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NMR backbone assignments of the cyanobacterial transcriptional factor, SmtB, that senses the zinc concentration in the cell

Eugene H. Morita[a], Takashi Kosada[b], Toshio Yamazaki[b], Yoshimasa Kyogoku[b] and Hidenori Hayashi[a,*]

[a]*Department of Chemistry, Faculty of Science, Ehime University, 2-5 Bunkyo-cho, Matsuyama, Ehime 790-8577, Japan;* [b]*Institute for Protein Research, Osaka University, 3-2 Yamada-oka, Suita, Osaka 565-0871, Japan*

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Biological context

In *Synechococcus* PCC7942, the *smt* locus is responsible for the tolerance to zinc and cadmium. This was verified by deletion of the *smt* locus, which caused a reduction of the Zn/Cd tolerance (Turner et al., 1993). The *smt* locus consists of two divergently transcribed genes, *smtA* and *smtB.* The *smtA* gene encodes the class II metallothionein (Olafson et al., 1988; Shi et al.,1992), and the *smtB* gene encodes the repressor of *smtA* transcription, which consists of 122 amino acid residues (Huckle et al., 1993; Morby et al., 1993). Considering the sequence homology, SmtB is thought to be a member of the ArsR family of metalloregulatory proteins, which includes ArsR as well as CadC and CadX involved in resistance to arsenate/arsenite/antimonite and Cd, respectively. It has been proposed that, in addition to a predicted helix-turn-helix motif for DNA binding, each member of the ArsR family contains a metal binding box.

In the absence of heavy metal ions, the transcription of *smtA* is repressed by the binding of SmtB to the operator-promoter region between the *smtA* and *smtB* genes (Erbe et al., 1995), while the transcription is stimulated by trace amounts of heavy metal ions (Zn, Cu and Cd). This is thought to be caused by inhibition of complex formation between the recognition DNA sequence and SmtB, as the heavy metal binds to SmtB (Erbe et al., 1995). Recently, it was found that SmtB is predominantly a dimer and binds two Zn ions per subunit (Kar et al., 1997). Mutation work has indicated candidate ligand amino acids for the Zn ion but they have not been confirmed yet (Turner et al., 1996).

*To whom correspondence should be addressed.

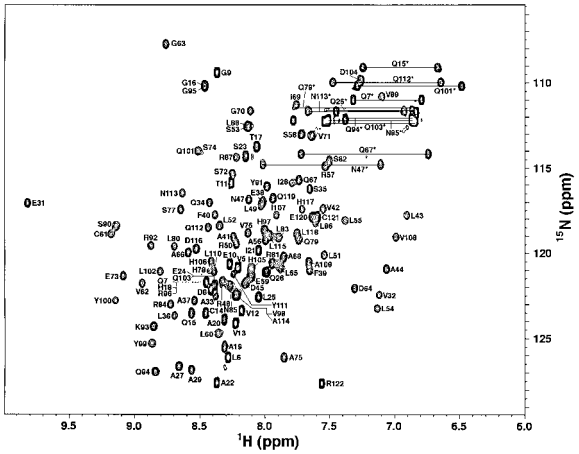

Figure 1. Two-dimensional HSQC spectrum of SmtB (0.6 mM, NMR buffer, pH 6.0, 40 °C) collected at a ^{1}H resonance frequency of 500 MHz. To optimize the resolution in the nitrogen dimension, a ^{15}N spectral width of 1500 Hz was used (sw1=1500 Hz, sw=8000 Hz, nt=32, ni=64, np=1024). The cross-peak assignments denoted by asterisks are the side-chain resonances of Asn or Gln.

To understand how the conformational change following the heavy metal ion sensing reduces the affinity to the recognition DNA sequence in SmtB, the structural change of SmtB followed by the heavy metal binding is being studied by NMR spectroscopy. For this purpose, we report the NMR backbone assignments of SmtB in this paper.

Methods and results

A gene fragment coding the complete SmtB sequence was amplified by PCR and cloned into the vector, pET-

21d, which was transfected into the host *E.coli* bacterial strain, BL21(DE3). SmtB, bound with no zinc ion, was overexpressed in minimal medium (Miller) containing $^{15}NH_4Cl$ (0.5 g/l)and [$^{13}C_6$]-D-glucose (2 g/l) with 1 mM IPTG and 1 mM rifampicine. The majority of SmtB was expressed in the soluble fraction, with a yield of approximately 10 mg/l. The purified SmtB was obtained using a two-step protocol on HiTrap-SP strong cation exchange (5 ml; Pharmacia) and HiLoad Superdex75$_{pg}$ gel filtration (26 mm $\times$ 60 cm; Pharmacia) columns. N-Terminal amino acid sequence analysis of the purified SmtB showed that the N-terminal methionine residue was deleted. The size of the purified SmtB was determined by SDS-PAGE and sedimentation equilibrium analyses, it being found that SmtB is a dimeric 14 kDa protein. This result is in good agreement with that reported by Kar et al. (1997). A gel shift assay showed that the overexpressed SmtB exhibits almost the same affinity and specificity as to the recognition DNA sequence as the native SmtB.

A 0.6 mM NMR sample of SmtB was prepared in 250 µl of 90%H_2O/10%D_2O or 99% D_2O in the following NMR buffer: 50 mM K_2HPO_4/KH_2PO_4, 50 mM KCl, 1.1 mM DTT, 1 mM EDTA, pH 6.0. 2D $^1H-^{15}N$ HSQC and 3D HSQC-NOESY ($^1H-^{15}N$ and $^1H-^{13}C$), HNCA, HN(CO)CA, HNCO, HCACO, CBCA(CO)NH, HNCACB, HNCO and HCCH-TOCSY data were collected at 40 °C using Bruker DMX 500 spectrometer. The data were processed using NMRPipe (Delaglio, 1995) on an SGI workstation. The ^1H, ^{13}C, and ^{15}N chemical shifts were referenced according to Wishart et al. (1995).

Extent of assignments and data deposition

Figure 1 is a 2D HSQC spectrum of SmtB obtained at a ^1H resonance frequency of 500 MHz. The ^1HN 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($^1H-^{15}N$). The HN(CO)CA and HNCA spectra were used to verify the assignments. The HSQC spectra for SmtB in which the amino acids were specifically ^{15}N-labelled (Ala, Val, Leu, Lys, Cys, Arg, and His) were also used to verify the assignments, respectively. Using these assignments, the ^{13}CO and Hα resonances were determined from the results of the HNCO, HCACO, and HCCH-TOCSY experiments. Only two pairs of the cross peaks for the side-chain resonances (Asn and Gln) in Figure 1 could not be unambiguously assigned.

In total, 116 of the 117 possible ^1HN resonances (121 residues minus three prolines and the terminal amino group) were observed (99%), and all of these were assigned (100%). The list of chemical shifts was deposited with the BioMagResBank (accession number 4128). On the basis of the backbone assignments for SmtB, 3D structure calculations are in progress.

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