

Metal-Driven Allosteric Regulation of the MtsR Metalloregulator: Mechanistic Insights into Activation and DNA Binding

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Streptococcus pyogenes is a human pathogen responsible for infections ranging from mild to life-threatening, rendering its virulence mechanisms important targets for therapeutic intervention. Manganese homeostasis in this organism is regulated by MtsR, a DtxR-family metalloregulatory transcription factor whose activation mechanism remains incompletely understood. Members of the DtxR family share a conserved modular architecture and undergo metal-induced allosteric transitions that reorganize their DNA-binding domains in response to fluctuations in intracellular metal availability [1–3]. Here, we combine molecular dynamics (MD) simulations with EPR spectroscopy, circular dichroism (CD), and differential scanning calorimetry (DSC) to characterize Mn²⁺-dependent conformational changes in MtsR. Comparative analysis of *apo*, partially metallated, and fully metallated states indicates that Mn²⁺ binding progressively restricts conformational heterogeneity rather than inducing a discrete structural transition. The fully metallated form adopts a compact arrangement of DNA-binding domains, in which the $\alpha 3$ recognition helices are positioned at a separation compatible with simultaneous engagement of adjacent major grooves in B-form DNA. In contrast, the *apo* ensemble samples expanded and misaligned conformations that are structurally incompatible with productive DNA binding. At the molecular level, Mn²⁺ coordination stabilizes an Arg157-centered interaction network that allosterically couples the regulatory core to the DNA-binding domains. These interactions are persistent in the metallated state, but weak or absent in the *apo* form. Experimental biophysical measurements support this model.

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References:

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