BACKBONE DYNAMICS OF THE INTRA-MOLECULAR LIGAND BINDING BY THE SH3-LIKE DOMAIN OF THE DIPHTHERIA TOXIN REPRESSOR

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Introduction

Diphtheria toxin repressor (DtxR) regulates the expression of iron-sensitive genes in *Corynebacterium diphtheria*. DtxR contains an N-terminal metal and DNA-binding domain that is connected by a proline-rich flexible peptide segment (Pr) to a C-terminal src homology 3 (SH3)-like domain. The proline-rich segment forms an intra-molecular complex with the SH3-like domain in the apo-state of the protein. In the active or holo repressor the SH3 domain dissociates from the Pr segment and participates in metal coordination. Thus, intra-molecular binding and release of the Pr segment functions as a regulatory switch that modulates the activation of repressor [1]. A dynamics study of the intra-molecular interaction of the flexible ligand would contribute to understanding the 'switch mechanism', causing the activation of such proteins. Our approach is to use two Diphtheria toxin repressor constructs, one that contains the SH3-like domain and its proline-rich peptide ligand in a single polypeptide chain (Pr-SH3) and the other having just the SH3–like domain (SH3). The Pr-SH3 domain represents the bound state of the protein and the SH3 the unbound state. A comparison of the dynamics of these two forms will give us information on any induced dynamics in the backbone of the SH3-like domain on being bound to the proline-rich segment.

Experimental

Backbone amide ^{15}N Spin-lattice (T_1), spin-spin (T_2) and ^{1}H-^{15}N Nuclear Overhauser enhancements (NOE) experiments were performed for the constructs on the 600MHz and the 720MHz magnet systems at the NHMFL. The ^{15}N relaxation rate was analyzed according to the Model Free formalism [2] of Lipari and Szabo and the reduced spectral density mapping. These studies were combined with a computational analysis for the intra-molecular complex.

Results and Discussion

Compared to the unbound state, intra-molecular complex formation increases the amplitude of high frequency (ps/ns) motions and slightly reduces low frequency (ms/ms) motions with little change in the average order parameter for the two constructs ($\langle S2 \rangle = 0.88 \pm 0.04$ and 0.87 ± 0.03 for Pr-SH3 and SH3, respectively). The overall correlation time for the Pr-SH3 and the SH3 construct was calculated to be 6.3 ns and 4.57 ns, respectively. The R_1 for the Pr segment was the same as that of the SH3 like domain, suggestive of a bound state, whereas the R_2 and NOE for these same residues was very low, suggestive of an unbound species. Consequently, the residues of the Pr segment were poorly fit using the standard model-free analysis, which may be due to its complex backbone motions in the bound state. Reduced spectral density mapping show this segment to be rich in nano-second motions. Using the R2 data for the SH3 domain and a small peptide corresponding to the Pr segment, we estimate that the Pr segment occupies the binding site roughly 50% of the time.

Conclusions

The average order parameter of the SH3 domain in SH3 and Pr-SH3 show this domain to be ordered in both protein constructs. The backbone flexibility of the Pr segment in Pr-SH3 is high indicating that this protein has regions with dramatically different dynamics. The protein has a periodic 'open-close', motion with the SH3 domain being bound by the Pr-segment 50% of the time.

Acknowledgements

We acknowledge the NHMFL and NIH (AI021628) for support.

References

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