MEASURING 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 intramolecular complex with the SH3-like domain in the apo-state of the protein. The physical interaction between Pr-rich segment and the SH3-like domain contributes to the activation of the protein and proper biochemical function [1]. The dynamics study of the intramolecular interaction of such a flexible ligand is of much interest and would contribute to obtaining the time-scale on which such interactions occur.

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 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 system in NHMFL. The ^{15}N relaxation rate was analyzed according to Model Free formalism [2] to determine the general order parameters to quantify the internal dynamics. Overall correlation times and diffusion tensors for the two constructs were calculated using quadric and r2r1_diffusion programs [2].

Results and Discussion

The ^{15}N T_1, T_2 and ^{1}H-^{15}N NOE are sensitive to high frequency motions (ps-ns) and to slow motions (µs-ms). Protein dynamics was studied by the 'Model Free', spectral density formalism of Lipari and Szabo [3] as implemented by Palmer [2]. The relaxation parameters obtained from the ^{15}N relaxation experiments are fitted to five different models in Model free for each residue. In the Pr-SH3 construct the low or negative values for NOE for the proline-rich segment shows that this region is highly flexible and the relatively higher values for T_1/T_2 for the SH3-like domain depicts a more ordered state. Most of the residues from the flexible proline-rich region were fitted to a model incorporating the overall tumbling and the internal fast motions of the residues. The flexibility for this region was in the range of 0.36-0.68 (S2). The SH3 domain had a generalized order parameter in the 0.78-0.94 ranges. The overall correlation time for the Pr-SH3 construct was calculated to be 6.4 ns and 5.1 ns for SH3. The SH3 construct has relatively higher order parameters then the Pr-SH3 construct, showing that the flexibility of the backbone and the internal dynamics of the residues increase on going from bound to the unbound state.

Conclusions

The backbone of proline-rich segment of the Pr-SH3 construct is highly flexible and exhibits extensive dynamics on the pico-nanosecond time scale. Motion on the micro-millisecond timescale is not seen. These results suggest that the proline-rich ligand shows mobility while bound in the crevice and that association-dissociation motions on a slower (µs-ms) time scale do not contribute significantly to the relaxation.

References

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