.5 ± 0.14 nM, respectively, showing more than 50-fold higher affinity than that to the 1GC substrate. The K_d of Sp1ZF6(Arg)8 to the 2GC(10) substrate was 3.1 ± 0.94 nM, which shows 22-fold higher affinity than that to the 1GC substrate. On the other hand, that of Sp1ZF6(Arg)8 to the 2GC(0) substrate was 71 ± 7.7 nM, which is almost the same as the affinity to the 1GC.

DNA binding region of artificial six-zinc finger peptides

Figs. 3A and B demonstrate the results of the DNase I footpriniting analyses against the 2GC(0) and 2GC(10) substrates, respectively. The entire 2GC(0) sequence was protected by the binding of Sp1ZF6(Gly)10, though the binding of Sp1ZF6(Arg)8 to 2GC(0) induced weak protection. In both cases, the hypersensitive cleavages at the 3′-outside of the GC[N], typically induced by binding of the Sp1 zinc finger [15,23,27], were detected. Regarding the 2GC(10) sequence, both GC-box regions were protected by binding of Sp1ZF6(Arg)8 and Sp1ZF6(Gly)10, and the hypersensitive cleavages at the 3′-outside of each GC-box sequence were detected.

Zinc finger-base interaction of Sp1ZF6(Gly)10 and Sp1ZF6(Arg)8

Fig. 4 shows the results of methylation interference assays. This method detects specific guanine bases playing an important role in the formation of the peptide–DNA complex. Targeting the 2GC(0) sequence (Fig. 4A), Sp1ZF6(Gly)10 showed strong contacts with the guanine bases, G(2), G(3), G(4), G(6), G(9), G(1′), G(2′), G(3′), G(4′), and G(6′), that ranged through both GC[N] and GC[C]. On the other hand, Sp1ZF6(Arg)8 made strong contacts mainly with the guanine bases in the GC[N], G(9), G(1′), G(2′), G(3′), G(4′), and G(6′), and no strong contact was observed in the GC[C]. In the case of binding to the 2GC(10) sequence (Fig. 4B), Sp1ZF6(Gly)10 demonstrated base contacts with G(1), G(2), G(3), G(4), G(6), G(1′), G(2′), G(3′), G(4′), G(6′), G(8′), and G(9′), and Sp1ZF6(Arg)8 showed contacts with G(2), G(3), G(4), G(6), G(2′), G(3′), and G(4′) included in both GC[N] and GC[C].

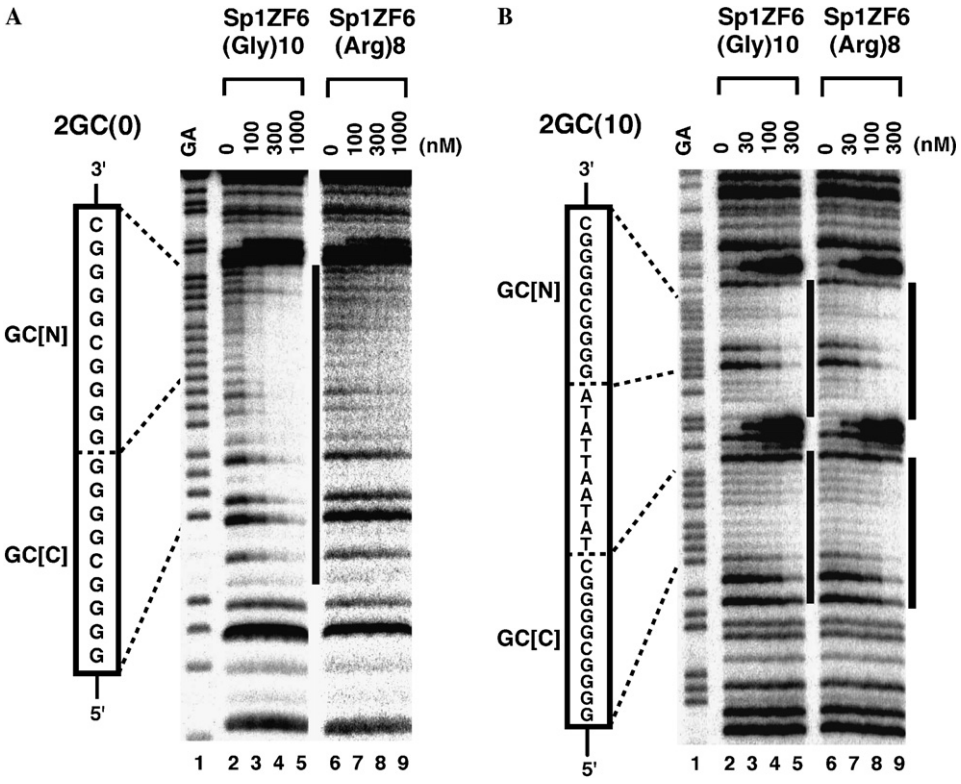

Fig. 3. DNase I footprinting analyses of Sp1ZF6(Gly)10 and Sp1ZF6(Arg)8 binding to the 2GC(0) substrate (A) and the 2GC(10) substrate (B). Lane 1, G + A (Maxam–Gilbert reaction products). Every peptide concentration is noted in the figure (lanes 1–9). The vertical lines indicate the protected regions. The analyses were repeated more than three times and consistent footprinting patterns were obtained.

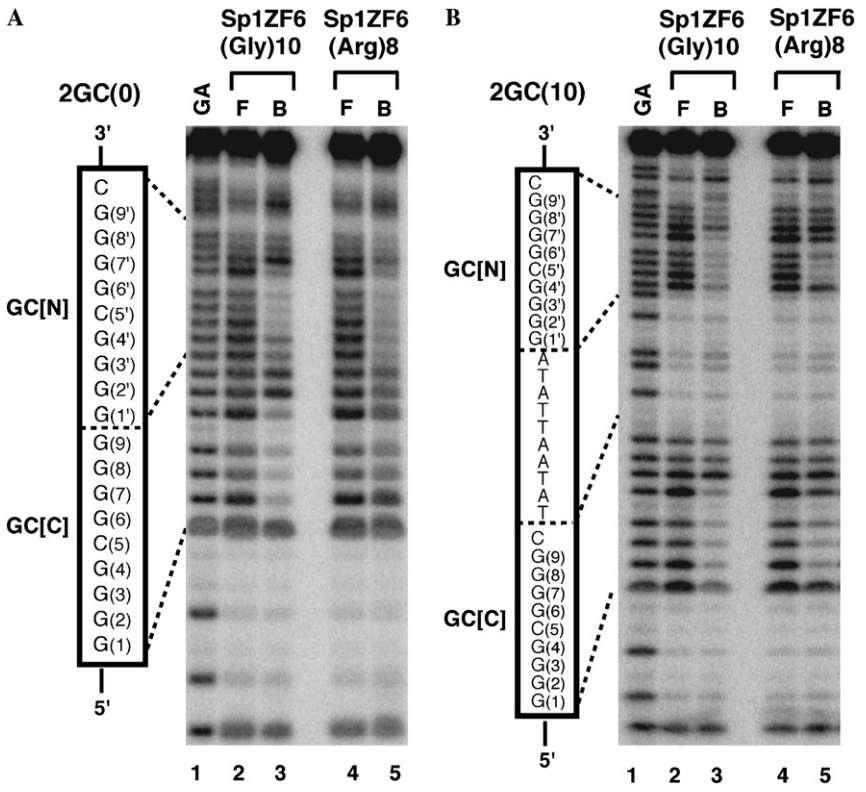

Fig. 4. Methylation interference assays of the binding of Sp1ZF6(Gly)10 and Sp1ZF6(Arg)8 to the 2GC(0) substrate (A) and 2GC(10) substrate (B). Lane 1, G + A (Maxam–Gilbert reaction products); lanes 2 and 4 marked with F, free DNA samples; and lanes 3 and 5 marked with B, peptide-bound DNA samples. The assays were repeated more than three times and consistent results were obtained.

Discussion

The gel mobility shift assays revealed an obvious preference to the 2GC(10) sequence versus the 2GC(0) sequence for the binding of Sp1ZF6(Arg)8 but not for that of Sp1ZF6(Gly)10. Previous reports showed that six-zinc finger peptides that consist of two 3-zinc finger units have more than 10-fold higher affinity to its full target site than that to the single target site for the original three-zinc finger peptide [11,14,22]. The observed significant increase in binding affinity as compared with those to the 1GC substrate supports that the binding modes of Sp1ZF6(Gly)10 to the 2GC(10) and 2GC(0) substrates and of Sp1ZF6(Arg)8 to the 2GC(10) substrate have almost the same binding pattern as that of a six-zinc finger peptide to its full target site. In the case of the binding of Sp1ZF6(Arg)8 to the 2GC(0) substrate, however, it is suggested that not all six zinc finger motifs function to bind to the 2GC(0) substrate.

According to the previous reports on DNase I footprinting experiments, Sp1ZF3 covers the GC-box sequence and its 5′-outside region, and induces hypersensitive cleavages at the 3′-outside region of a GC-box [15,23,27]. Therefore, the footprinting pattern of Sp1ZF6(Gly)10 to the 2GC(0) substrate indicates that Sp1ZF6(Gly)10 binds to the whole 2GC(0) region. On the contrary, the weak protection of the 2GC(0) by Sp1ZF6(Arg)8 suggests that DNA binding of Sp1ZF6 (Arg)8 is mostly non-specific. In the case of binding to the 2GC(10) sequence, Sp1ZF6(Gly)10 and Sp1ZF6(Arg)8 are demonstrated to bind to both GC-box sites.

The methylation interference assays for the 2GC(0) substrate revealed that Sp1ZF6(Arg)8 mainly recognizes guanine bases only in the GC[N], while Sp1ZF6(Gly)10 shows recognition of the guanine bases within both GC[N] and GC[C]. The result supports the hypersensitive DNA cleavages at the 3′-outside region detected by the DNase I footprinting assay of Sp1ZF6(Arg)8 to the 2GC(0) substrate. The interference patterns of the 2GC(10) sequence also support that Sp1ZF6(Arg)8 binds to both GC-box sites of the 2GC(10) as well as Sp1ZF6(Gly)10.

A linker of the zinc finger proteins plays an important role in DNA binding as shown by the fact that linker phosphorylation reduces the DNA binding affinity of the zinc finger peptides [28,29]. In our previous report, Sp1ZF6(GR)4, which has the GRGRGRGRGQ sequence as a linker, binds to the 2GC(10) [19] and also to the 2GC(0) (data not shown). The kinetic analysis suggested that the (GR)4 linker makes contact with the DNA phosphate backbone during the initial stage

of DNA binding [19]. In the case of Sp1ZF6(Arg)8, the following reasons might cause DNA binding selectivity to the 2GC(10) not to the contiguous targets, 2GC(0): (i) electrostatic repulsion between cationic side chains within the polyarginine linker, (ii) low conformational freedom of the polyarginine linker, and/or (iii) non-sequence-specific electrostatic interaction between the polyarginine linker and DNA phosphate backbone at the contiguous target sequence.

To establish the binding to two discontinuous sites, Moore et al. [22] prepared six-zinc finger peptides with a non-sequence-specific zinc finger as a linker, which consists of about 30 amino acid residues and folds into a compact globular structure. In comparison to the six-zinc finger peptides with flexible linkers, the peptides with structured linkers display increases in the DNA binding affinity. However, the difference in affinity between the continuous and discontinuous two target sequences is only a few-fold. In this study, the novel peptide, Sp1ZF6(Arg)8, obviously discriminated between the continuous and discontinuous two GC-box sequences. Of special interest is that such a short linker could give the DNA binding selectivity of a six-zinc finger peptide.

Recent progress in understanding the DNA binding mechanism of the zinc finger proteins has enabled us to create artificial zinc finger peptides with desired sequence specificities by targeting specific amino acid residues important for base recognition [6–12]. However, information on the linker region is still developing [13,18,19,22,28–31]. Toward universal regulation of the gene expression, more adaptability to various DNA sequences and structures such as bending or looping is required for the artificial zinc finger peptides. Our results provide helpful information for the linker design of future zinc finger peptides targeting various DNA states in addition to the sequence.

Acknowledgment

We thank Yasuhisa Shiraishi for his critical reading of the manuscript.

References

[1] A.C. Jamieson, J.C. Miller, C.O. Pabo, Drug discovery with engineered zinc-finger proteins, Nat. Rev. Drug Discov. 2 (2003) 361–367.
[2] R.R. Beerli, C.F. Barbas III, Engineering polydactyl zinc-finger transcription factors, Nat. Biotechnol. 20 (2002) 135–141.
[3] C.O. Pabo, E. Peisach, R.A. Grant, Design and selection of novel Cys2His2 zinc finger proteins, Annu. Rev. Biochem. 70 (2001) 313–340.
[4] M. Imanishi, Y. Hori, M. Nagaoka, Y. Sugiura, Design of novel zinc finger proteins: towards artificial control of specific gene expression, Eur. J. Pharm. Sci. 13 (2001) 91–97.
[5] N.P. Pavletich, C.O. Pabo, Zinc finger-DNA recognition: crystal structure of a Zif268–DNA complex at 2.1 Å, Science 252 (1991) 671–673.
[6] E.J. Rebar, C.O. Pabo, Zinc finger phage: affinity selection of fingers with new DNA-binding specificities, Science 263 (1994) 671–673.
[7] H.A. Greisman, C.O. Pabo, A general strategy for selecting high-affinity zinc finger proteins for diverse DNA target sites, Science 275 (1997) 657–661.
[8] A.C. Jamieson, H. Wang, S.-H. Kim, A zinc finger directory for high-affinity DNA recognition, Proc. Natl. Acad. Sci. USA 93 (1996) 12834–12839.
[9] Y. Choo, A. Castellanos, B. García-Hernández, I. Sánchez-García, A. Klug, Promoter-specific activation of gene expression directed by bacteriophage-selected zinc fingers, J. Mol. Biol. 273 (1997) 525–532.
[10] C.V. Lund, P. Blancafort, M. Popkov, C.F. Barbas III, Promoter-targeted phage display selections with preassembled synthetic zinc finger libraries for endogenous gene regulation, J. Mol. Biol. 340 (2004) 599–613.
[11] Q. Liu, D.J. Segal, J.B. Ghiara, C.F. Barbas III, Design of polydactyl zinc-finger proteins for unique addressing within complex genomes, Proc. Natl. Acad. Sci. USA 94 (1997) 5525–5530.
[12] L. Magnenat, P. Blancafort, C.F. Barbas III, In vivo selection of combinatorial libraries and designed affinity maturation of polydactyl zinc finger transcription factors for ICAM-1 provides new insights into gene regulation, J. Mol. Biol. 341 (2004) 635–649.
[13] J.-S. Kim, C.O. Pabo, Getting a handhold on DNA: design of poly-zinc finger proteins with femtomolar dissociation constants, Proc. Natl. Acad. Sci. USA 95 (1998) 2812–2817.
[14] R.R. Beerli, D.J. Segal, B. Dreier, C.F. Barbas III, Toward controlling gene expression at will: specific regulation of the erbB-2/HER-2 promoter by using polydactyl zinc finger proteins constructed from modular building blocks, Proc. Natl. Acad. Sci. USA 95 (1998) 14628–14633.
[15] T. Kamiuchi, E. Abe, M. Imanishi, T. Kaji, M. Nagaoka, Y. Sugiura, Artificial nine zinc-finger peptide with 30 base pair binding sites, Biochemistry 37 (1998) 13827–13834.
[16] M.I. Ordiz, C.F. Barbas III, R.N. Beachy, Regulation of transgene expression in plants with polydactyl zinc finger transcription factors, Proc. Natl. Acad. Sci. USA 99 (2002) 13290–13295.
[17] X. Guan, J. Stege, M. Kim, Z. Dahmani, N. Fan, P. Heifetz, C.F. Barbas III, S.P. Briggs, Heritable endogenous gene regulation in plants with designed polydactyl zinc finger transcription factors, Proc. Natl. Acad. Sci. USA 99 (2002) 13296–13301.
[18] M. Imanishi, Y. Hori, M. Nagaoka, Y. Sugiura, DNA-bending finger: artificial design of 6-zinc finger peptides with polyglycine linker and induction of DNA bending, Biochemistry 39 (2000) 4383–4390.
[19] M. Imanishi, Y. Sugiura, Artificial DNA-bending six-zinc finger peptides with different charged linkers: distinct kinetic properties of DNA bindings, Biochemistry 41 (2002) 1328–1334.
[20] J.L. Pomerantz, S.A. Wolfe, C.O. Pabo, Structure-based design of a dimeric zinc finger protein, Biochemistry 37 (1998) 965–970.
[21] S.A. Wolfe, R.A. Grant, C.O. Pabo, Structure of a designed dimeric zinc finger protein bound to DNA, Biochemistry 42 (2003) 13401–13409.
[22] M. Moore, Y. Choo, A. Klug, Design of polyzinc finger peptides with structured linkers, Proc. Natl. Acad. Sci. USA 98 (2001) 1432–1436.
[23] M. Yokono, N. Saegusa, K. Matsushita, Y. Sugiura, Unique DNA binding mode of the N-terminal zinc finger of transcription factor Sp1, Biochemistry 37 (1998) 6824–6832.

[24] M. Brenowitz, D.F. Senear, M.A. Shea, G.K. Ackers, Quantitative DNase footprint titration: a method for studying protein–DNA interactions, Methods Enzymol. 130 (1986) 132–181.
[25] J. Kuwahara, A. Yonezawa, M. Futamura, Y. Sugiura, Binding of transcription factor Sp1 to GC box DNA revealed by footprinting analysis: different contact of three zinc fingers and sequence recognition mode, Biochemistry 32 (1993) 5994–6001.
[26] M. Nagaoka, Y. Shiraishi, Y. Uno, W. Nomura, Y. Sugiura, Interconversion between serine and aspartic acid in the alpha helix of the N-terminal zinc finger of Sp1: implication for general recognition code and for design of novel zinc finger peptide recognizing complementary strand, Biochemistry 41 (2002) 8819–8825.
[27] Y. Shiraishi, M. Imanishi, Y. Sugiura, Exchange of histidine spacing between Sp1 and GLI zinc fingers: distinct effect of histidine spacing-linker region on DNA binding, Biochemistry 43 (2004) 6352–6359.
[28] S. Dovat, T. Ronni, D. Russell, R. Ferrini, B.S. Cobb, S.T. Smale, A common mechanism for mitotic inactivation of C2H2 zinc finger DNA-binding domains, Genes Dev. 16 (2002) 2985–2990.
[29] D. Jantz, J.M. Berg, Reduction in DNA-binding affinity of Cys2His2 zinc finger proteins by linker phosphorylation, Proc. Natl. Acad. Sci. USA 101 (2004) 7589–7593.
[30] E. Peisach, C.O. Pabo, Constraints for zinc finger linker design as inferred from X-ray crystal structure of tandem Zif268–DNA complexes, J. Mol. Biol. 330 (2003) 1–7.
[31] W. Nomura, Y. Sugiura, Effects of length and position of an extended linker on sequence-selective DNA of zinc finger recognition peptides, Biochemistry 42 (2003) 14805–14813.