FUNCTIONAL DYNAMICS OF ARGININE KINASE

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Introduction

Arginine kinase undergoes major conformation 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 and ternary complexes with non-hydrolyzable substrate, transition state analogue complexes, and, most importantly, during catalytic turnover. A general appreciation of the importance of enzyme dynamics is lacking and while experiment and computation have hinted at its influence, a fundamental understanding is absent [1,2]. Using NMR and X-ray crystallography, we are proposing AK as a model system to examine the linkage between dynamics and catalysis: Several high resolution crystal structures of AK on the reaction path are available and others are in the pipeline and AK is amenable to NMR despite its 42 kDa molecular weight.

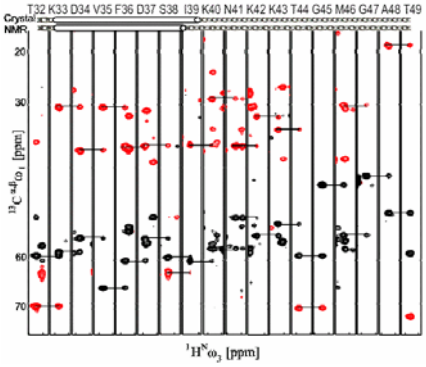

Fig 1: HNCACB strips showing $^{13}C^{\alpha,\beta}$ connectivity for residues T32-T49

Experimental

2D and 3D spectra have been recorded using the NHMFL Inova600 and Inova720 spectrometers: 2D [^{15}N,^{1}H]-TROSY, 3D [^{15}N,^{13}C,^{1}H]-HNCACB (Fig. 1), -HNcoCACB, -HNCA, -HNcoCA, -HNcaCB and -HNcocaCB on a uniformly ^{2}H,^{13}C,^{15}N labeled AK for backbone resonance assignment. To this end, we have also prepared 8 aa-type selective (D,N,R,C,K,N,L,V) samples (Fig. 2). Titration, using 2D [^{15}N,^{1}H]-TROSY, with each substrate/product has been performed to give individual binding constants and extension to ternary complexes will follow.

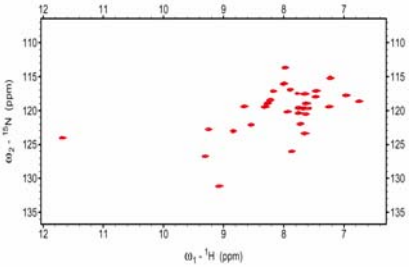

Fig 2: [^{15}N, ^{1}H]-TROSY of ^{15}N-Lysine AK

Results and Discussion

Sequence specific main-chain resonance assignments are complete for about 90% of AK. Fig. 1 shows HNCACB strips for a short helix and loop region and comparison of secondary structure observed in the crystal structure (top) and calculated from chemical shifts (bottom). Fig. 2 shows the ^{15}N-K specific label resolving all lysine NHs. Incomplete assignments from a core β-sheet and "extra" NH correlations in the aa-type selective spectra revealed a core of NDs that could only be exchanged to NHs with unfolding AK with urea and then refolding in H_2O (data not shown).

Conclusions

Resonance assignment for AK has been challenging due to the large mass and the need to unfold the protein to exchange trapped NDs. Datasets of single substrate and product are being interpreted regarding to chemical shift mapping and binding constants. Relaxation experiments (^{15}N-T_1, -T_2, -relaxation dispersion, and ^{1}H-^{15}N NOE) are in progress or queued for extracting dynamical information. Present efforts are also directed towards validation of X-ray structures with dipolar couplings and pKa determinations of catalytically important residues (C274, E225/314).

Acknowledgements

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References

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[2] Frauenfelder, H., *et al.*, Science, **229**, 337-345 (1985).