

## Artificial Nine Zinc-Finger Peptide with 30-Base Pair Binding Sites<sup>†</sup>

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Newly designed zinc-finger peptide Sp1ZF9 containing nine Cys<sub>2</sub>-His<sub>2</sub> type motifs has been manipulated. The DNA binding property of Sp1ZF9 was compared with those of native three zinc-finger Sp1(530-623) and artificial six zinc-finger Sp1ZF6 peptides. Although the equilibrium time was less than 0.5 hr for Sp1(530-623)-DNA complex, Sp1ZF6 and Sp1ZF9 required approximately 48 and 72 hrs respectively for full complex formation. Evidently, the footprinting analysis demonstrated that Sp1ZF9 and Sp1ZF6 bind at least 27 and 18 contiguous base pairs of DNA sequence, respectively. Sp1ZF9 showed two step bindings to DNA, namely first the recognition of GC (5'-GGG-GCG-GGGCC-3') sequence by the N-terminal Sp1 domain and next the recognition of the corresponding target sequences by the middle and C-terminal Sp1 domains. In contrast with unimolecular binding of Sp1ZF9 and Sp1ZF6, two Sp1(530-623) molecules bind to one GCIII (5'-GGG-GCG-GGG-GGG-GCG-GGG-GGG-GCG-GGGCC-3') site region. Of special interest is the fact that new nine zinc-finger peptide Sp1ZF9 can bind to DNA sequence of approximately 30-base pairs. Such multi zinc-finger peptides may be useful as genome-specific transcriptional switches in future.

Key Words: Zinc-finger protein/ transcription factor/DNA recognition/ multi finger/ artificial protein

DNA binding proteins selectively bind to specific DNA sequence, and play an important role in biological systems.<sup>1</sup> Zinc-finger domain of Cys<sub>2</sub>-His<sub>2</sub> type is a typical class of DNA binding protein, and contains the sequence of (Tyr, Phe)-X-Cys-X<sub>2,4</sub>-Cys-X<sub>3</sub>-Phe-X<sub>5</sub>-Leu-X<sub>2</sub>His-X<sub>3,5</sub>-His, usually in tandem arrays.<sup>2-5</sup> The X- ray

crystal structures of the Zif268- and GLI-DNA complexes revealed the characteristic DNA binding mode of zinc finger proteins as follows: (1) recognition of three bases per one finger motif, (2) structure of tandemly repeated finger domain, and (3) binding to the sequence of asymmetric base pairs.<sup>6-9</sup> Transcription factor Sp1 involves

### BIOORGANIC CHEMISTRY- Bioactive Chemistry-

#### Scope or research

The major goal of our laboratory is to elucidate the molecular basis of the activity of various bioactive substances by biochemical, physicochemical, and synthetic approaches. These include studies on the mechanism of sequence-specific DNA cleavage by antitumor or carcinogenic molecules, studies on the DNA recognition of zinc-finger proteins, and model studies on the action of ion channels. In addition, artificial designed peptides have also been developed as useful tools in molecular biology and potentially in human medicine.



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three Cys<sub>2</sub>-His<sub>2</sub> type zinc-finger motifs at the C-terminus of protein,<sup>10</sup> and is closely related to Zif268.<sup>11</sup> Indeed, Sp1 strongly binds to GGG-GCG-GGG sequence. On the basis of the nature of Cys<sub>2</sub>-His<sub>2</sub> type zinc-finger motif and recognition bases of Sp1, we designed novel nine zinc-finger peptide Sp1ZF9 and also its DNA binding properties were compared with those of three finger Sp1(530-623) and six finger Sp1ZF6 peptides. DNA binding of nine zinc-finger protein TFIIIA is well known to be dominated by interaction of select few fingers.<sup>2, 8, 13-15</sup> Therefore, it is of special interest to create new nine zinc-finger peptide that can bind to DNA sequence over an extended region of 30-base pairs. Such multi zinc-finger peptides may be hopeful in future gene therapy strategies. Certainly, molecules with high DNA binding affinity and long sequence specificity in the human genome are useful tools in molecular biology and potentially in human medicine.<sup>16-18</sup>

**Design of multiple zinc-finger proteins:** Novel multiple zinc-finger peptides, Sp1ZF6 and Sp1ZF9, were newly created from zinc-finger motif of transcription factor Sp1. These peptides were constructed by connecting C-terminal Sp1 molecule to N-terminal of a following one. The *Krüppel-type* linker (Thr-Gly-Glu-Lys-Pro) which is conserved in many zinc-finger proteins, was selected for connection of Sp1 finger domains. This linker plays in controlling the orientation and spacing of adjacent finger and also is involved in nonspecific interaction with phosphate backbone of DNA.<sup>6, 8, 19, 23, 24</sup>

**Binding specificity of Sp1(530-623), Sp1ZF6, and Sp1ZF9.** In gel mobility shift assays, the binding sites were predicted from Sp1 recognition site (GGG GCG GGG).<sup>10</sup> The equilibrium time was less than 0.5 hr for Sp1(530-623)-DNA complex. By contrast, Sp1ZF6 and Sp1ZF9 required approximately 48 and 72 hrs, respectively. To determine binding specificity of the multiple zinc-finger peptides, we performed the gel mobility shift assays with two DNA fragments. The binding affinity of Sp1(530-623) was no significant difference in three DNA fragments. Sp1ZF6 showed about 20-fold preferential binding to GCII compared with GC. Sp1ZF9 gave approximately 30-fold higher affinity with GCIII than GC. The results reveal that the length of the binding DNA sequence is dependent on

the number of these zinc-finger motifs. On the other hand, the binding affinities for GCIII complexes of Sp1ZF6 and Sp1ZF9 were considerably close. Probably, this is because GCIII sequence contains both GC and GCII sequences. Two Sp1(530-623) molecules bind to one GCIII fragment but Sp1ZF9(or Sp1ZF6) does with unimolecule.

**DNA binding of multiple zinc-finger peptides:** In order to examine the DNA binding site of Sp1ZF6 and Sp1ZF9 on GCIII, DNase I footprinting assays were performed. Under lower peptide concentration, Sp1(530-623) bound the GC-box of 3'-portion. With increasing the peptide concentration, the GC-box of 5'-portion was protected and also the hypersensitive breakages were detected at C(14) and G(15) within the middle GC-box. Clearly, two Sp1(530-623) peptides bound to GCIII. On the other hand, Sp1ZF6 and Sp1ZF9 exhibited different binding features from Sp1(530-623). Sp1ZF6 bound to longer sites than 18 bp of 3'-end in the GCIII. Sp1ZF9 protected slightly longer binding sites than the 27 bp target site.

**Binding affinity of Sp1ZF9 to GCIII.** To estimate accurately the binding affinity, the active peptide concentration should be calculated on the basis of only the preparation fraction which is active to bind to DNA. However, the larger peptide may be expected to be less likely to fold and more likely to be oxidized in long equilibrium period. By using the peptide prepared freshly, therefore, we determined approximate DNA binding affinity of Sp1ZF9. In the case of 72 hr, apparent equilibrium dissociation constant ( $K_d$ ) was  $1.2 \pm 0.3$  nM. Recently, we determined that the dissociation constant ( $K_d$ ) of three zinc-finger Sp1(530-623) peptide for GC-box DNA is  $3.5 \pm 0.5$  nM.<sup>25</sup>

In conclusion, newly designed nine zinc-finger peptide Sp1ZF9 binds a contiguous 27-bp DNA. The multiple zinc-finger peptide has two steps of the sequence recognition and binding for peptide-DNA complex formation. Recently, zinc-finger motifs contacting with various sequences were selected by the technique of phage display.<sup>29-32</sup> The present results would provide good information for design of new DNA binding proteins to recognize long DNA sequence. Indeed, human Y-box binding protein gene promoter<sup>33</sup> and human immune activation (Act-2) gene<sup>34</sup> include GCIII-like long GC sequences. In future gene therapy, such multi zinc-finger proteins may be useful as genome-specific transcriptional switches.