Designer Zinc Finger Proteins: Tools for Creating Artificial DNA-Binding Functional Proteins

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ABSTRACT
The design of artificial functional DNA-binding proteins has long been a goal for several research laboratories. The zinc finger proteins, which typically contain many fingers linked in tandem fashion, are some of the most studied DNA-binding proteins. The zinc finger protein's tandem arrangement and its the ability to recognize a wide variety of DNA sequences make it an attractive framework to design novel DNA-binding peptides/proteins. Our laboratory has utilized several design strategies to create novel zinc finger peptides by re-engineering the C_2H_2-type zinc finger motif of transcription factor Sp1. Some of the engineered zinc fingers have shown nuclease and catalytic functional properties. Based on these results, we present the design strategies for the creation of novel zinc fingers.

Introduction

Zinc finger proteins have fascinated many research groups because of their modular assembly and broad range of biological functions.[1] Especially, their DNA-binding ability made the zinc finger domain a potential molecule to be re-engineered into DNA-binding functional proteins. The classical C_2H_2-type zinc finger proteins are the most common DNA-binding domain found in human transcription factors, and this domain constitutes 2% of the entire human genome.[2] Individual zinc fingers are short stretches of approximately 30 amino acids folded into a $\beta\beta\alpha$ compact structure with the aid of the Zn(II) ion. The Zn(II) ion pins together the β-hairpin and α-helix in a tetrahedral coordination with the two conserved cysteines (located in the β-hairpin) and two histidine residues (located in the α-helix) as shown in Figure 1.[3] The Zn(II) metal is not directly involved in the DNA-binding activity but is essential for the stability of the $\beta\beta\alpha$ architecture of the zinc finger domain. Most natural zinc finger proteins have three fingers, but some have as many as 37, linked one after another via small peptide linkers, like fingers on a hand, positioned in such a way that they can readily recognize adjacent base pairs along the DNA double helix.[1,4] Two to three tandem zinc fingers are necessary and sufficient for specific binding without participation of any other domain. Each finger recognizes three to four base pairs in the major groove of the DNA via the N-terminal α-helix. The key DNA-contacting residues present in the helical region are responsible for the discrimination of the base pairs.[4] Moreover, zinc finger proteins bind to a sequence of asymmetric base pairs unlike other nucleic acid recognition motifs such as the basic leucine zipper and helix−turn−helix(αtα).[5] Thus the C_2H_2-type zinc finger domain offers an attractive framework to design novel DNA-binding proteins with a high specificity and affinity by linking together several fingers to recognize DNA sequences of different lengths, and by mixing and matching zinc fingers, one can logically construct zinc finger proteins that bind virtually any gene in any cell.

The field of zinc-finger engineering has remarkably progressed in the past few years. This progress has been fueled by the pioneering work of Klug from the Medical Research Council, Cambridge, U.K., Barbas of the Scripps Research Institute, Pabo of the Massachusetts Institute of Technology, Berg of the Johns Hopkins University School of Medicine, and the scientists from Sangamo Biosciences.

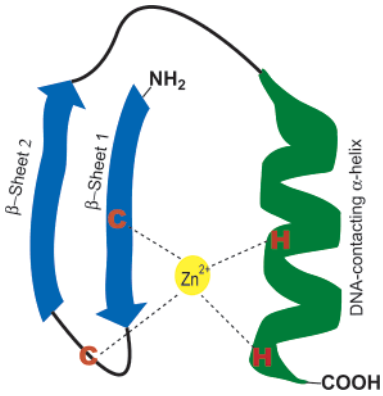

FIGURE 1. Schematic representation of classical C_2H_2-type zinc finger motif displaying the $\beta\beta\alpha$ fold. Zinc-coordinating amino acids are shown in red.

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The engineered novel DNA-binding proteins can be utilized to carry out a variety of cellular activities by combining them with different functional domains. Researchers have successfully designed novel nucleases and transcription factors using the C_2H_2-type zinc finger domain as a scaffold.[6] The potential uses of such engineered zinc finger proteins in drug discovery have been summarized in recent review articles by eminent scientists in the field.[7] Over the past few years, our laboratory has been focusing on creating novel DNA-binding proteins by re-engineering the C_2H_2-type zinc finger domain of the human transcription factor Sp1. We have successfully designed several novel DNA-binding proteins based on the C_2H_2-type zinc finger motif. Some of them have shown functional properties such as hydrolytic and nuclease properties. We now summarize some of our design strategies to create artificial functional zinc fingers possessing a high-affinity specific DNA-binding property.

DNA Sequence Recognition of Human Transcription Factor Sp1

The human transcription factor Sp1, which was originally isolated from HeLa cells, is well-known to be an ubiquitous factor that activates transcription by RNA polymerase II.[8] Sp1 contains three contiguous repeats of a classical C_2H_2-type zinc finger motif as a DNA binding domain, and a peptide containing only this domain can bind to the GC box DNA with almost the same affinity as the full-length Sp1.[9] The crystal structure of the DNA binding domain of this family, Zif268, bound to its target DNA site has been determined.[4a] In the complex, the three zinc fingers occupy the major groove of the DNA in series, each making specific contacts to the overlapping four base pair subsites. The protein interacts with both strands of the DNA, but the majority of the contacts are with the guanine-rich strand. On the basis of the similarity of the amino acid sequences and their target DNA sequences between Sp1 and Zif268, a model of the three-zinc finger Sp1−DNA interaction was originally proposed.[9,10] However, alanine scanning mutagenesis studies combined with an electrophoretic analysis and the NMR structure of the Sp1−DNA complex showed minor variations in the interaction mechanism. The mode of the putative interaction of Sp1 with the GC box DNA is given in Figure 2.[11]

Novel Zinc Fingers by Swapping α-Helices and β-Sheet Elements

Sp1 contains a DNA-binding domain consisting of three C_2H_2-type zinc fingers (fingers 1−3) in the C-terminal region, which preferably binds to the GC box, 5′-GGG GCG GGGC-3′ (Figure 2B).[12] On the other hand, the C-terminus in three of the six C_2H_2-type zinc fingers of the *Drosophila* transcription factor CF2-II possesses a sequence preference for the AT-rich element 5′-GTA TAT A-TA-3′.[13] A novel artificial zinc finger peptide, Sp1HM, binding to the AT-rich sequence was created by α-helix swapping between these proteins.[14] In the chimeric Sp1HM zinc finger, the original GC specific α-helices of

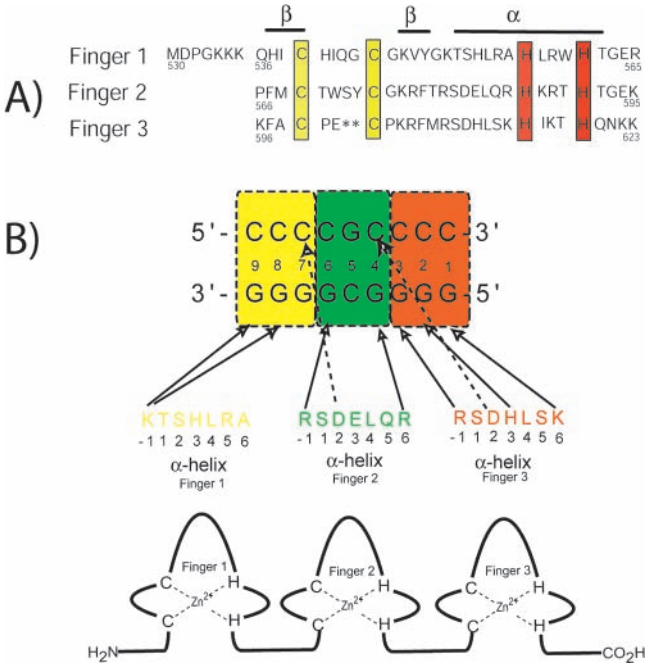


FIGURE 2. (A) Primary sequence of Sp1(530−623). The numerals indicate the positions of the amino acids in native Sp1. The zinc-coordinating cysteines and histidines are boxed. (B) Putative base recognition mode of Sp1(530−623).[11] The GC box sequence, which is divided into subsites I−III, is shown with numbering in the 5′→3′ direction on the guanine-rich strand.

the Sp1 fingers were swapped with the AT specific α-helices of CF2-II as shown in Figure 3. The folding property of Sp1HM was analyzed by circular dichroism (CD). The CD spectrum of Sp1HM was comparable with those of the wild-type Sp1(zf123) and the single zinc finger of Sp1. Negative Cotton effects with a minimum at 206 nm and a shoulder around 222 nm suggest that Sp1HM has an ordered secondary structure, namely, α-helices. Sp1HM binds to AT with 3.2 and 1330 nM K_d values in the absence and presence of the competitor DNA, respectively. In contrast, the K_d values of Sp1(zf123) for GC in the absence and presence of the competitor DNA (calf thymus) were 4.0 and 46.1 nM, respectively. No evident binding complex for Sp1(zf123)−AT or Sp1HM−GC was detected under our experimental conditions. These results suggest that the engineered Sp1HM binds to AT with a high affinity and specificity. Thus, it could be possible to alter the DNA specificity of a particular zinc finger protein by rational redesign. The zinc finger peptide for the TATA box (5′-GCT ATA AAA-3′) was created by Greisman and Pabo.[15] They used the three-zinc-finger domain derived from Zif268 as a framework and succeeded in selecting the $TATA_{ZF}$ by a sequential selection strategy based on the phage display technique. The $TATA_{ZF}$ binds to the TATA box with a 0.12 nM dissociation constant, indicating that the DNA binding affinity of the zinc finger peptides for the AT-rich sequence is estimated to be on the order of less than nanomolar. These results imply that our rational design is also effective and convenient for creating zinc finger peptides with a high affinity comparable to that of a zinc finger selected by the phage display technique.

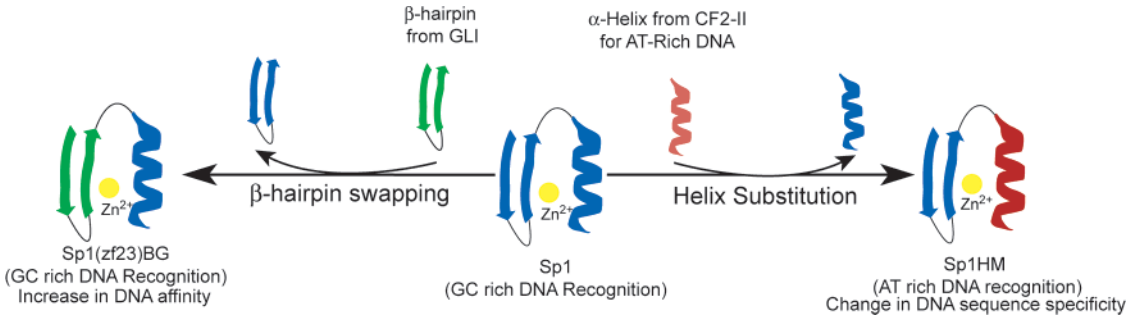


FIGURE 3. Swapping of the α-helix and *β*-hairpin regions alters the DNA binding properties of Sp1.[14,16]

According to our recent findings, swapping of the *β*-hairpin between two fingers alters the DNA binding properties.[16] The *β*-hairpin elements were thought to play only a structural role because the DNA-contacting amino acid residues are in the α-helical region. Therefore, most of the design strategies focused on the α-helical region. By swapping the *β*-hairpin elements between the Sp1 and GLI zinc fingers, we investigated the role of the *β*-hairpin region of the C_2H_2-type zinc fingers on the DNA binding. The Sp1 and GLI zinc fingers were chosen because both zinc fingers show distinct base and phosphate interactions, and their structural and functional information is well documented.[17] The engineered zinc fingers, Sp1(zf23)-BG and GLI(zf45)BS, showed CD spectra similar to those of the C_2H_2-type zinc finger peptides. However, the values at 222 nm of GLI(zf45)BS ($[\theta]_{222} = -9403$) being greater than those of Sp1(zf23)BG ($[\theta]_{222} = -3029$) suggest a structural difference in the histidine−histidine spacing.[17b] It is surprising to note that the Sp1 mutant Sp1(zf23)BG with the GLI *β*-hairpin had a higher DNA-binding affinity for the GC box than that of the wild-type Sp1. In contrast, the GLI mutant with the Sp1 *β*-hairpin GLI(zf45)BS completely lost its DNA-binding ability for GLIseq despite the existence of the DNA-recognizing helical region. The result of the DNase I footprinting analyses showed that the DNA-binding mode of the Sp1-type mutants is evidently affected by the substitution of the *β*-hairpin region. These results indicate that the *β*-hairpin region appears to participate in the DNA binding of the non-Zif268-type zinc fingers such as GLI. A detailed structural analysis using NMR and X-ray would provide additional information on the binding mode of the mutants. However, the present results suggest that the *β*-hairpin should also be considered when engineering novel zinc fingers.

Unnatural H_2H_2-type Zinc Finger Protein with Specific DNA Hydrolysis Ability

Although C_2H_2, C_3H, C_4, and C_6-type zinc finger proteins exist in nature,[18] a novel H_2H_2-type zinc finger protein has never been observed. We have created the first artificial H_2H_2-type zinc finger protein (H_4Sp1) engineered by cysteine to histidine mutations of the C_2H_2-type zinc finger transcription factor Sp1 (Figure 4).[19] The engineered zinc finger consists of three zinc fingers, and in each finger, the cysteine residues were substituted with a histidine residue in the metal coordination site. Conformational analysis by CD and ^{1}H 2D-NMR study suggested that the

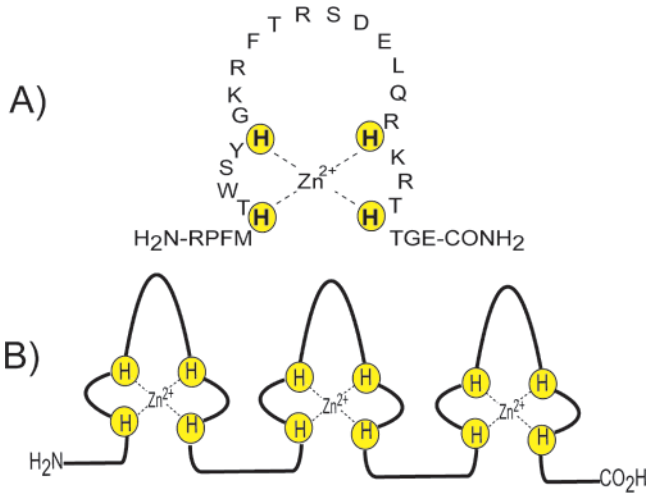
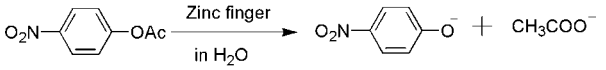


FIGURE 4. Artificial H_2H_2-type zinc finger proteins: (A) zf(HHHH) designed from the second finger of Sp1; (B) the three-tandem zinc finger protein H_4Sp1.

engineered zinc finger retains the general *ββ*α fold of the wild-type protein in the presence of Zn(II). The gel mobility shift assay and DNase I footprinting analysis reveal the binding of Zn(II)−H_4Sp1 to the DNA fragment containing an Sp1 recognition site GC-box (5′-GGG GCG GGG CC-3′). Methylation interference analysis suggests a similar DNA-binding mode for H_2H_2Sp1 and C_2H_2-Sp1.

In an another study aimed at creating a single finger novel metallopeptide with catalytic zinc sites, a series of zinc finger peptides of 30 amino acids in length were designed based on the second finger of Sp1.[20] The peptide mutants differed only by the amino acids in the zinc-coordinating site, which change the coordination number. The folding studies reveal that the mutants retain the ability to bind 1 equiv of zinc ion and fold into compact structures, like the wild-type zinc finger peptide, despite their tridentate coordination. In addition, an electronic spectral study of the Co(II)−zf(HHHH) complex indicates a coordination geometry containing two vacant sites.[20a] 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. The hydrolysis ability of the zinc finger mutant was investigated using 4-nitrophenyl acetate (NA) as the substrate, Scheme 1. All the

Scheme 1. Hydrolytic Cleavage by Engineered Zinc Fingers



zinc complexes of the mutant peptides displayed an appreciable hydrolytic activity. The hydrolytic activity increased when the number of histidine residues was increased at the coordination site. In general, a zinc complex with a higher Lewis acidity exhibits a higher hydrolytic activity.[21] The zinc-coordinating cysteine residue decreases the Lewis acidity of the zinc ion through its electron-donating ability and, consequently, reduces the hydrolytic reactivity. As expected, among the mutants, the zf(HHHH) peptide showed a better hydrolytic activity than the other mutants. The catalytic activity was evidently higher than that of the zinc−cyclen (1,4,7,10-tetraazacyclododecane) complex, which is one of the best model complexes for hydrolytic zinc enzymes.[22] This mutant also catalyzed the hydrolysis of amino acid esters (Boc-glutamine 4-nitrophenyl ester, D- or L-Gln-ONp) with a significant enantioselectivity. It is interesting to note that the apopeptides also displayed a hydrolysis reactivity. The reactivity of the apopeptides was increased when the number of histidine residues increased in the coordination site, which is in contrast with those of the wild-type Zn(II)−peptide complexes.[20b] This result unequivocally supports the belief that the hydrolysis by apopeptides is caused by the histidine residues, while the hydrolysis by the Zn(II)−peptide complex is caused by the zinc center of the peptides. In the Zn−peptide complex system, the hydrolytic reaction is affected by the environmental factors of the zinc coordination center such as the electron-donating ability of the ligands, the coordination structure, and the folding structure. These results demonstrate that the redesign of a structural metal site in proteins is promising for the creation of novel catalytically active metalloproteins.

Since zinc finger motifs have been known to bind to the major groove of the DNA duplex in a sequence-specific fashion, we expected that our zinc finger mutants would function as a novel type of nuclease with a high sequence specificity for DNA duplexes. Therefore, we examined the ability of the zf(HHHH) mutant to hydrolyze the model DNA compound bis(4-nitrophenyl) phosphate (BNP). It was revealed that the mutant catalyzed the hydrolysis of BNP to afford 4-nitrophenolate and 4-nitrophenyl phosphate. This result encouraged us to examine the DNA hydrolytic ability of the zf(HHHH). A supercoiled plasmid DNA, pUC19GC, was used for this purpose because pUC19GC contains a GC box to which Sp1 specifically binds.[10b] The DNA pUC19, which contains no GC box, was used as the control to examine the specificity. Usually, DNA cleavage converts the supercoiled plasmid DNA (form I) to the nicked circular form (form II) and then to the linear form (form III). The zf(HHHH) zinc finger converted pUC19GC to form II but not to form III. The cleavage efficiency linearly increased as the zinc concentration increased and reached a plateau at the point of 1 equiv of zinc ion. These results emphasize that the zinc ion plays a critical role in the DNA hydrolysis. However, the hydrolytic reactivity decreased along with an increase in the ionic strength, 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 was also seen for pUC19, which suggested the zf(HHHH) single finger's inability to discriminate the DNA, that is, it was nonselective. The logical extension of this work was to link the single fingers to make a multifinger protein in a tandem fashion like the native Sp1. As expected, the tandem array of this peptide to form a three-tandem zinc finger protein (H_4Sp1) displayed a drastic enhancement in the DNA affinity and sequence-selective cleavage of DNA and converted the plasmid DNA to the form III DNA.[23] Moreover, in sharp contrast to the single-finger zf(HHHH), the reactivity of the three-finger H_4Sp1 for pUC19GC increased as the ionic strength increased. On the other hand, it showed only a small hydrolytic activity for pUC19 even 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 H_4Sp1 was achieved through specific interactions, such as hydrogen bonding with DNA bases of the GC box, like the wild-type Sp1.

Multiple-Zinc Fingers and DNA Bending Fingers

Recognition of a long DNA sequence is a rare function in the natural DNA-binding proteins. The naturally occurring nine zinc finger protein TFIIIA binds to a shorter DNA sequence because it uses only a few select fingers for their DNA bindings.[4b,24] A protein consisting of three zinc fingers recognizes a DNA sequence of nine base pairs in length. Similarly, six fingers linked together would recognize a DNA sequence of 18 base pairs in length, which would be a rare sequence in the human genome. There is much interest in engineering sequence-specific binding proteins that bind to the longer regions of DNA.[25] Other groups have demonstrated that such polydactyl zinc finger peptides should be broadly applicable as genome-specific transcription switches in gene therapy strategies and the development of novel transgenic plants and animals.[6c,26] To achieve this ambitious goal, we have designed novel six- and nine-finger peptides (Sp1ZF6 and Sp1ZF9) based on Sp1, Figure 5.[27] These peptides were constructed by connecting the three finger domains of Sp1 by a Kruppel-type linker (Thr-Gly-Glu-Lys-Pro) peptide. The resulting nine zinc finger peptide, Sp1ZF9, binds a contiguous 27-base pair DNA with a high affinity and specificity. The affinity of the nine-finger peptide had increased by 30-fold compared to the wild-type three-finger Sp1.

On the basis of the multi-zinc-finger described above, we designed a DNA bending finger to regulate gene expression.[28] DNA structural changes, such as bending, play an important role in various biological reactions, such as transcription, recombination, and replication. A ligand that induces DNA bending in a site-specific manner would be expected to be an artificial regulator of a specific gene

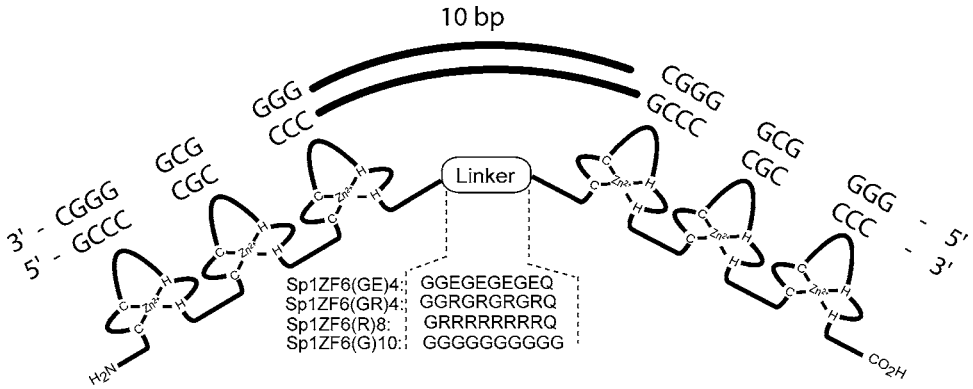


FIGURE 5. Schematic representation of artificial DNA bending fingers and their target DNA sequences.

transcription. Therefore, an artificial protein that induces a DNA conformational change is interesting as a transcriptional regulator of a specific gene. We created six-zinc-finger proteins, Sp1ZF6(Gly)$_n$ (n = 4, 7, or 10), by connecting two units of Sp1 through flexible polyglycine peptide linkers. The gel mobility shift and footprinting analyses revealed that Sp1ZF6(Gly)$_7$ and Sp1ZF6(Gly)$_{10}$ bind to two distal GC boxes with a 10 bp intervening sequence resulting in DNA bending. The phasing assays strongly suggested that the induced DNA bending was directed toward the major groove and that Sp1ZF6(Gly)$_7$ caused the most significant directional change in the DNA bending. Of special interest are the facts that the designed six-finger peptides, Sp1ZF6(Gly)$_7$ and Sp1ZF6(Gly)$_{10}$, can induce DNA bending in the intervening region between the two distal binding sites and that the linker length between the two three-finger motifs plays a crucial role in the entire DNA bending direction.

The 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.[29] To investigate the effect of the linker type on the DNA-binding properties, we have designed six-zinc-finger peptides, Sp1ZF6(GE)$_4$ and Sp1ZF6(GR)$_4$, with charged linkers (Figure 5).[30] These proteins are also able to induce DNA bending similar to the Sp1ZF6(Gly)$_n$ protein. The DNA recognition mode and the induced DNA structural change were similar among these proteins. However, the kinetic aspects of their DNA bindings were different in each case. In particular, the dissociation rate of Sp1ZF6(Gly)$_{10}$ was the fastest and that of Sp1ZF6(GR)$_4$ was the slowest. The Sp1ZF6(Arg)$_8$ peptide, which has a cationic polyarginine (RRRRRRRR) peptide as a linker between the two three-finger units of Sp1 preferably binds to discontinuous DNA sequences, whereas the Sp1ZF6-(Gly)$_{10}$, which has a structurally flexible polyglycine (GGGGGGGGGG) peptide sequence as linker, binds to both continuous and discontinuous DNA targets.[31] The DNA-binding selectivity of Sp1ZF6(Arg)$_8$ toward discontinuous targets could be due to (i) the electrostatic repulsion between the cationic side chains within the polyarginine linker, (ii) the low conformational freedom of the polyarginine linker, or (iii) the non-sequence-specific electrostatic interaction between the polyarginine linker and DNA phosphate backbone in the contiguous target sequence. These findings clearly indicate that the DNA sequence preferences of the engineered zinc fingers can be altered by the choice of linker sequences with different charges.

Designer Zinc Fingers as DNA Cutters

The complete analysis of the human genome reveals close relationships between the DNA sequences and diseases. Therefore, the application of this knowledge to gene therapy has become very important. To achieve a high selectivity for a target DNA sequence, the construction of an artificial nuclease has been widely investigated as a promising tool.[32] Chandrasegaran and co-workers have shown that a zinc finger protein can be coupled to the nonspecific DNA cleavage domain of the Type IIS restriction enzyme *Fok*I to produce a zinc-finger nuclease.[33] Conversion of a DNA-binding protein to a DNA-cleaving functional protein by attachment of a metal-chelating ligand is one of the most versatile methods for affinity cleaving. These reported chimeric proteins have largely utilized helix−turn−helix or b-zip-type motifs and interacted with DNA as a dimer.[34] Therefore, their target sites are limited to palindromic base sequences with a dyad or a pseudodyad axis. On the other hand, DNA sequences recognized by the C_2H_2-type zinc finger proteins are almost asymmetric because of their monomeric binding mode. We designed a novel highly selective DNA cutter peptide Sp1GGH based on Sp1.[35] The primary sequence of the Sp1GGH comprising two functional domains is shown in Figure 6. The DNA-binding domain contains the C-terminal region (residues 529−696) of Sp1, which consists of three tandem repeats of the C_2H_2-type zinc finger motif. In an effort to give DNA-cleaving activity to the zinc finger protein, the tripeptide Gly-Gly-His was attached to the N-terminus. It was shown that the Ni(II) complex of the tripeptide attached to the DNA-binding protein can successfully cut DNA in the presence of a peracid.[36] Incorporation of tripeptide GGH at the N-terminal region of the designed protein does not affect the structure. It was confirmed the zinc finger domain binds to Zn(II) ion, whereas the N-terminal tripeptide binds to Ni(II) selectively. Sp1GGH showed a highly selective DNA cleavage.[35]

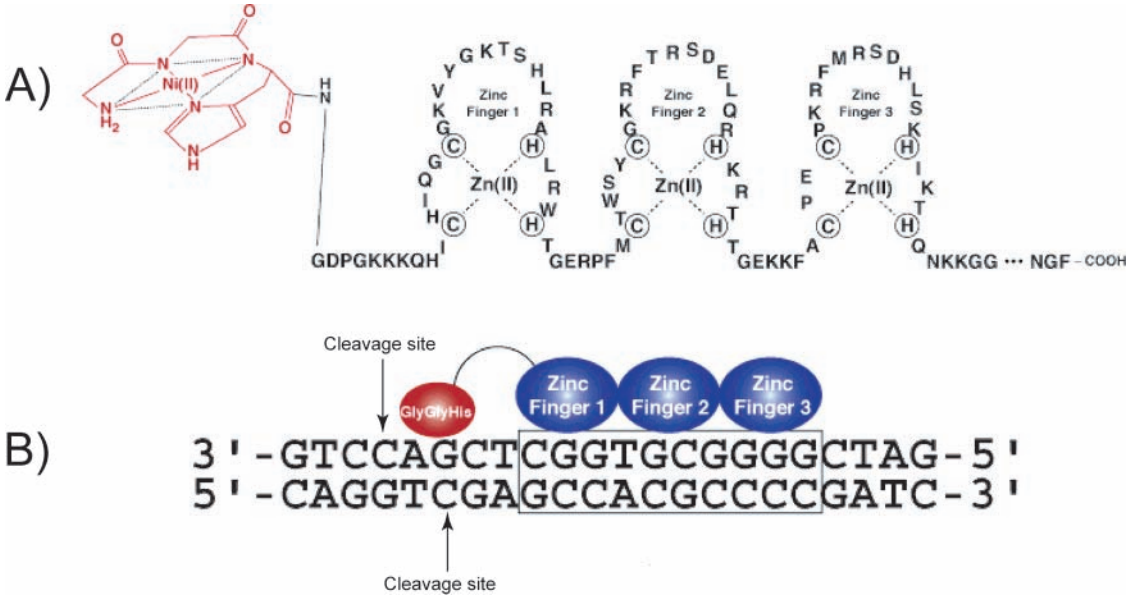


FIGURE 6. (A) Schematic representation of DNA cutter Sp1GGH and (B) DNA substrate showing the cleavage sites. The box indicates the GC box.

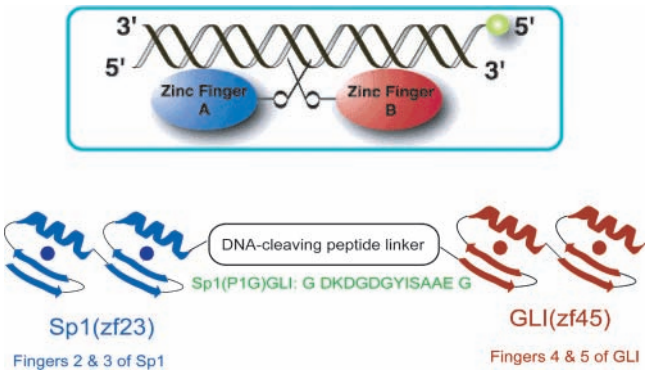


FIGURE 7. Schematic representation of artificial zinc-finger-type nuclease Sp1(P1G)GLI.

Recently, we reported an artificial zinc finger-type nuclease, Sp1(P1G)GLI, in which we linked two different zinc fingers using a metal-chelating linker peptide possessing a DNA-cleaving property.[37] We found that a synthetic peptide spanning fingers 2 and 3 of the Sp1 zinc finger [sp1(zf45)] has a sufficient DNA-binding affinity for the binding site.[10] Likewise, a synthetic peptide corresponding to fingers 4 and 5 of the GLI zinc finger [GLI-(zf45)] was shown to have a sufficient DNA-binding affinity for the binding site.[17b] The two peptide segments were connected using a cerium-binding peptide linker in a novel attempt to create an artificial nuclease. A schematic representation of the designed proteins is shown in Figure 7. The engineered zinc finger SP1(P1G)GLI, when bound to Ce(IV), showed a site-specific DNA-cleavage pattern inside its DNA-binding sites. This designed nuclease has a high potential for application to any DNA sequences based on the following features: (i) the usefulness of the zinc finger domains for almost all of the DNA triplets and (ii) the facilitated engineering of multi-zinc-finger-type nucleases by converting any normal linkers into P1 linkers.

Summary and Outlook

This Account has described some of our strategies to create novel DNA-binding proteins by re-engineering the DNA-binding domain of the human transcription factor Sp1. The C_2H_2 domain of Sp1 provided an attractive framework for the design of artificial zinc finger proteins with a high DNA specificity. The DNA binding properties of the designed zinc fingers were customized by altering the secondary structure elements, namely, the α-helix and β-sheet, present in the domain. Catalytically active metalloproteins were created by manipulating the zinc-coordinating site. Replacement of cysteines with histidine produced an unnatural H_2H_2-type zinc finger, which showed specific hydrolytic cleavage of DNA.

The results described here and in the literature from other groups suggest that the zinc finger protein design field has reached a stage where it is possible to create novel zinc fingers proteins for diverse DNA target sites by design or phage display or by the combination of both.[6] The engineered zinc finger proteins that recognize preselected DNA sites have numerous potential applications especially when other functional domains are attached. The most natural application is the generation of novel transcription factors by attaching silencing or activation domains to up or down regulate the expression of a target gene. This approach has been utilized by other groups and demonstrated the use of zinc finger transcription factors in gene regulation in mammalian cells or plants.[26,38] Researchers at Sangamo Biosciences have created a zinc finger transcription factor to activate vascular endothelial growth factor, which is currently in clinical trials.[39] Apart from the designer transcription factors, novel nucleases have been reported in which the DNA-binding zinc finger domain is attached to the DNA hydrolysis domain. The recent report of human gene correction using designed zinc-finger nucleases is particularly exciting.[40] Recently,

it was shown that artificial zinc finger proteins can be used to inhibit virus replication in human cells and plants.[41] These demonstrations suggests that designer zinc finger proteins have diverse applications in medicine and agriculture.

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