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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N NMR assignments for a carbon monoxide generating metalloenzyme from *Klebsiella pneumoniae*

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Biological context

E2 is a 179-residue soluble monomeric metalloenzyme from the methionine salvage pathway in *Klebsiella pneumoniae*. In this pathway, metabolism of an acid-reductone (1,2-dihydroxy-3-keto-5-thiomethylpentene) is a branch point. E2, with a Ni^{2+} as cofactor, catalyzes the oxidative cleavage of acid-reductone generating carbon monoxide, formate and methylthiopropionic acid (Wray and Abeles, 1995). If the Ni^{2+} cofactor is replaced by Fe^{2+}, the enzyme (now called E2′) converts the same substrate to a methionine precursor and no CO is formed. E2 and E2′ can be interconverted by denaturation by guanidium chloride and reconstitution with the proper metal. The apoenzyme does not exhibit any catalytic activity with the acid-reductone substrate. E2 and E2′ are novel enzymes, in that different functions are observed for proteins with the same primary sequence (Dai et al., 1999).

We have been unable to find any structurally characterized protein or even known gene products with significant homology to E2/E2′. In fact, there are only a limited number of nickel-containing enzymes of known structure. We are using NMR to determine the structure of E2 and to understand the differences between E2 and E2′. A protein functionally similar to E2′ has been found in rat liver, but no such analog of E2 has yet been found. However, there is evidence suggesting that carbon monoxide, one of the enzymatic products of E2, acts as a neurotransmitter in mammals (Verma et al., 1993). The control of the methionine salvage pathway, via E2 and E2′ or their analogs in mammals, may also provide a mechanism for regulating the levels of polyamines, which is associated with cell proliferation and cancer.

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Methods and results

The gene encoding E2 was expressed in *E. coli* BL21(DE3) in a modified minimal medium containing $^{15}NH_4Cl$ and ^{13}C-U-glucose and supplemented with 1 μM of Ni^{2+} after IPTG induction (Dai et al., 1999). E2 was separated from E2′ using gel filtration and anion exchange columns and assayed to be functionally equivalent to E2 isolated from its natural host *Klebsiella*.

Two 2 mM samples were prepared, one labeled with ^{15}N and one uniformly labeled with ^{15}N, ^{13}C. Samples were dissolved in 30 mM potassium phosphate buffer (pH 7.4, D_2O:H_2O = 1:9) and placed into a 5 mm Shigemi micro NMR tube. NMR spectra were acquired at 298 K on Bruker AMX-500 (Brandeis), DMX-600 and Varian Unity Plus 750 (MIT FBML) spectrometers equipped with three axis gradients and triple resonance probes. NOESY-^{1}H,^{15}N-HSQC and TOCSY-^{1}H,^{15}N-HSQC experiments were performed on the uniformly ^{15}N labeled sample with both TOCSY and NOESY mixing times set at 50 ms.

A fast acquisition ^{1}H NMR experiment identifies four downfield shifted ^{1}H resonances at 50.8, 59.9, 68.9 and 70.1 ppm, confirming the existence of a paramagnetic center in E2. 2D NOESY experiments (with water suppression obtained using either presaturation or flip-back) were acquired using a ^{15}N labeled sample with ^{15}N decoupling during evolution and acquisi-

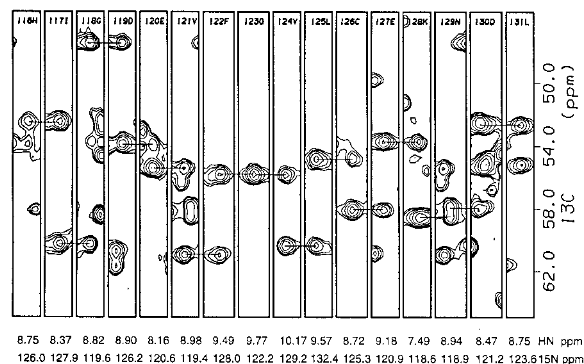

Figure 1. Strip plot of the HNCA spectrum of ^{15}N, ^{13}C uniformly labeled E2(Ni^{2+}) showing Cα connectivity from residue His^{116} to Leu^{131}.

tion. HNCO, HNCA, HN(CO)CA (Grzesiek and Bax, 1992a), CBCA(CO)NH (Grzesiek and Bax, 1992b), HBHA(CO)NH (Grzesiek and Bax, 1993), HCCH-TOCSY and gd-HCACO (Zhang and Gmeiner, 1997) were acquired for the ^{13}C, ^{15}N sample. Data were processed using NMRPipe (Delaglio et al., 1995) and exported to Felix97 (MSI, Inc.) for analysis.

Backbone sequential assignments were made using a combination of HNCA and HN(CO)CA. Assignments were confirmed using HCCH-TOCSY and HBHA(CO)NH and were extended to side chains. Backbone carbonyl assignments were mainly derived from HNCO and confirmed by gd-HCACO. For residues preceding prolines, gd-HCACO was used to make the assignments.

Since the protein contains paramagnetic Ni^{2+}, resonances from residues surrounding the metal binding site are broadened. Those residues are Phe^{92} to Val^{103}, Val^{134} to Asp^{143} and Trp^{162} to Phe^{166} (15% of total residues). Preliminary structural calculations indicate that the metal binding site contains four histidines (His^{96}, His^{98}, His^{137} and His^{140}), suggesting that those are ligands for the metal. Based on secondary structural considerations, we speculate that a disulfide bridge may be formed between the only two cysteines in E2, Cys^{114} and Cys^{126}.

In summary, sequential backbone assignments were made for E2, as shown in an HNCA strip plot (Figure 1). Approximately 15% of the residues in E2 are close enough to the paramagnetic center to render their sequential assignment impossible using standard methods. We are currently attempting to reconstitute the protein with diamagnetic metals in order to make full ^{1}H, ^{15}N and ^{13}C resonance assignments for the paramagnetic region.

Extent of assignments and data deposition

Of a total of 179 residues in E2, complete ^{15}N, NH, H^{α} and $^{13}C^{\alpha}$ assignments have been made for 140 non-proline residues, along with 141 CO assignments. H^{α} and C^{α} chemical shifts for six prolines and the N-terminal serine were also assigned. Complete or nearly complete ^{1}H side chain assignments have been made for 138 residues. In most cases, ^{13}C and/or ^{15}N resonances correlated with assigned side chain ^{1}H resonances have been assigned as well. Partial assignments (backbone and/or side chain) have been made for another 16 residues. No assignments could be made for 25 residues in E2. In almost all cases where assignments are either missing or incomplete, paramagnetic broadening due to the presence of Ni^{+2} appears to be responsible. The chemical shifts for Ni^{+2} E2 have been deposited in the BioMagResBank database (accession no. # 4313).

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