Journal of Biomolecular NMR, **14:** 191–192, 1999.
KLUWER/ESCOM

Letter to the Editor: Backbone NMR assignments of a cyanobacterial transcriptional factor, SmtB, that binds zinc ions

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Received 18 February 1999; Accepted 12 March 1999

Key words: backbone assignments, heavy metal ion sensing, SmtB, transcriptional factor, zinc ion binding

Biological context

In *Synechococcus* PCC7942, the *smt* locus is responsible for tolerance to zinc and cadmium. This was verified by deletion of the *smt* locus, which caused a reduction in zinc/cadmium tolerance (Turner et al., 1993). In the *smt* locus, there are two divergently transcribed genes, *smtA* and *smtB*. The *smtA* gene encodes class II metallothionein (56 amino acid residues) (Shi et al., 1992), and the *smtB* gene encodes the repressor of *smtA* transcription (122 amino acid residues) (Morby et al., 1993).

In the absence of heavy metal ions, the transcription of *smtA* is repressed on binding of SmtB to the 100 bp operator-promoter region lying between the *smtA* and *smtB* genes (Erbe et al., 1995), while the transcription is stimulated by trace amounts of heavy metal ions (especially zinc and cadmium). This stimulation is thought to be caused by inhibition of the complex formation between SmtB and the recognition DNA sequence, as a result of the heavy metal ion binding to SmtB (Erbe et al., 1995).

It was found that SmtB predominantly forms a dimer and binds two zinc ions per subunit (Kar et al., 1997). Mutation work has suggested candidates for the amino acid residues ligating zinc ions (Turner et al., 1996). Recently, the crystal structure of the SmtB dimer was solved by X-ray crystallographic analysis and it was found that SmtB has a helix-turn-helix motif that might bind to the recognition DNA sequence (Cook et al., 1998). Furthermore, based on the results of soaking experiments with mercury ions and SmtB crystals, the ligand amino acids which might bind zinc ions were proposed. However, the following remain to be elucidated: the mechanism underlying the ion selective sensing by SmtB, and the relationship between the structural changes following the zinc ion binding with SmtB and the loss of the affinity of SmtB to the recognition DNA sequence.

To solve these problems, we have studied the solution structure of SmtB, which had bound zinc ions, by NMR spectroscopy. We report here the NMR backbone assignments of zinc-bound SmtB for the first step. By comparing the present results with our previous results for zinc-free SmtB (Morita et al., 1998), we identified the ligand amino acids for zinc ions, and have shown for the first time that a cysteine residue in the N-terminal flexible region of SmtB acts as a ligand for zinc ions.

Methods and results

In the minimal medium (Miller) containing $^{15}NH_4Cl$ (0.5 g/l) and [$^{13}C_6$]-D-glucose (2 g/l), SmtB was expressed in *E. coli* BL21(DE3)/pLysS harboring a plasmid containing the complete nucleotide sequence corresponding to SmtB. The purified SmtB was obtained by the same method as described previously (Morita et al., 1998). A 0.5 mM NMR sample of SmtB, that had bound zinc ions, was prepared in 250 μl of 90% H_2O/10% D_2O or 99% D_2O in the following NMR buffer: 50 mM PIPES, 50 mM KCl, 4 mM β-mercaptoethanol, 1.05 mM zinc acetate, pH 6.0.

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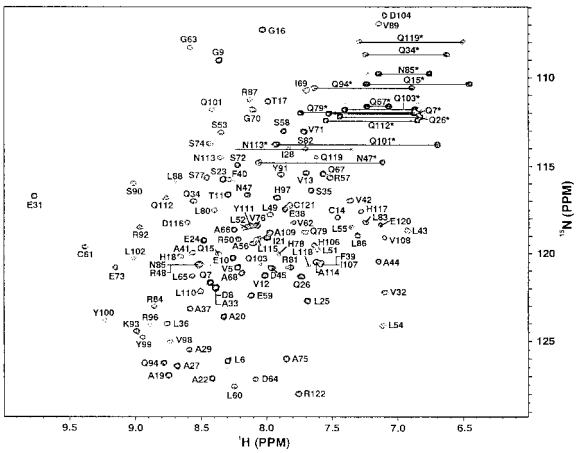

Figure 1. Two-dimensional ^{1}H-^{15}N HSQC spectrum of SmtB (0.6 mM; 50 mM PIPES buffer, pH 6.0; 40 °C), that has bound two equimolar zinc ions, measured at a ^{1}H resonance frequency of 500 MHz. To optimize the resolution in the nitrogen dimension, a ^{15}N spectral width of 1500 Hz was used ($sw_1 = 1500$ Hz, $sw_2 = 8000$ Hz, $n_{scan} = 32$, $n_1 = 64$, $n_2 = 1024$). The cross-peak assignments denoted by asterisks are the side-chain resonances of Asn and Gln.

2D ^{1}H-^{15}N HSQC and 3D HSQC-NOESY (^{1}H-^{15}N and ^{1}H-^{13}C), ^{1}H-^{15}N HSQC-TOCSY, HNCA, HN(CO)CA, CBCA(CO)NH, HNCACB, and HCCH-TOCSY data were collected at 40 °C with Bruker DMX 500 and DRX 600 spectrometers. The data were processed using NMRPipe (Delaglio et al., 1995) on SGI workstations (Indigo and O2). The ^{1}H, ^{13}C, and ^{15}N chemical shifts were referenced according to the method of Wishart et al. (1995).

Extent of assignments, data deposition and physiological meaning

In the presence of zinc ions, the exchange rate between free and zinc-bound SmtB is slow, and so we have assigned the signals in the 2D ^{1}H-^{15}N HSQC spectra of zinc-free and zinc-bound SmtB independently. Figure 1 shows a 2D ^{1}H-^{15}N HSQC spectrum of zinc-bound SmtB obtained at a ^{1}H resonance frequency of 500 MHz. The ^{1}HN resonances in Figure 1 were assigned primarily using the HNCACB and CBCA(CO)NH data in conjunction with the amide-to-amide region of the HSQC-NOESY(^{1}H-^{15}N) spectrum. The HN(CO)CA and HNCA spectra were used to verify the assignments. The ^{1}H-^{15}N HSQC spectra for the zinc-bound SmtB, in which the amino acids were specifically ^{15}N-labelled (Ala, Val, Leu, His and Arg), were also used to verify the assignments. Using these assignments, the Hα resonances were identified from the results of the ^{1}H-^{15}N HSQC-TOCSY and HCCH-TOCSY experiments. All pairs of cross peaks for the side resonances (Asn and Gln) in Figure 1 could be unambiguously assigned.

In total, 112 of the 117 possible ^{1}HN resonances (121 residues minus three prolines and the terminal amino residues) were observed (96%), and all of them were assigned (100%). A list of the chemical shifts has been deposited in the BioMagResBank under accession number 4306. By comparing these assignment results with our previous results for zinc-free SmtB, the chemical shift changes following the zinc ion binding were analyzed, and it was found that the cysteine residue (Cys14) in the N-terminal flexible region acts as a ligand for zinc ions. This result is the first experimental evidence regarding the function of the N-terminal flexible region of SmtB, and is supported by gel shift assay results with point mutated SmtB in which Cys14 is replaced with a serine residue (data not shown).

Acknowledgements

This work was supported by a Grant-in-Aid from the Ministry of Education, Science and Culture of Japan (No. 10179214).

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