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Backbone resonance assignments of the 42 kDa enzyme arginine kinase in the transition state analogue form

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Abstract

Nearly complete backbone resonance assignments for the 357 residue, 42 kDa enzyme arginine kinase in a transition state analogue (TSA) complex are presented. The TSA is a quaternary complex of arginine kinase, MgADP, arginine, and nitrate. About 93% (320 of 344) of the non-proline backbone amides were assigned using an enzyme enriched with ²H, ¹³C, and ¹⁵N in combination with three enzyme samples prepared with a single ¹⁵N-labeled amino acid (K, L, and R). The amide assignments will provide the foundation for investigating the dynamics of arginine kinase when in a TSA complex.

Keywords

Arginine kinase; phosphagen kinase; NMR spectroscopy; resonance assignment; enzyme dynamics; transition state analogue

Biological Context

Arginine kinase (E.C. 2.7.3.3), a member of the phosphagen kinase family of enzymes, catalyzes reversible phosphoryl transfer between ATP and arginine. Found primarily in invertebrates, arginine kinase buffers ATP levels in cells with high and/or variable energy demands. In addition to their important physiological roles, phosphagen kinases have served as paradigms for bimolecular enzyme catalysis (Ellington 2001). Monomeric arginine kinase from the Atlantic horseshoe crab *Limulus polyphemus* is a particularly good model for investigating the connections between enzyme dynamics and catalysis, due to its amenability to both high resolution X-ray crystallography and NMR spectroscopy, and a size (357 residue, 42 kDa) that exemplifies a rich repertoire of loop and domain motions

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(Davulcu et al. 2005; Davulcu et al. 2009; Davulcu et al. 2011; Niu et al. 2011; Yousef et al. 2003; Zhou et al. 1998).

Crystal structures of arginine kinase in substrate-free and transition state analogue forms demonstrate that the enzyme undergoes large conformational changes during the catalytic cycle, including rigid body rotation of the N-terminal domain and folding of two loops over the active site (Yousef et al. 2003; Zhou et al. 1998). NMR spin relaxation analyses indicate substrate-free arginine kinase exhibits dynamics across a wide, ns-ms timescale (Davulcu et al. 2009). Furthermore, intrinsic motions in the N-terminal domain and the loop-spanning residues 182-201 have experimental activation barriers commensurate with those of catalysis, indicating that one or both of these motions are rate-limiting on turnover, as opposed to the chemical step of phosphoryl transfer (Davulcu et al. 2011). Changes to the dynamics of arginine kinase when substrates are bound, however, are uncharacterized. The transition state of arginine kinase is emulated by binding of arginine, MgADP, and nitrate, the planar nitrate group mimicking the γ -phosphoryl being transferred between arginine and nucleotide. Backbone resonance assignment of arginine kinase in the transition state analogue form lays the foundation for NMR spin relaxation studies of enzyme complexes through which changes to the enzyme dynamics can be characterized through an enzyme's catalytic cycle.

Materials and Experiments

Protein preparation

Isotope-enriched arginine kinase was expressed and purified using previously published protocols, including urea-based unfolding and refolding in H₂O to facilitate exchange of amide deuterons with protons in the enzyme core (Davulcu et al. 2005; Pruett et al. 2003). Briefly, *E. coli* strain BL21(DE3) possessing the arginine kinase cDNA on the pET-22b vector were grown in M9 minimal media supplemented with 0.2% ¹³C₆-D-glucose, 0.1% N-ammonium sulfate, and 99% D₂O to an OD_{600nm} of 0.8 at 37 °C, induced with 1 mM IPTG, and subsequently allowed to grow at 37 °C overnight. Cells were then harvested, lysed, and arginine kinase was purified from the soluble lysate fraction by ion exchange chromatography on a DEAE column, equilibrated with 10 mM Tris (pH = 8.0), 1 mM EDTA, and 1 mM dithiothreitol, followed by size exclusion on an S100 column, equilibrated with 10 mM Tris (pH = 8.0), 100 mM KCl, 1 mM EDTA, and 1 mM dithiothreitol. The unfolding and refolding occurred between these chromatographic steps, by initial incubation in 6 M urea followed by sequential dialysis into 4, 2, 1, 0.5 and 0 M urea. The TSA complex was formed by exchanging purified arginine kinase with a TSA buffer containing 10 mM d₄-imidazole (pH = 6.5), 1.0 mM dithiothreitol, 200 μ M NaN₃, 6 mM ADP, 8 mM MgCl₂, 60 mM arginine, 20 mM NaNO₃, 90% H₂O, and 10% D₂O. Exchange was accomplished with repeated dilution and concentration of enzyme with TSA buffer using a Vivaspin 500 concentrator (Sartorius) to a final enzyme concentration of $\sim$ 1.8 mM. Enzyme prepared in this manner was uniformly isotope enriched with ¹³C and ¹⁵N and $\sim$ 80% enriched in ²H.

Three amino-acid-type specific arginine kinase samples were also prepared, which were ¹⁵N-labeled at amino acids R, K, or L. These specific labeled samples were prepared

using media and methods described elsewhere and purified as described above, omitting the refolding process, as these samples were not deuterated (Davulcu et al. 2005).

NMR Spectroscopy

NMR experiments were recorded at 25 °C on a Varian INOVA 600 NMR spectrometer equipped with a cryogenic HCN probe and also on an 800 MHz spectrometer with a TCI cryoprobe. NMR data were processed with FELIX 2007 (FELIX NMR) and analyzed using Sparky 3 (Goddard and Kneller 2004). ^1H chemical shifts were referenced to the DSS methyl proton at 0 ppm and ^{13}C and ^{15}N were referenced indirectly (Markley et al. 1998). Backbone assignments were obtained from TROSY versions of 3D [^{13}C , ^{15}N , ^1H] HNCACB, HN(CO)CACB, HN(CO)CA, HN(CA)CO, and HNCO experiments. A 3D [^1H , ^{15}N , ^1H] NOESY (80 ms mixing time) assisted assignment of backbone amides for which efficient NMR spin relaxation precluded assignment using through-bond experiments. 2D [^{15}N , ^1H] TROSY spectra of arginine kinase specific labels assisted in resolving resonance degeneracies in the reference TROSY spectrum.

Extent of Assignment and Data Deposition

The 2D [^{15}N , ^1H] TROSY spectrum of arginine kinase in the transition state analogue form is shown in Figure 1. Backbone amide resonances have been assigned for 320 of 344 non-proline residues with M1-V2, L61, F136-N137, E150, F188, R193-F194, E224-H227, T273-N274, G310-T316, G320, and L331 unassigned. There are only a few unassigned amide resonances in the TROSY spectrum suggesting most of the unassigned amide resonances are significantly line broadened and are not observed. Of 357 total residues in arginine kinase, C_α resonances have been assigned for 331 residues, with M1, Y68-P70, P100, P135-N137, F186, R193, E224-D226, F270-N274, D293, G310-H315, and R330 unassigned. Of 328 non-glycine residues, C_β resonances have been assigned for 300 residues, with M1, T6, Y68-P70, S74, P100, P135-N137, P169, F186, R193-F194, E224-D226, F270-N274, D293, T311-R312, E314-H315, E319, and R330 unassigned. Finally, of 357 total residues, carbonyl carbon resonance assignments have been made for 333 residues, with M1, N60, A69, S74, P100, P135-N137, E160, R193, E224-D226, C271-T273, R309-H315, and R330 unassigned.

Secondary chemical shifts (δ) were calculated for $^{13}\text{C}_\alpha$, $^{13}\text{C}_\beta$, and $^{13}\text{C}_{\text{co}}$ (backbone carbonyl carbon) nuclei by subtraction of random coil chemical shifts from the experimentally observed values for the respective nuclei (Figure 2). Secondary shifts are a predictor of secondary structure in solution and there is good agreement with the crystal structure of the arginine kinase transition state analogue complex (Wishart and Sykes 1994; Zhou et al. 1998). Only seven residues exhibit backbone ^1H and/or ^{15}N chemical shifts that deviate significantly from average values obtained from the Biological Magnetic Resonance Data Bank: L50, I67, L131, L275, A281, S282, and G332. Without exception, these residues are within 5 Å of aromatic side chains or bound substrates, indicating that their unusual chemical shifts likely arise from ring current effects or proximity to substrates (Zhou et al. 1998).

The chemical shift assignments reported here have been deposited in the Biological Magnetic Resonance Data Bank (<http://www.bmrb.wisc.edu>) with accession number 19208.

Acknowledgments

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Fig. 1. 2D [^{15}N , ^1H] TROSY spectrum of ^2H , ^{13}C , and ^{15}N enriched arginine kinase in a transition state analogue complex with arginine, MgADP, and nitrate. Backbone amide resonance assignments are indicated.

Fig. 2. Deviations from random coil $^{13}\text{C}_\alpha$ (top panel), $^{13}\text{C}_\beta$ (middle panel), and $^{13}\text{C}_{\text{co}}$ (backbone carbonyl carbon; bottom panel) chemical shifts. Locations of secondary structure observed in the crystal structure of the transition state analogue complex of arginine kinase are depicted in the lower panel.