SEQUENCE AND STRUCTURAL CHARACTERIZATION OF METAL BINDING IN ACTIVATION OF DIPHTHERIA TOXIN REPRESSOR (DTXR)

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Introduction

Diphtheria toxin repressor (DtxR) is a two domain Fe²⁺-activated transcriptional regulator of iron homeostasis genes in Corynebacterium diphtheriae, and co-regulates expression of the diphtheria toxin gene. Transition metal ions are bound in two structurally and functionally distinct sites, and metal binding effects a disordered-to-ordered structural transition, changes the interactions between the two domains, and increases the affinity for dimer formation.

We are investigating how metal binding leads to activation. In this project, we use paramagnetic relaxation enhancement data to identify the order of metal binding into the two sites.

Experimental

Backbone amide ¹⁵N longitudinal (T₁) relaxation times were measured using the 720 MHz magnet system at the NHMFL. The ¹⁵N relaxation rates were used to extract distances using the Solomon–Bloembergen equation [1]. We specifically probed binding sequence by measuring the enhancement in T₁ relaxation of backbone amides in the presence of a paramagnetic Mn²⁺, using Cd²⁺ as a diamagnetic control for the metal bound state.

Results and Discussion

NMR gives direct evidence of metal binding to the ancillary site by measuring distances from the backbone amides of E170 and Q173 to the bound metal. At 1 equivalent of metal added, the distances to E170 and Q173 in the wild type protein are 12.9, and 13.6Å, respectively, whereas at 2 equivalents of metal, the distances are 8.8, and 8.3Å. We measured distances with an ancillary site mutant, H79A. H79A mutant reduces the repressor activity by ~50%. The distances measured were found them to be 12.8, and 9.8Å at 1 equivalent, and 12.5, and 10.1Å at 2 equivalents of metal added.

Conclusions

Metal binding sequence by the wt DtxR is primary, followed by ancillary site binding. H79A shows binding by the primary site first, followed by binding to the ancillary site, consistent with the wild type repressor. We are currently investigating metal binding by primary site mutants. Mutating any residues involved in coordination of the primary metal site abolishes the activity of the repressor.

References