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# The Conformations of the Manganese Transport Regulator of *Bacillus subtilis* in its Metal-free State

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The manganese transport regulator (MntR) from *Bacillus subtilis* binds cognate DNA sequences in response to elevated manganese concentrations. MntR functions as a homodimer that binds two manganese ions per subunit. Metal binding takes place at the interface of the two domains that comprise each MntR subunit: an N-terminal DNA-binding domain and a C-terminal dimerization domain. In order to elucidate the link between metal binding and activation, a crystallographic study of MntR in its metal-free state has been undertaken. Here we describe the structures of the native protein and a selenomethionine-containing variant, solved to 2.8 Å. The two structures contain five crystallographically unique subunits of MntR, providing diverse views of the metal-free protein. In apo-MntR, as in the manganese complex, the dimer is formed by dyad-related C-terminal domains that provide a conserved structural core. Similarly, each DNA-binding domain largely retains the folded conformation found in metal bound forms of MntR. However, compared to metal-activated MntR, the DNA-binding domains move substantially with respect to the dimer interface in apo-MntR. Overlays of multiple apo-MntR structures indicate that there is a greater range of positioning allowed between N and C-terminal domains in the metal-free state and that the DNA-binding domains of the dimer are farther apart than in the activated complex. To further investigate the conformation of the DNA-binding domain of apo-MntR, a site-directed spin labeling experiment was performed on a mutant of MntR containing cysteine at residue 6. Consistent with the crystallographic results, EPR spectra of the spin-labeled mutant indicate that tertiary structure is conserved in the presence or absence of bound metals, though slightly greater flexibility is present in inactive forms of MntR.

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The manganese transport regulator (MntR) controls manganese homeostasis in *Bacillus subtilis*.[1] When cellular levels of manganese are high, MntR represses the transcription of genes encoding manganese uptake transporters by binding to cognate operator sequences.[1–3] Manganese is an essential nutrient in bacteria and plays an important role in cellular defense against reactive oxygen species. In several pathogenic species of bacteria, the regulation of manganese uptake has been linked to virulence.[4] Notably, *Bacillus anthracis*, the causative agent of anthrax, possesses a close homolog of MntR,[5] and mutants of *B. anthracis* lacking proteins involved in manganese uptake have weakened virulence.[6] Furthermore, MntR is a member of the DtxR/IdeR family of metalloregulators, named for the diphtheria toxin repressor (DtxR) of *Corynebacterium diphtheriae* and the iron-dependent regulator (IdeR) of *Mycobacterium tuberculosis*, both of which play key

Abbreviations used: DtxR, diphtheria toxin repressor; EPR, electron paramagnetic resonance; HTH, helix-turn-helix; IdeR, iron-dependent regulator; MntR, manganese transport regulator; RMSD, root mean square deviation.

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roles in the virulence of the organisms from which they have been isolated.[7–10] Because of the importance of these regulatory proteins in bacterial physiology and virulence, the mechanism of their action is of interest. In the past several years, the structural changes that relate to the activation of DtxR have received considerable attention,[11–16] but little is known about how its distantly related structural homolog, MntR, is activated by manganese binding.

Structures of MntR have been solved in the presence of $Mn^{2+}$ or $Cd^{2+}$, another strongly activating metal ion.[17,18] MntR is a functional homodimer of 142-residue subunits, each composed of two domains. The 71-residue N-terminal DNA-binding domain consists of three α-helices and two strands of antiparallel β-sheet (Figure 1), the latter forming the flexible "wing" of a winged helix-turn-helix (HTH) motif that interacts with DNA.[17,19] The C-terminal domain contains four α-helices and forms the dimerization interface with its dyad-related mate. The N and C-terminal domains are connected by a long linker helix (α4; residues 64–86) that extends from the wing to the dimer interface. Two manganese ions bind at the interface of the two domains in a single subunit, forming a binuclear complex that involves residues from the DNA-binding domain (Asp8 and Glu11), the dimerization domain (Glu99, Glu102, and His103) and from the linker helix (His 77; Figure 1). The geometry of the binuclear complex with manganese appears to be sensitive to crystal packing forces. However, multiple lines of evidence suggest that the physiological complex places two $Mn^{2+}$ at a separation of 4.4 Å, bridged by Glu99 and Glu102.[5,17,18] Cadmium binds in a similar geometry to the two sites, which

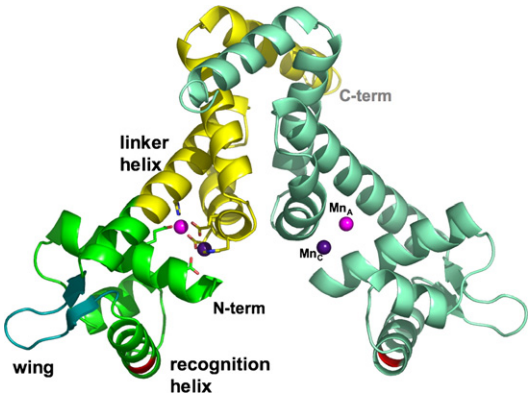


**Figure 1.** The structure of the MntR•$Mn^{2+}$ complex (PDB ID 2F5F). Two subunits form a homodimer. The left subunit is colored to show the C-terminal dimerization domain in yellow and the N-terminal DNA-binding domain in green. The N and C termini are also labeled. The flexible "wing" is shown in turquoise. The linker helix, α4, and recognition helix of the helix-turn-helix motif are identified. The $Mn^{2+}$ ions occupying the A and C sites of both subunits are colored light and dark purple, respectively. The backbone positions of Lys41 residues are shown in red. The residues that comprise the metal-binding site of the left subunit are shown in stick representation. Figures are drawn with PyMOL.[36]

have been identified as A and C (Figure 1). Interestingly, the structure of MntR complexed with $Zn^{2+}$, a weakly activating metal, reveals only a single bound metal ion, in the A site.[3,18]

Although structures of complexes of MntR with strongly and weakly activating metals have been obtained, there is little variation between these forms. The chief difference involves helix α1, which includes residues 3–20 in the $Mn^{2+}$ and $Cd^{2+}$ complexes. In the zinc complex of MntR, the helix unwinds at its N terminus so that α1 is shortened to include only residues 5–20. While this structural difference hints at greater flexibility in the MntR•$Zn^{2+}$ complex than in the MntR•$Mn^{2+}$ or MntR•$Cd^{2+}$ complexes, there are no other substantial changes in backbone conformation, perhaps due to restraints imposed by crystal packing. As evidence that the crystalline environment can influence the conformation of MntR, a comparison of structures of the MntR•$Mn^{2+}$ complex from monoclinic and hexagonal crystal forms shows that the DNA-binding domains can move somewhat independently of the dimeric core formed by dyad-related C-terminal domains, *via* slight flexibility in the linker helix, α4.[18] This conformational shift repositions the metal-binding residues in the N-terminal domain (Asp8 and Glu11) by as much as 1.5 Å, and the recognition helices of the HTH motif can move by roughly 3 Å with respect to each other in the MntR dimer.

In addition to the conformational plasticity observed in the MntR•$Mn^{2+}$ complex, there is presumably a unique set of structural features associated with the metal-free form of MntR that renders it inactive and distinct from the metal-bound form. A spectroscopic investigation into the activation of MntR revealed that structural reorganization takes place upon metal binding, but it remains unclear whether the reorganization originated from a stably folded conformation or from a molten globule-like state.[20] Thus, there is no explicit description of the tertiary and/or quaternary changes that take place upon activation of MntR by metal binding. To further investigate the mechanism of activation, we have determined the structure of MntR in its metal-free state and investigated the conformation of the N-terminal domain using site-directed spin labeling.[21,22] The results presented here are consistent with a disorder to order transition in the tertiary structure of MntR that orients independent, folded domains in positions to promote high affinity interactions with DNA.

## The structure of Apo-MntR

The crystal structures of native apo-MntR (apo-$MntR_{nat}$) and the selenomethionine-containing derivative (apo-$MntR_{SeMet}$) have been solved to 2.8 Å resolution. Apo-$MntR_{SeMet}$ crystallized in the orthorhombic space group $P2_12_12_1$ and the structure was solved using the anomalous signal from five of the 12 selenium atoms from the two dimers in the asymmetric unit (Table 1). Dimer 1 is composed of subunits A and B, and dimer 2 is composed of

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