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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N chemical shift assignments of an enolase-phosphatase, E1, from *Klebsiella oxytoca*

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Biological context

More than 60% of known human cancer cell lines exhibit methionine dependent cell growth (Quash et al., 2003). It has been shown that these tumor cell lines retain the ability to synthesize methionine from homocysteine but are unable to efficiently recycle the thiomethyl moiety of methionine via the methionine salvage pathway (Dumontet et al., 1996). The methionine salvage pathway is ubiquitous, present in both prokaryotic and eukaryotic cells. It converts 5′-*S*-methyl-5′-thioadenosine (MTA), a side product of polyamine biosynthesis, back to methionine through a series of steps including the conversion of 1-phospho-2,3-diketo-5-*S*-methyl-5-thiopentane to an acireductone (1,2-dihydroxy-3-keto-5-S-methyl-5-thio-1-pentene). This reaction is catalyzed by E1 enolase-phosphatase in *Klebsiella oxytoca* (Myers et al., 1993). E1 is a bi-functional monomeric 26 kDa enzyme that requires Mg^{2+} as a co-factor for activity (Myers et al., 1993). E1 represents the first enzyme with established enolase-phosphatase activity. The first reaction catalyzed by E1 is enolization of substrate at the C_1-C_2 bond, with the enol intermediate transiently accumulating prior to the second step, dephosphorylation of O_1. Based on primary sequence homology, E1 is a member of the haloacid dehalogenase (HAD) superfamily of enzymes and crystal structures of several members of this superfamily of enzymes have been reported (Shin et al., 2003). Members of the HAD superfamily fold in two domains, with a highly conserved core domain assuming a Rossmann fold and a more divergent 'cap' domain. The active site in all structurally characterized HAD enzymes is located between the two domains. There is no crystal structure for E1, and we are pursuing both crystallographic and NMR approaches to the structure of this enzyme. The first step towards an NMR structure is obtaining sequence specific resonance assignments, which we report here. This work represents the first report of NMR chemical shift assignments for a member of HAD superfamily of enzymes, providing necessary information for investigations of dynamics on different timescales accessible by NMR. Such investigations will further our understanding of function and specificity of E1 in particular and HAD superfamily enzymes in general.

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Methods and results

The gene encoding E1 was expressed in *E. coli* BL21(DE3)pLysS in minimal medium containing $^{15}NH_4Cl$ and U-^{13}C-glucose (Cambridge Isotope Labs) in order to obtain uniformly ^{13}C, ^{15}N labeled protein sample or $^{15}NH_4Cl$ and unlabeled glucose for obtaining ^{15}N labeled protein. In addition, a selectively ^{15}N-threonine sample was prepared by adding ^{15}N-threonine to the cell growth in minimal media as part of an amino acid mixture immediately prior to induction. Expression was induced by addition of IPTG. E1 was isolated following the previously reported procedure (Myers et al., 1993). The enzyme obtained has identical activity to E1 enzyme isolated from *K. oxytoca*. Samples were originally purified in 20 mM Tris-HCl, pH 7.4 buffer containing 0.5 mM $MgSO_4$. Prior to NMR, buffer was exchanged for 20 mM *d*-Tris-HCl, in 90/10 H_2O/D_2O, with 0.5 mM $MgSO_4$ using P2 spin columns. All NMR samples were ∼1 mM in E1 concentration. After buffer exchange, samples were placed in 5 mm Shigemi micro NMR tubes.

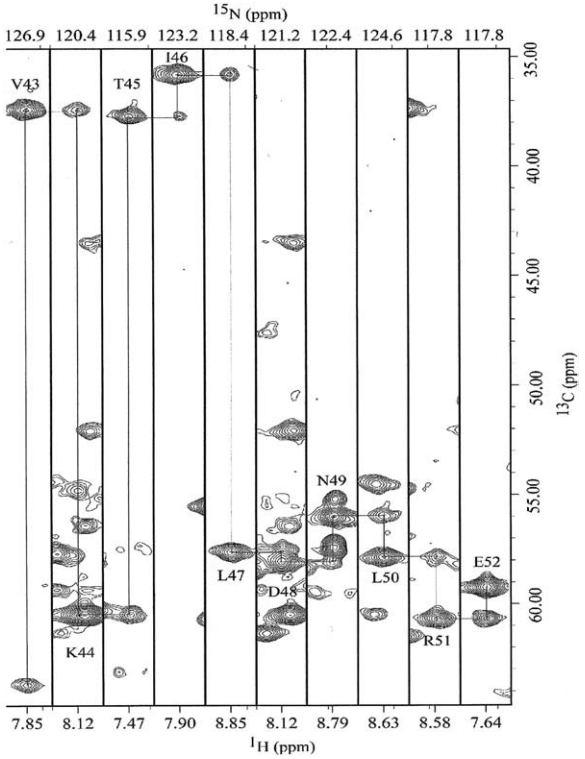

Figure 1. Strip plots of HNCA spectrum of ^{13}C, ^{15}N uniformly labeled enolase-phosphatase, E1, showing C_α connectivity from residue Val 43 to Glu 52. Note that the C_α resonances of residues 43, 45 and 46 are folded from their downfield positions.

NMR spectra were acquired at 298 K, on a Varian Unity Inova 600 14 T spectrometer, using a 5 mm $^1H\{^{13}C, ^{15}N\}$ probe equipped with pulsed field gradients. In order to obtain sequence specific backbone assignments a set of sensitivity-enhanced triple resonance experiments was performed, including HNCO, HN(CO)CA, HNCA, CBCA(CO)NH, HNCACB and HCCONH. In addition, $^1H,^{15}N$–HSQC, $^1H,^{15}N$ NOESY-HSQC and $^1H,^{15}N$ TOCSY-HSQC data sets were collected, in order to aid backbone assignment procedure and alleviate possible ambiguities. Side chain assignments were made using information contained in HCCH-TOCSY, and two ^1H, ^{13}C NOESY-HSQC data sets optimized for detection of ^{13}C resonances in aliphatic and aromatic regions, respectively. All experimental pulse sequences used are standard pulse sequences included in Varian ProteinPack (Varian Inc.) for INOVA spectrometers and were applied without modifications other than parameter calibration.

Data were processed using NMRPipe (Delaglio et al., 1995), and analyzed using XEASY (Bartels et al, 1995). Proton chemical shifts were referenced directly to internal DSS, while ^{15}N and ^{13}C shifts were indirectly referenced (Wishart et al., 1995). For preliminary assignments, an automated assignment program, GARANT (Bartels et al., 1997), was used that provided tentative assignments for ∼20% of the backbone. A computer-assisted manual assignment approach was used to further assign backbone resonances. An example of sequential connectivities as seen in HNCA spectrum along with appropriate residue assignments is given in Figure 1.

Extent of assignments and data deposition

E1 enolase-phosphatase consists of 229 residues, including 11 prolines, and 95% of all backbone resonances and 75% of all side chain resonances have been assigned. Although most residues that are expected to be in the active site have been identified, backbone assignments for several residues in this region are missing, most likely due to dynamic broadening. Most of the unassigned side chain resonances are in heavily overlapped regions of the spectrum (phenylalanine aromatic resonances and lysine/arginine methylene groups). All of the current assignments were made using fully protonated 26 kDa E1. The ^1H, ^{13}C and ^{15}N chemical shifts for E1 have been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under the BMRB accession number 6125.

Acknowledgements

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