Journal of Biomolecular NMR, **13:** 195–196, 1999.
KLUWER/ESCOM

Letter to the Editor: Sequence-specific ^{1}H, ^{15}N and ^{13}C assignment of adenylate kinase from *Escherichia coli* in complex with the inhibitor AP_5A

Eva Meirovitch*, Michael A. Sinev & Elena V. Sineva
Department of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel

Received 20 August 1998; Accepted 7 October 1998

Key words: adenylate kinase, dynamic properties and enzyme function, NMR assignment

Biological context

Adenylate kinase (AK) is a small multi-domain monomeric enzyme catalyzing the transfer of a phosphoryl group from ATP to AMP. Crystallographic studies on AKs suggest closure of enzyme domains upon formation of the enzyme–substrate ternary complex (Vonrhein et al., 1995).

Time-resolved fluorescence energy-transfer studies of *E. coli* AK (AKeco) revealed that, unlike the pseudo-ternary complex AKeco*AP_5A [P^1,P^5-di(adenosine-5′)pentaphosphate], the ligand-free enzyme features substantial inter-domain flexibility (Sinev et al., 1996). The latter data address the role of *dynamic* phenomena in AK catalysis. The nearly complete sequence-specific assignment of AKeco*AP_5A provides the basis for elucidating dynamic and structural properties that control kinase catalysis.

It should be noted that ^{1}H, ^{15}N and ^{13}C assignments and the secondary structure of an AK variant from chicken muscle in complex with AP_5A have been published by Byeon et al. (1993). A set of (mainly backbone) ^{1}H assignments of AKeco were determined recently by Burlacu-Miron et al. (1998).

Methods and results

Recombinant plasmid pEAK91, containing the intact gene coding for *E. coli* AK (Reinstein et al., 1988), was a gift of Prof. Wittinghofer (Max-Planck Institute for Molecular Physiology, Dortmund, Germany). For the preparation of ^{15}N,^{13}C-labeled AKeco, *E. coli* HB101 cells, transformed with the pEAK91 plasmid, were grown at 37 °C in Celtone-CN medium (Martek Biosciences Corp.). The protein was purified as described previously (Sinev et al., 1996). The yield of purified enzyme was about 110 mg per liter of cell culture.

AKeco stock solutions were prepared in 40 mM sodium-phosphate buffer (pH 6.8). To prepare the 100% D_2O sample, 290 μl of the AKeco*AP_5A solution in sodium phosphate buffer was freeze-dried, dissolved in 400 μl of D_2O, incubated for 24 h at room temperature, freeze-dried again, and dissolved in D_2O to a final volume of 290 μl. AKeco/AP_5A concentrations were 2.7 mM/5.7 mM (2.1 mM/5.6 mM) in the 95% H_2O/5% D_2O (100% D_2O) sample. Sample volumes of 250 μl were used in Shigemi cells.

^{15}N-HSQC, ^{13}C-HSQC, 3D CBCA(CO)NH, 3D HNCACB, 3D HNCO, 3D HBHA(CBCACO)NH, 3D C(CO)NH, 3D H(CCO)NH, 3D HCCH-TOCSY, 3D HCCH-COSY, 3D ^{15}N-edited NOESY, and 3D ^{13}C-edited NOESY experiments (Bax and Grzesiek, 1993) were carried out at 303 K on a Bruker DMX 600 MHz spectrometer, in the phase-sensitive mode. Pulse sequences developed by Bax and co-workers, with typical acquisition parameters as outlined in the original papers, were used. The spectra were processed using NMRDraw/NMRPipe (Delaglio et al., 1995). Forward (mirror-image) linear prediction was used for semi-constant (constant) indirect evolution time periods. Apodization with a cosine-bell function in the acquisition dimension, and a cosine function in the indirect dimensions, was applied. The time-domain data were zero-filled to the next power of two.

The ^{15}N-HSQC plane contained 202 out of the 203 expected $\delta(^{1}H_i^N)$, $\delta(^{15}N_i)$ correlations. Establishing sequential connectivity among these coordinates, and determining the respective amino acid type, was carried out simultaneously using 3D HNCACB and 3D

*To whom correspondence should be addressed.

C(CO)NH. Sequence-specific assignment of the backbone and non-aromatic side-chain carbon and proton nuclei was achieved primarily with 3D C(CO)NH and 3D H(CCO)NH. In some cases CBCA(CO)NH and HBHA(CBCACO)NH data were used.

Except for very few cases (in which 3D CBCA(CO)NH provided the missing $^{13}C^{\alpha}_{i-1}/^{13}C^{\beta}_{i-1}$ assignments), both the $^{13}C^{\alpha}_{i}/^{13}C^{\beta}_{i}$ and $^{13}C^{\alpha}_{i-1}/^{13}C^{\beta}_{i-1}$ pairs were observed in the 3D HNCACB spectrum. All of the intra-residue ^{13}C spin systems expected to feature C(CO)NH peaks did so.

The assignment procedure was carried out using the program ANSIG 3.3 (Kraulis, 1989). Strips along the carbon dimension were extracted from the 3D HNCACB spectrum at a given coordinate $\delta(^1H^N)_i$, $\delta(^{15}N)_i$. Connectivity with $\delta(^1H^N)_{i+1}$, $\delta(^{15}N)_{i+1}$ was established by locating the matching pair $^{13}C^{\alpha}_{i}/^{13}C^{\beta}_{i}$, common to $(i+1)$ and i, in the 3D HNCACB matrix. Connectivity with $\delta(^1H^N)_{i-1}$, $\delta(^{15}N)_{i-1}$ was established by locating the matching pair $^{13}C^{\alpha}_{i-1}/^{13}C^{\beta}_{i-1}$, common to $(i-1)$ and i, in the 3D HNCACB matrix. Potentially matching $^{13}C^{\alpha}/^{13}C^{\beta}$ pairs were identified, and the selection of the correct pair was based on C(CO)NH. In this manner sequence-specific $\delta(^1H^N)$, $\delta(^{15}N)$ assignment of polypeptide chain segments, flanked by proline or terminal residues, was accomplished. Non-aromatic side-chain proton assignments were determined based on 3D H(CCO)NH, and carbonyl carbons were assigned based on 3D HNCO.

The unassigned backbone protons included at this stage $^1H^{\alpha}$ and $^1H^{\beta}$ of Lys^{13} and Gly^{214}; $^{13}C'$, $^1H^{\alpha}$ and $^1H^{\beta}$ of the residues preceding proline; and $^1H^{\beta}$ of Pro^{87}. 3D ^{15}N-edited NOESY and 3D ^{13}C-edited NOESY were used to assign all of the missing $^1H^{\alpha}$ and $^1H^{\beta}$ resonances.

All of the ^1H and ^{13}C spin systems, except for those pertaining to residues preceding proline, could be identified in the 3D C(CO)NH and 3D H(CCO)NH spectra. Complete spin systems were observed for all the shorter amino acids, whereas 8 methyne, 20 methylene and 30 methyl signals beyond the γ position were missing for the long amino acids.

Most of the missing aliphatic ^1H resonances were assigned based on 3D HCCH-TOCSY, 3D HCCH-COSY and 3D ^{13}C-edited NOESY. ^{13}C-HSQC was used to help pair methyl proton and carbon resonances. Few ^{13}C chemical shifts exceeded the expected ranges; in these cases potential alternatives were examined with special care, and excluded. The 3D ^{15}N-edited NOESY spectrum was only used to assign $^1H^{\alpha}$ and $^1H^{\beta}$ of residues preceding proline. 3D ^{13}C-edited NOESY and HCCH-TOCSY tuned to aromatic protons were used to assign aromatic ^1H resonances. 3D HNCACB was used to assign labile side-chain protons of Gln, Asn and Arg.

Extent of assignments and data deposition

All of the ^1H polypeptide backbone resonances, except for Met^1 NH_2 and Gly^{14} H^N, were assigned. All of the non-aromatic ^1H side-chain resonances, except for 1 β, 9 γ, 7 δ and 2 ϵ methylene positions, and 5 methyl positions, were assigned. The resonances of several methylene and isopropyl groups were found to be degenerate. Unassigned aromatic resonances included 4 out of 12 $^1H^{\epsilon}$ of Phe/Tyr, $^1H^{\zeta}$ of all the Phe residues, and $^1H^{\epsilon 1}$ of all the His residues. Among the labile side-chain protons 5 out of 12 Gln/Asn amide protons, and 5 out of 13 Arg $^1H^{\epsilon}$ protons, were not assigned. All of the backbone heteronuclei, except for ^{15}N of Gly^{14}, C$'$ of Lys^{13}, Gly^{214} and residues preceding proline, and $^{13}C^{\alpha}$ of Pro^{139}, were assigned.

The assignments have been deposited at the BioMagRes Bank, accession number 4193.

Acknowledgements

We thank Prof. Elisha Haas for supporting this project, and Drs. R.A. Byrd, A.S. Altieri, P.J. Domaille and Mr. V. Tugarinov for advice and fruitful discussions. Financial support from ISF grant 728/96, and several visiting positions at SCRI, Florida State University, Tallahassee, FL, are gratefully acknowledged.

References

Bax, A. and Grzesiek, S. (1993) *Acc. Chem. Res.*, **26**, 131–138.

Burlacu-Miron, S., Perrier, V., Gilles, A.-M., Pistotnick, E. and Craescu, C.T. (1998) *J. Biol. Chem.*, **273**, 19102–19107.

Byeon, I-J.L., Yan, H., Edison, A.S., Mooberry, E.S., Abildgaard, F., Markley, J.L. and Tsai, M.-D. (1993) *Biochemistry*, **32**, 12508–12521.

Delaglio, F., Grzesiek, S., Vuister, G.W., Zhu, G., Pfeifer, J. and Bax, A. (1995) *J. Biomol. NMR*, **6**, 277–293.

Kraulis, P.J. (1989) *J. Magn. Reson.*, **24**, 627–633.

Reinstein, J., Brune, M. and Wittinghofer, A. (1988) *Biochemistry*, **27**, 4712–4720.

Sinev, M.A., Sineva, E.V., Ittah, V. and Haas, E. (1996) *Biochemistry*, **35**, 6425–6437.

Vonrhein, C., Schlauderer, G.J. and Schulz, G.E. (1995) *Structure*, **3**, 483–490.