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^{1}H, ^{13}C, and ^{15}N resonance assignments of NmtR, a Ni(II)/Co(II) metalloregulatory protein of *Mycobacterium tuberculosis*

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Abstract NmtR is a Ni(II)/Co(II)-specific repressor expressed in *Mycobacterium tuberculosis,* which regulates the transcription of a membrane transporter proposed to mediate cytoplasmic Ni(II)/Co(II) efflux. Here we report the backbone and side chain resonance assignments of the apo-NmtR and the backbone assignments of Ni(II)-bound form of NmtR.

Keywords Metalloregulatory protein · Winged helix · Nickel sensor · Allosteric regulation · *Mycobacterium tuberculosis*

Biological context

Mycobacterium tuberculosis (*Mtb*) is an obligate pathogen of human tuberculosis that encodes a remarkable diversity of known and putative metal ion transporters. These include multiple putative P-type ATPases, ABC transporters, and cation diffusion facilitators (CDF) which collectively allow the bacterium to respond to a range of host-killing mechanisms while maintaining intracellular metal homeostasis (Agranoff and Krishna 2004). A large number of metalloregulatory proteins in *Mtb* regulate the expression of a variety of transporters. Among them, NmtR represses the transcription of *ctpJ* which encodes a P-type ATPase metal transporter at low Ni(II) concentrations. At high Ni(II) concentrations, NmtR binds Ni(II) and dissociates from the DNA operator, allowing for expression of CtpJ which exports Ni(II) from the cytoplasm (Agranoff and Krishna 2004; Campbell et al. 2007). NmtR belongs to the arsenic repressor (ArsR) family of metal sensor proteins that adopt a typical winged-helix homodimeric fold and is classified as an "α5" sensor within this large family of repressors (Osman and Cavet 2010). NmtR binds Ni(II) and Co(II) to a pair of interprotomer sites adopting octahedral coordination geometry that is proposed to include four metal ligands from the C-terminal α5 helix (Asp91′, His93′, His104 and His107) and two ligands from the extreme N-terminus, including the α-amine of Gly2 (the N-terminal Met is processed) and the side chain of His3 (Reyes-Caballero et al. 2011). Although previous studies have provided detailed insights into metal-induced allosteric switching mechanisms of several ArsR family proteins (Arunkumar et al. 2009) the precise details of the allosteric regulation of NmtR by Ni(II) remain unclear. Elucidating the solution structure and dynamical properties of NmtR is critical to understanding the molecular mechanism of Ni(II)-mediated negative allosteric regulation of DNA operator binding (Pennella et al. 2003). Here we report sequence specific ^{1}H, ^{13}C and ^{15}N assignments of apo- and Ni(II)-bound NmtR.

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Methods and experiments

Protein expression and purification

NmtR expression and purification was essentially carried out as described previously (Pennella et al. 2003; Reyes-Caballero et al. 2011). In brief, the *nmtR* coding region was amplified by polymerase chain reaction (PCR) from

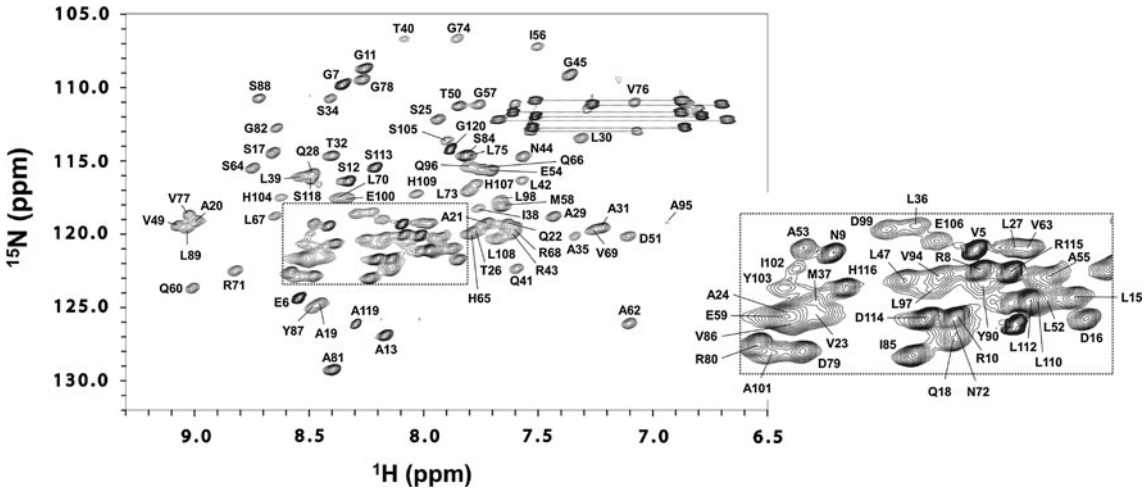

Fig. 1 ^{1}H–^{15}N HSQC spectrum of apo-NmtR. Residue numbers are labeled on the *crosspeaks*. *Crosspeaks* from the middle of the spectrum are shown in the inserted *box*, *right*

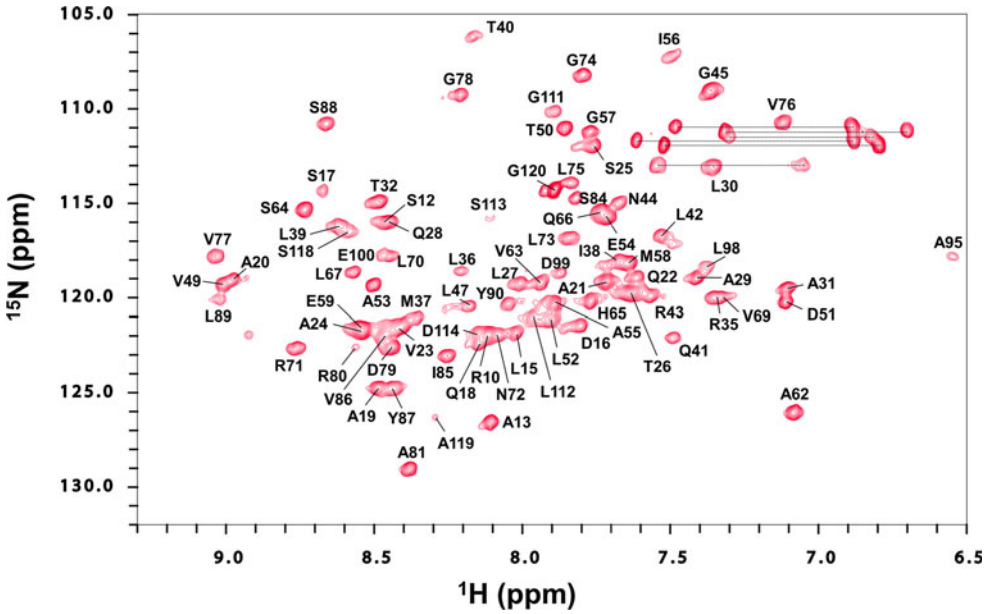

Fig. 2 ^{1}H–^{15}N HSQC spectrum of Ni(II)-bound NmtR. Residue numbers are labeled on the *crosspeaks*

M. tuberculosis H37Rv DNA ligated to pGEM-T (Promega) prior to subcloning into the *Nde*I/*Eco*RI sites of pET29a (Novagen). NmtR was expressed in *E. coli* BL21(*DE3*) and purified by polyethylenimine (PEI) precipitation of the lysis supernatant, followed by two successive ammonium sulfate fractionations (35 and 70 %) and dissolution of the 70 % ammonium sulfate pellet in 25 mM MES buffer (pH 6.0), 0.1 M NaCl, and 5 mM EDTA in preparation for a SP (sulfopropyl)-Sepharose chromatography. Peak SP factions were pooled and subjected to Q-Sepharose chromatography in 25 mM Tris (pH 8.0) and 0.05 M NaCl, with a final polishing on a G75 preparative grade gel filtration column run in 10 mM Hepes (pH 7.0) and 0.2 M NaCl buffer. The uniformly ^{13}C- and ^{15}N-labeled protein was expressed in M9 media containing $^{15}NH_4Cl$ and ^{13}C-glucose and purified in exactly the same way.

NMR spectroscopy

Samples for NMR spectroscopy were prepared in 10 mM Hepes, pH 7.0, 100 mM NaCl as 10 % $^{2}H_2O$/90 % H_2O mixture or 100 % $^{2}H_2O$ at protomer protein concentrations ranging from 0.35 to 0.50 mM. All NMR experiments were performed on a Varian DDR 600 or 800 MHz spectrometer fitted with cryogenic probe systems at 310 K in the METACyt Biomolecular NMR Laboratory at Indiana University. NMR spectra were referenced to external DSS. NMR data processing and analysis were performed using

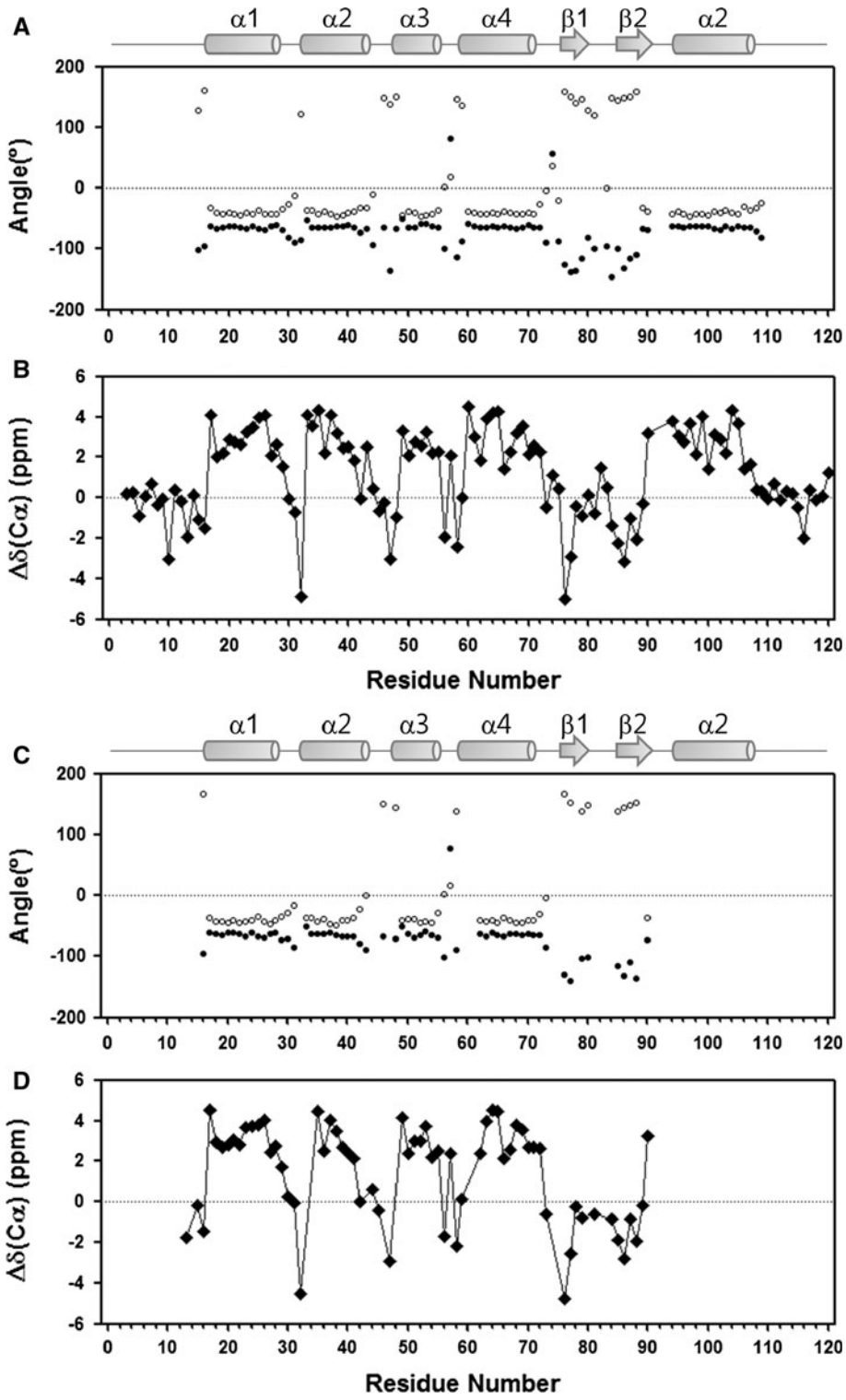

Fig. 3 TALOS+ derived backbone torsion angles and secondary ^{13}Cα chemical shifts of apo-NmtR (**a**, **b**) and Ni(II)-NmtR (**c**, **d**). ^{13}Cα shifts calculated by subtraction of published random coil values from the experimental ^{13}Cα chemical shifts for apo-NmtR (**b**) and Ni(II)-NmtR (**d**). *Filled circles* phi angles, *open circles* psi angles, *filled diamonds* secondary ^{13}Cα chemical shifts. A secondary structure schematic based on TALOS+ analysis is shown at the *top panels* **a** and **c** for the apo- and Ni(II)-loaded NmtR, respectively

NMRPipe (Delaglio et al. 1995) and NMRView (Johnson and Blevins 1994). Sequential backbone resonance assignments were obtained using standard triple-resonance NMR spectroscopy: HNCA (Grzesiek and Bax 1992), HN(CO)CA, HNCACB (Wittekind and Mueller 1993), and CBCA(CO)NH (Grzesiek and Bax 1992). Assignments of side chain resonances were made using three-dimensional HCCH-COSY (Bax et al. 1990), H(CCO)NH-TOCSY (Montelione et al. 1992; Grzesiek et al. 1993) and C(CO)NH-TOCSY (Grzesiek et al. 1993) experiments.

Assignments and data deposition

Apo-NmtR (119 residues; residues 2–120) purifies as a dimer on a G75 gel filtration column with the N-terminal Met1 processed (Reyes-Caballero et al. 2011). The ^{1}H–^{15}N HSQC spectrum (Fig. 1) shows $\approx$123 cross peaks consistent with a symmetric homodimeric state under the solution conditions used for NMR studies. Backbone ^{1}H, ^{13}C, and ^{15}N assignments of NmtR have been completed to 96 %. A total of 108 resonances of the 113 expected non-proline residues were assigned, with backbone amide resonances corresponding to Gly2, His3, Asp91, Thr92, and His93 not assignable in this state. Figure 2 shows the ^{1}H–^{15}N HSQC spectrum of Ni(II)-bound NmtR. The ^{1}H–^{15}N correlations for 25 residues (residues 4–10, 34, 60, 61, 82, 83, 94, 96, 97, 101–110) disappear upon binding of the paramagnetic Ni(II) ion to each site on the dimer. Backbone ^{1}H, ^{13}C, and ^{15}N assignments of Ni(II)-NmtR have been completed for 83 of the 113 expected non-proline residues. A TALOS+ (Shen et al. 2009) analysis of apo-NmtR (Fig. 3a, b) and the allosterically inhibited Ni(II) form (Fig. 3c, d) each reveals the presence of five α-helices and two short β-strands; this close correspondence between the two conformers is consistent largely with a Ni(II)–induced quaternary structural transition that is most strongly linked to allostery (Arunkumar et al. 2009). The ^{1}H, ^{13}C, ^{15}N resonances for both apo- and Ni(II)-NmtR have been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under the accession numbers 18003 and 18326 for apo- and Ni(II)-NmtR, respectively.

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