

# Artificial Zinc Finger Peptide Containing a Novel His<sub>4</sub> Domain

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Zinc finger constitutes one of the most common DNA binding motifs. Although zinc finger proteins consisting of Cys<sub>2</sub>His<sub>2</sub>, Cys<sub>3</sub>His, Cys<sub>4</sub>, and Cys<sub>6</sub> domains are known in nature, a novel His<sub>4</sub> zinc finger protein has never been observed. Herein, we have created the first artificial His<sub>4</sub>-type zinc finger protein (H<sub>4</sub>Sp1) engineered by Cys to His mutations of the Cys<sub>2</sub>His<sub>2</sub>-type zinc finger transcription factor Sp1. The CD features of the single finger H<sub>4</sub>Sp1f2 and three-finger H<sub>4</sub>Sp1 clearly demonstrate the folding of the mutant His<sub>4</sub> peptides by complexation with Zn(II). The NMR study of Zn(II)-H<sub>4</sub>Sp1f2 reveals that some distortions of the helical region occur due to Zn(II) coordination. The gel mobility shift assay and DNase I footprinting analysis strongly show the binding of Zn(II)-H<sub>4</sub>Sp1 to the GC-box site of duplex DNA. The methylation interference pattern of Zn(II)-H<sub>4</sub>Sp1 binding significantly resembles that of the corresponding C<sub>2</sub>H<sub>2</sub>Sp1 binding. The present artificial peptide H<sub>4</sub>Sp1 is the first example of a zinc finger containing the His<sub>4</sub> domain. Of special interest is the fact that the zinc finger domains of H<sub>4</sub>Sp1 are folded (although not identical to the native structure) and bind DNA similar to wild type C<sub>2</sub>H<sub>2</sub>Sp1.

*Key words:* Zinc finger/ Sp1/ Designed metallofinger/ DNA binding

Metalloproteins play important roles in gene regulation, and some metal ions also participate in the transcriptional stage by protein-mediated metallation. In particular, zinc ion is essential as a structural factor for zinc finger proteins, which constitute one of the most common DNA binding motifs. Zinc finger proteins acquire DNA binding ability by Zn(II)-complexation. In nature, Cys<sub>2</sub>His<sub>2</sub>-, Cys<sub>3</sub>His-, Cys<sub>4</sub>-, and Cys<sub>6</sub>-type zinc fingers exist. Among them, the Cys<sub>2</sub>His<sub>2</sub>-type zinc finger motif especially possesses the following fascinating characteristics; (1) a compact ββα fold is acquired by Zn(II)-coordination to bind the asymmetric DNA sequence, (2) one finger recognizes 3 to 4 base pairs by the side chains of amino

acids located on the recognition helix, and (3) extended recognition can be attained by tandem repeating. The mini ββα fold of the Cys<sub>2</sub>His<sub>2</sub>-type zinc finger motif is an attractive framework for designing a novel metallofinger motif. At the present stage, no newly designed metallofingers that have evident DNA recognition ability are known. In addition, zinc finger proteins containing the His<sub>4</sub> domain have never been found or engineered. Herein, we have created the first novel His<sub>4</sub>-type zinc finger protein by Cys to His mutations of a Cys<sub>2</sub>His<sub>2</sub>-type zinc finger in transcription factor Sp1.

In order to clarify the structural alteration of the zinc finger domain by Cys to His mutations, the H<sub>4</sub>Sp1f2-Zn(II)

## BIOORGANIC CHEMISTRY — Bioactive Chemistry —

### Scope of 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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complex was investigated using two-dimensional NMR techniques. In contrast to  $C_2H_2Sp1f2$ , a 3D structural model of  $H_4Sp1f2$  showed the twisting of an extended strand in the Pro2-Trp7 region and helical unwinding around Asp18-Gln21, as indicated by the disappearance of the  $C_\alpha H_1-NH_{1+3}$  NOEs between them. The twisting detected in the Pro2-Trp7 region probably occurs in order to accommodate a tetrahedral binding geometry enforced by zinc coordination constraints.

To examine whether the folding of  $H_4Sp1$  is Zn(II)-dependent as well as that of  $H_4Sp1f2$ , CD studies were carried out. The mutant protein  $H_4Sp1$  was constructed from  $C_2H_2Sp1$  by Cys to His mutations of all three of the zinc finger domains. The CD spectrum of  $H_4Sp1$  in the Zn(II)-free buffer was characteristic of the random coil. In the presence of Zn(II), the negative CD signal bands near 203 nm and 222 nm clearly increased, demonstrating the folding of  $H_4Sp1$  by Zn(II)-complexation. The CD feature of Zn(II)- $H_4Sp1$  was different from that (207 and 226nm) of Zn(II)- $C_2H_2Sp1$  to some degree, suggesting that the zinc finger domain of  $H_4Sp1$  did not form the structure identical to that of the wild type  $C_2H_2Sp1$ .

The results of the gel mobility shift assays clearly showed that Zn(II)- $H_4Sp1$  was bound to the DNA fragment (41 bp) containing an Sp1 recognition site GC box (5'-GGGGCGGGGCC-3'). From the evidence for the monomeric binding of the  $C_2H_2$ -type zinc finger to the single binding site, the binding mode of Zn(II)- $H_4Sp1$  is also monomeric because the mobility of the shifted band was the same as that of Zn(II)- $C_2H_2Sp1$ . However, the DNA binding affinity of  $H_4Sp1$  for the GC box was lower than that of  $C_2H_2Sp1$ . Furthermore, the Zn(II)-dependent DNA binding ability of  $H_4Sp1$  was shown by the decrease in the DNA binding by EDTA. From the result of the DNase I footprinting analysis of Zn(II)- $H_4Sp1$  for a 148-bp DNA fragment containing the GC box,  $H_4Sp1$  almost protected the residues of the GC box at 2.4  $\mu$ M from DNase I cleavage in the presence of Zn(II). These results strongly indicate that the  $H_4Sp1$  still retained the DNA binding ability in spite of the Cys to His mutation.

Previous studies have demonstrated that  $C_2H_2Sp1$  can bind DNA by interacting with Co(II), Cd(II), and Ni(II) as well as Zn(II). To clarify whether the present  $H_4Sp1$  gains the DNA binding ability by various metal complexations,  $H_4Sp1$  was reconstructed with Zn(II), Ni(II), Cu(II), Co(II), or Cd(II) from insoluble fractions during the purification step, and a gel mobility shift assay was conducted. While Zn(II)-reconstituted  $H_4Sp1$  was really bound to a GC-box fragment, under this experimental condition, the DNA binding activity was not detected in the case of Ni(II)-, Cu(II)-, Co(II)-, and Cd(II)-reconstituted  $H_4Sp1$ . From the CD studies of the  $H_4Sp1f2$ , only the Zn(II) binding to its  $His_4$  domain highly induced an ordered conformation with the secondary structure. Therefore, these results indicate that effective

folding by Zn(II) binding is essential for the DNA binding ability of  $H_4Sp1$ .

When Zn(II)- $H_4Sp1$  was bound to the GC-box fragment (41 bp), its methylation interference pattern was compared with that of Zn(II)- $C_2H_2Sp1$ . In the case of  $H_4Sp1$ , strong base contacts with G(2), G(3), G(4), and G(6) in the guanine-rich strand (G-strand) and G(5') in the cytosine-rich strand (C-strand) were detected and also weak contacts with G(1) and G(7) in the G-strand and G(11') in the C-strand were observed. The interference feature remarkably resembled that of the wild type  $C_2H_2Sp1$ , indicating similar DNA binding modes between  $H_4Sp1$  and  $C_2H_2Sp1$ .

The Cys to His conversion generates a larger metal coordination sphere because the size of the His residue is larger than that of the Cys residue as opposed to the case. Although the Cys to His mutation results in a decreased DNA binding affinity, the present peptide  $H_4Sp1$  maintains the DNA binding ability. In spite of the structural alteration or distortion of the zinc finger domain as clearly indicated by the CD and NMR evidence of  $H_4Sp1f2$ , it is of special interest that specific interactions between the recognition helix of the finger 2 and its subsite, 5'-GCG-3', are retained. In addition, fingers 1 and 3 of  $H_4Sp1$  also preserve the DNA recognition ability of the wild type  $C_2H_2Sp1$ . Several reasons are considered for the fact that  $H_4Sp1$  and  $C_2H_2Sp1$  show similar DNA recognition, despite the somewhat different helical conformation between their zinc finger domains. First, the DNA binding to Zn(II)- $H_4Sp1$  may cause an additional structural change and result in a specific DNA recognition analogous to Zn(II)- $C_2H_2Sp1$ . Indeed, it is known that the specific DNA binding induces a structural alteration in the basic region of GCN4. Second, Zn(II)- $H_4Sp1$  may recognize DNA bases in a different manner from Zn(II)- $C_2H_2Sp1$ . Namely, the mutant peptide  $H_4Sp1$  compensates for specific contacts with particular bases by a directional change in the side chain that is essential for DNA recognition. As a result, the recognition helix of Zn(II)- $H_4Sp1$  may be located in a different orientation to DNA from that of Zn(II)- $C_2H_2Sp1$ .

In conclusion, this paper describes the preparation, structure, and DNA binding properties of an engineered  $His_4$  mutant of the zinc finger protein Sp1. We present the NMR structure for a single mutated zinc finger ( $H_4Sp1f2$ ) from Sp1 and the DNA binding data for a three domain mutant ( $H_4Sp1$ ). The CD characteristics of  $H_4Sp1f2$  and  $H_4Sp1$  are also compared with those of  $C_2H_2Sp1f2$  and  $C_2H_2Sp1$ . Interestingly, the  $His_4$  domain is folded and recognizes the GC-box DNA. The development of such a novel peptide may also lead to a new zinc finger type or other metal finger domain. Additionally, the present results suggest that a  $His_4$ -type zinc finger protein may also exist in nature.