Structural and Dynamic Studies of Endonuclease V-DNA Interactions

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Introduction: Endonuclease V (EndoV) is a ubiquitously expressed DNA repair enzyme found in multiple organisms including eubacteria, archaea, and eukaryotes. All deaminated DNA lesions are substrates for EndoV, yet the exact mechanism remains unknown. Recent investigations have shown that this enzyme exhibits both endonucleases and exonuclease activity as well (Feng et al., 2005). Both structural and dynamic relaxation studies with varied DNA substrates and substrate mimics may provide mechanistic insight into the differential activities of this multifaceted enzyme. In an effort to unravel both the structural and mechanistic features of EndoV, we have begun studying this enzyme derived from the thermophilic bacterium *Thermotoga maritime* (Tma). Tma EndoV consists of 225 residues and is shares approximately 50% identity to other bacterial homologues, including Yersinia pestis and other highly infectious bacteria. Preliminary NMR studies indicate that the enzyme is correctly folded and exhibits inherent micro-millisecond timescale motions that may allow for the conformational changes necessary for catalysis.

Experimental: N15-edited Nuclear Overhauser Spectroscopy (NOESY) spectra have been collected on the 720 MHz Varian spectrometer and initial transverse relaxation data (R2 rates) have been collected on the 600 MHz Varian spectrometer. All titrations with inosine were also conducted at 600 MHz with 0.3 mM Tma EndoV both with and without 5 mM inosine present.

Results and Discussion: From 15N-HSQC and NOESY data, the high dispersion and numerous NOEs indicate that we have reconstituted a well-folded protein. Moreover, specific binding to inosine has been confirmed by NMR through preliminary titration data, indicating this protein does target deaminated nucleosides as expected (Fig. 1)

To establish a foundation for future studies that will probe protein/DNA complexes, we have begun to collect NMR relaxation data on free EndoV. Although the resonance assignments have yet to be made, preliminary R2 relaxation rates indicate a wide disparity in local motions. Thus, like the subset of enzymes that have been dynamically characterized (Eisenmesser et al., 2005), EndoV exhibits inherent motions and this may allow for the conformational changes required for its multi-functional activities.

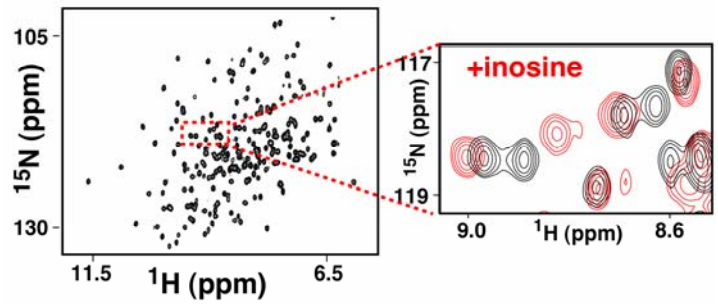

Figure 1. The 15N-HSQC of purified Tma EndoV (left panel) is well folded and capable of binding free inosine (right panel).

Conclusions: We have expressed, refolded, and purified Tma EndoV and shown that this protein is both well folded and competent in inosine binding, confirming that we have reconstituted the functional form of this enzyme. Complete titration data with inosine as well as larger DNA substrates that contain inosine will reveal the explicit affinities. Preliminary dynamics experiments indicate this enzyme is highly flexible as has been found for other enzymes (Eisenmesser et al., 2005), yet both the resonance assignments and solution structure will allow for the identification of inherent motions that are important for enzyme activity. Such inherent flexibility may be important for the multifunctional role of EndoV as both an endonucleases and an exonuclease.

Acknowledgements: We wish to thank Dr. Fengli Zhang (NHMFL) for his continued effort in setting up all NMR experiments.

References:

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