DNA binding of a basic leucine-zipper protein with novel folding domain

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ABSTRACT
DNA-binding proteins frequently utilize short α-helices as their critical DNA recognition elements. In this research, we have employed the structure-based design to construct a small domain that could target the specific DNA sequences recognized by the yeast transcriptional activator GCN4. The new DNA binding motif recognizes specific DNA sequences as a dimer with high affinity and specificity under the physiological conditions.

INTRODUCTION
The yeast transcriptional factor GCN4 is one of a large family of DNA binding proteins identified by the basic leucine zipper (bZIP) structural motif [1]. When GCN4 binds a specific DNA sequence as a dimer, the leucine zipper region of two monomers is assembled into a parallel coiled-coil [2], whereas structure of the basic region transits from the random coil to an α-helix [3]. This induced-fit mechanism is considered to play an important role in the sequence-specific DNA binding of GCN4 [4]. It has been demonstrated that the folding/unfolding transition of GCN4 is highly cooperative with protein dimerization and DNA binding. The GCN4 involves the electrostatic repulsion within the basic region in the absence of DNA, whereas it is electrostatically neutralized when bound at DNA.

As the first step to investigate the role of the 'induced-fit' mechanism on the sequence-specific DNA recognition by GCN4, we have attempted to design a small domain that can target the specific DNA sequences and can fold into stable even with such an electrostatic repulsive force. Our design strategy utilizes a well-folded small domain of villin headpiece [5] as a scaffold for the structure-based design of a new DNA binding region (Fig. 1). Appropriate substitutions of the α-helix at the C-terminal of villin with amino acid residues necessary for the sequence-selective binding of GCN4 would afford a novel DNA binding domain with a folded structure.

Here, we report the design and DNA binding of a new domain that can target a specific DNA sequence recognized by GCN4. The new domain binds the expected DNA sequences as a dimer at the physiological condition at room temperature.

Fig. 1. Possible model for the interaction between the bVIL-K71 DNA contacting region (ribbon) and the API DNA sequence (wire). Only the DNA contacting region of a monomeric bVIL-K71 is shown.

MATERIAL AND METHOD
Preparation of the Protein
The new DNA binding proteins (bVIL-W64 and bVIL-K71) were prepared by E.coli expression system.
The proteins were purified by using cation exchange and reversed phase chromatography. The purity of proteins was confirmed by SDS-PAGE and amino acid analyses.

**Electrophoretic Mobility Shift Assay**

DNA binding activity of proteins was monitored by the electrophoretic mobility assay. Binding reaction was performed in the presence of the indicated amount of the proteins with 20 pM 32P-labeled oligonucleotide duplex in binding mixture containing 20 mM Tris-HCl (pH 7.5), 4 mM KCl, 2 mM MgCl2, 1 mM EDTA, 100 µg/ml BSA, 0.1% NP-40 and 6% sucrose. The binding mixtures were incubated at room temperature for 15 minutes, and then were loaded on a 10% native polyacrylamide gel. The increase of the mobility-shifted band was analyzed by autoradiography and quantitated by the densitometry of the autoradiogram. The concentration of the proteins was determined by quantitative amino acid analysis.

**Circular Dichroism Studies**

CD experiments were performed on a J-720 CD spectrometer at 18 °C using 0.1 cm cuvette. Spectra of the proteins in the presence of oligonucleotides were calculated as the difference between the bound spectrum and a spectrum of the respective free oligonucleotide. Samples contained 20 mM Tris-HCl (pH 7.5), 4 mM KCl, 2 mM MgCl2, 1 mM EDTA, 8 µM protein monomer, and 5 µM oligonucleotide duplex when present. Spectra were the average of 32 scans and were corrected with a spectrum of buffer alone. Temperature scans were performed with 10 µM of the protein by scanning continuously from 4 to 80 °C.

**RESULT AND DISCUSSION**

**Expression and Purification**

The genes encoding the chimeric proteins, bVIL-W64 and bVIL-K71 were synthesized and cloned into pBLZ vector. The proteins were expressed in BL21(DE3)pLysS cells. SDS-PAGE analysis of the cell lysate after an induced culture indicated target protein band. After large-scale expression, induced cells were harvested and then extracted by using sonicator. The extracts were purified with an ion exchange and a reversed phase chromatography. The final yields of the bVIL-W64 and bVIL-K71 were 6 mg and 4 mg per 500 ml of culture, respectively.

**DNA Binding of bVIL Proteins**

Both bVIL-W64 and bVIL-K71 bind DNA sequences recognized by GCN4 as dimers. The equilibrium dissociation constants (Kd) were determined for binding complexes of GCN4, bVIL-W64 and bVIL-K71 to the specific and non-specific DNA. The Kd was measured from titration of the gel shift and by using BIAcore. bVIL-K71 formed a specific DNA complex as stable as that with GCN4. The DNA sequence selectivity of the chimeric protein toward the CRE (5'-ATGACGTCAT-3') and API (5'-ATGACTCAT-3') sequences is different from that observed for GCN4. In addition, the binding specificity of bVIL-K71 was temperature dependent.

**CD Studies on bVIL-W64 and bVIL-K71**

Circular dichroism difference spectra were measured for the chimeric proteins. Each CD spectrum shows the signal characteristic of a helical conformation in the presence of the specific DNA sequence. However, the way increasing the helicity in the presence of a particular DNA sequence for the chimeric proteins was different from that for GCN4. The results indicate that the structural transition mechanism is altered for the chimeric protein. It is likely that the chimeric protein forms a new folding domain.

**Thermal stability of Chimeric Proteins**

Analyses of the thermal stability of bVILs and GCN4 indicate that the DNA contacting region of bVIL-K71 is more stable than that of GCN4. It is likely that the bVIL-K71 DNA recognition domain is stabilized by the hydrophobic core formed in a similar manner as observed for the villin headpiece. Neither chimeric protein formed a well-folded structure in absence of DNA. However, thermostability of bVIL-K71 chimera was improved as compared to GCN4.

**REFERENCES**