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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N backbone resonance assignment of *Escherichia coli* adenylate kinase, a 23.6 kDa protein

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Biological context

Adenylate kinase (AK) is a key enzyme in the nucleotide metabolism from bacteria to humans. It catalyses the reversible transfer of a phosphate group from ATP:Mg^{2+} to AMP with no phosphorylated intermediate. According to the amino acid sequence one can distinguish two major forms: a short one of about 180 amino acids and a longer one containing 30–40 additional residues.

Escherichia coli AK, with its 214 residues (23.6 kDa) belongs to the longer form. Its crystallographic three-dimensional structure comprises a central scaffold (a five-stranded parallel sheet surrounded by several α-helices) and two peripheral domains involved in ligand binding. The NMP_{bind} domain (residues 30–59) participates in the nucleotide monophosphate binding while the LID domain (residues 120–157) contacts the Mg^{2+}:ATP at the active site. In the catalytic process the two domains explore a large conformational space, moving like solid blocks relative to the protein main body (CORE domain) (Vonrhein et al., 1995). Thus, in the unliganded form, the LID moiety is situated away from the CORE domain while in the presence of ATP (or an analog) it moves closer to the core, shielding the binding site and the substrate from water. Other intermediate positions were also observed in the crystal, depending on the number and nature of ligands and the crystal-packing forces. Solution studies, like nonradiative excitation energy transfer (Sinev et al., 1996), suggested that, even in the absence of a ligand, the LID domain could have different positions defined by a given rotation/translation around a hinge region. NMR spectroscopy is a well-suited method for assessing the amplitude and time scale of such movement.

The present study was undertaken to determine the solution structure and dynamics of the *E. coli* AK in the apo form and in complex with substrates or inhibitors. Here we report the almost complete proton, carbon-13 and nitrogen-15 backbone assignment and 40% of the side chain resonance assignment of the apo enzyme in solution.

Methods and results

Uniformly labeled recombinant *E. coli* AK was produced by overexpression in *E. coli* HfrP418 strain using M63B1 minimal medium containing 1.5 g/l 99% [^{15}N]-ammonium sulfate and 2.0 g/l 99% [^{13}C]-glucose as the sole nitrogen and carbon source, respectively.

NMR samples (about 1 mM) were dissolved in potassium phosphate buffer (50 mM), pH 6.7 in 95% H_2O/5% D_2O or in 100% D_2O. The NMR spectra were acquired on Varian Unity-500 (Institut Curie, Orsay) or Bruker AMXII-600 spectrometers (European SON NMR Large-Scale Facility, Utrecht). Proton chemical shifts in ppm were referenced relative to the water signal that, at 308 K, resonates at 4.69 ppm from sodium 2,2-dimethyl-2-silapentane sulfonate (DSS). ^{15}N and ^{13}C references were set indirectly relative to DSS using frequency ratios (Wishart et al., 1995).

Standard methods were used to obtain pure absorption DQF-COSY, phase-sensitive NOESY and

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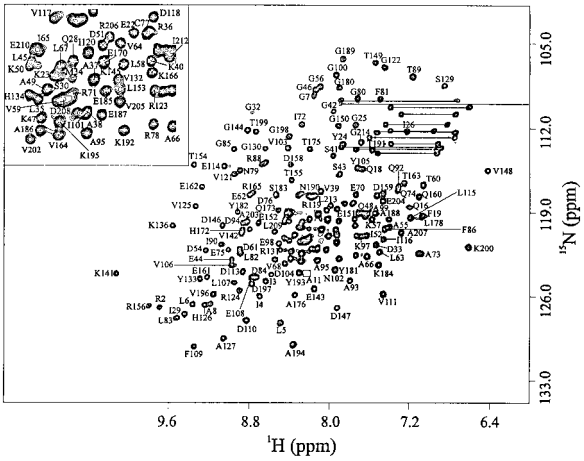

Figure 1. ^{1}H-^{15}N HSQC spectrum of *E. coli* AK in 50 mM phosphate buffer, pH 6.7, at 308 K. Assignment of cross-peaks is shown using the one-letter code for amino acids. Pairs of peaks connected by horizontal lines represent Asn and Gln side chain NH_2 groups.

TOCSY spectra (Wüthrich, 1986). 2D and 3D heteronuclear experiments (HSQC, NOESY-HMQC and TOCSY-HSQC) used the published pulse sequences (Bodenhausen and Ruben, 1980; Marion et al., 1989).

3D HNCA (Farmer II et al., 1992) and HN(CO)CA (Bax and Ikura, 1991) experiments were acquired at 500 MHz. The other spectral parameters can be obtained from the authors.

The wide dispersion of amide nitrogen frequencies and the quality of heteronuclear data are illustrated in the ^{1}H-^{15}N HSQC spectrum recorded at 308 K (Figure 1). Nevertheless, the region between 7.5–8.5 ppm in the proton dimension and between 117–124 ppm in the nitrogen dimension is very crowded. Many of the peaks in this area were assigned using HNCA/HN(CO)CA experiments. The analysis of the 2D homonuclear spectra and of the 3D NOESY-HMQC, TOCSY-HSQC, HNCA and HN(CO)CA spectra resulted in 72% backbone resonance assignments. Due to their high sensitivity and resolution, triple resonance experiments involving the β carbons were crucial for the achievement of the sequential assignment. Finally 95% of the ^{1}H, ^{15}N and ^{13}C backbone resonances were assigned. In addition, we were able to identify 40% of the side chain proton resonances by the detection of the $^{1}H_{\beta}$ signals in 3D HBHA(CBCACO)NH spectra and by the analysis of 2D COSY and TOCSY experiments.

Extent of assignments and data deposition

^{1}H, ^{15}N, and ^{13}C backbone resonances of 196 out of 204 non-proline residues and ^{1}H and ^{13}C resonances of 7 proline residues were assigned. Several amide ^{1}H/^{15}N cross peaks were not observed in the HSQC spectrum (Figure 1) but were assigned in the 3D triple resonance spectra. Among these are the residues forming the ATP binding P-loop (G10–T15). In the absence of any ligand or inhibitor, this fragment may be in intermediate exchange (on the NMR time scale) between two or more conformations, resulting in broader lines and low-intensity peaks. Similar difficulties were encountered in the assignment of the corresponding fragment in chicken muscle adenylate kinase (Byeon et al., 1993). The chemical shift values of the proton, nitrogen and carbon resonances are available as supplementary material (Table S1) and were deposited in the BioMagResBank in Madison, WI, U.S.A. (accession number 4152).

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