FUNCTIONAL DYNAMICS OF ARGININE KINASE

O. Davulcu (FSU, Chemistry), M.S. Chapman (FSU, Chemistry and OHSU, Biochemistry),
J.J. Skalicky (U.of Utah, Biochemistry)

Introduction

Arginine kinase undergoes large amplitude conformational changes during catalysis as seen when comparing crystal structures of substrate-free and transition state analogue bound AK. Here, we focus on the details of the underlying domain and sub-domain motions (and their rate constants) in substrate-free AK, binary, ternary, and transition state analogue complexes, and, most importantly, during catalytic turnover. A general appreciation of the importance of enzyme dynamics is lacking and while experiments and computations have hinted at its influence, a fundamental understanding is absent [1]. Using NMR and X-ray crystallography, we propose AK as a model system to examine the linkage between dynamics and catalysis.

Experimental

^{15}N transverse relaxation dispersion experiments have been collected at 600, 720, and 800 MHz and at both 15 and 25 ^{o}C. 600 and 720 MHz datasets were collected using the Inova600 and Inova720 spectrometers located at the NHMFL and repeated using spectrometers at the University of Utah. 800 MHz datasets were collected at the University of Colorado through shared time available to Dr. Jack Skalicky.

Results and Discussion

^{15}N transverse relaxation dispersion experiments identify ~40 residues undergoing dynamics in the µs-ms timescale in the substrate free form of AK. These residues cluster in secondary structure and many are in a loop that undergoes a large rearrangement on substrate binding. Fitting of these residues (Figure 1) to the following equation

$$R_{ex}(1/\tau_{CPMG}) = (p_A p_B \Delta\omega^2/k_{ex})[1 - \tanh(k_{ex}/8\nu_{CPMG})/(k_{ex}/8\nu_{CPMG})]$$

using data from all fields and temperatures simultaneously yields values of 825 ± 23 sec^{-1} and 435 ± 37 sec^{-1} for k_{ex}, the sum of forward and reverse rate constants, at 25 and 15 ^{o}C, respectively. Intriguing is that the rate constant for these slow motions are commensurate with turnover and may hint at a rate limiting motion.

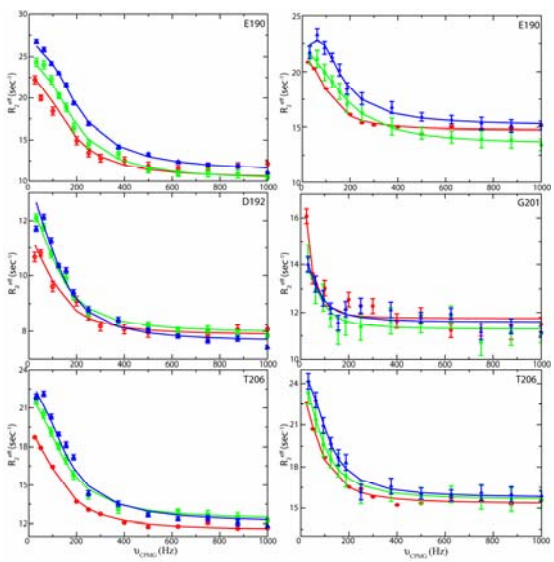

Figure 1: Relaxation dispersion profiles for selected residues in the 190 loop at 15 ^{o}C (left) and 25 ^{o}C (right) at static fields of 600 MHz (red circles), 720 MHz (green squares), and 800 MHz (blue triangles).

Conclusions

Dynamical information will be extracted from ^{15}N transverse relaxation dispersion data already in hand. Combining this with analysis of T_1, T_2, and NOE datasets will provide a complete description of dynamics in substrate free AK. Future goals include analysis of dynamics in enzyme complexed with substrate(s) and comparison of these with substrate free enzyme. Efforts are also directed towards structure determination of binary complexes of AK and pKa determinations of catalytically important residues (C274, E225/314).

Acknowledgements

The authors would like to acknowledge the NHMFL (IHRP), NIH and AHA for their generous funding of this project.

References

[1] Benkovic, S.J., *et al*., Science, **310**, 1196-1202 (2003).