ACTIVATION OF IRON-SENSITIVE BACTERIAL REPRESSORS

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Introduction

Bacteria require iron for survival, as do humans. However, bacteria can not store iron to the same extent as humans and are more dependent on obtaining iron from the environment than we are. This presents a problem for bacteria invading human hosts as the bacteria quickly become iron-starved, which triggers a stress response that includes increased expression of "iron acquisition proteins" and, in many cases, virulence factors that kill neighboring host cells. The iron released from the host cells is used by the bacteria. Significant human pathogens, including *Mycobacterium tuberculosis*, rely on this signaling pathway, so studies of iron sensing and regulation in *M. tuberculosis* may lead to novel therapeutic strategies.

The protein responsible for sensing iron levels and triggering the virulence response in *M. tuberculosis* is called IdeR, the iron-dependent regulator. IdeRs closest homologue is the diphtheria toxin repressor (DtxR), from *Corynebacterium diphtheriae*. We have studied the molecular mechanisms associated with metal sensing and regulation of gene expression in these proteins. A key part of this activation involves an intramolecular complex formed between a proline-rich peptide segment and an SH3-like domain in the repressor. We are using NMR spectroscopy and computational biophysics to characterize the dynamic nature of this key intermediate.

Experimental

Relaxation rates (R1 and R2) and NOE values for backbone nitrogens are measured using NMR spectroscopy at several magnetic field strengths (600 and 700 MHz at NHMFL; 900 MHz at University of Georgia) using standard pulse sequences for two constructs, one in which the intramolecular complex is formed and on in which it can not form. The experimental data are analyzed using the Modelfree formalism of Lipari and Szabo and by the reduced spectral density approach outlined by Kay, Torchia, and others. Molecular dynamics trajectories (50 ns) were calculated starting from NMR structures of these two constructs. The MD trajectory is used to calculate relaxation rates and the internal motions are analyzed using the iRED formalism developed by Bruschweiler.

Results and Discussion

The results indicate a highly dynamical nature for the intramolecular complex, in which the "ligand" is either very mobile in the ligand binding pocket or, more likely, is exhibiting an association – dissociation equilibrium on the time scale of the NMR experiments. The iRED analysis suggests that the internal motions may be closely coupled to the global tumbling of the protein, which is consistent with the Modelfree results.

Conclusions

We previously proposed that intramolecular complex functions as an energetic "sink" that competes with iron binding and repressor activation. The highly dynamic nature of the intramolecular complex is essential for fine-tuning the energetics of metal binding and therefore of iron sensing. We currently are extending our initial studies by comparing the backbone dynamics of various mutants that exhibit differential activation to further define how backbone motions in this part of the protein contribute to gene regulation.

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