Mutational analysis of the structure basis for the multimerization function of NifA central domain

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Abstract In *Klebsiella pneumoniae* (Kp) NifA central domain, when the conservative amino acid residue Thr-290 in C3 region was replaced by Val, the function of NifA for activating the transcription of *nif* genes was lost. Thus the conservative Thr-290 residue seems critical for the activation function of NifA central domain. This point mutant of NifA central domain is used to examine the putative multimerization function of NifA central domain by merodiploid experiment. The results showed that the NifA central domain bore the multimerization determinants of NifA protein. A series of truncated mutants of NifA were constructed to determine the structural elements at the central domain critical for multimerization. It demonstrates that amino acid residues 252—453 are involved in the multimerization function of NifA central domain.

Keywords: NifA protein, central domain, multimerization determinant.

NifA protein is the central positive regulator of nitrogen fixation (*nif*) genes. It activates transcription of *nif* operons by the alternative holoenzyme form of RNA polymerase, σ54-holoenzyme, in an NTP-dependent manner. The activity of NifA is inhibited by NifL protein under oxygen or in the excess of fixed nitrogen.

NifA is composed of three domains: the N-terminal domain, the central catalytic domain and the C-terminal DNA-binding domain.

We have less knowledge about the function of N-terminal domain of NifA. Berger et al. reported that the N-terminal deleted NifA protein showed higher activity in activating *nif* genes than the intact NifA[1]. Recently Gu et al. showed that the N-terminal domain of NifA determines the temperature sensitivity of NifA[2].

The purified C-terminal domain of NifA shows its ability to bind to the upstream activating sequences (UASs) of *nif* genes[3]. A helix-turn-helix motif responsible for the DNA-binding function was predicted in the C-terminal domain. It was supported by Missaillidis’ experiment, in which the conformation of the C-terminal domain of *Kp* NifA was probed by three spectroscopic techniques[4].

The central domain, bearing ATPase activity, plays an important role in transcription activation of *nif* genes. The purified central domain of *Kp* NifA itself can catalyze the
isomerization of closed complexes between $\sigma^{54}$-holoenzyme and a nif promoter to open complexes$^{[5]}$. NifA is an enhancer-binding protein (EBP). The amino acid sequences of EBPs, such as NifA, TyrR and NtrC, were analyzed and the central domains of EBP families were found to be highly homologous. Seven most conservative regions presented in the EBP central domains were named C1—C7, respectively. There were a Walker A motif in C1 and a Walker B motif in C4, which suggested that C1 and C4 took part in ATP binding and/or ATP catalysis$^{[6]}$. Deletion of C1 or mutation in C7 led to the loss of transcription activation function of NifA. The C3 region of Bradyrhizobium japonicum (Bj) NifA was predicted to interact directly with $\sigma^{54}$-holoenzyme, and the hydroxyl group of the conserved Thr residue in this region is essential for the activation function of NifA$^{[7]}$.

Lee and North et al. reported that NifA existed in form of dimer for activating the transcription of nif genes and predicted that major dimerization determinants of NifA would lie in the central domain$^{[3, 8]}$. Money’s result indicates that Azotobacter vinelandii NifA is possible to multimerize to tetramer in solution$^{[9]}$. In the present study, we examined the multimerization function of Kp NifA central domain by genetic means. Our results have demonstrated that the central domain bears the multimerization function, and the amino acid residue 252—453 region of that domain seems critical for the multimerization.

1 Materials and methods

1.1 Strains and plasmids

E. coli JM105 was used as the recipients for the plasmids. E. coli YMC11 (endA thi-1 hsdR17 supE44 ladU169 hutC gln(ALG)Δ2000) was used for determining the expression of introduced reporter gene in terms of $\beta$-galactosidase activity. Plasmid pMAL-cRI, the maltose-binding protein (MBP) expression vector, was provided by Prof. Xu Genjun, and pGEM-Teasy was from Promega Corporation. These two plasmids were used for construction of recombinant plasmids. Plasmid pST1021 carries the Kp nifBAL gene inserted into pACYC184$^{[10]}$. Plasmid pMB210$^{[11]}$ carries Rm nifH-lacZ fusion.

1.2 Medium

LB medium was used for E. coli growth. Antibiotics used were chloramphenicol (Cm) (40 $\mu$g/mL), ampicillin (Ap) (100 $\mu$g/mL) and tetracycline (Tc) (15 $\mu$g/mL).

1.3 DNA preparation

Plasmid DNAs were isolated by the alkaline lysis procedure. Bacterial cells were transformed by the CaCl$_2$ method.

1.4 PCR

The PCR procedure is: 94°C for 6 min, one cycle; 94°C for 1 min, 60°C for 1 min and 72°C for 2 min, 35 cycles; 72°C for 10 min, one cycle. Since the templates were GC-rich (62% or so),