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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C resonance assignments for the catalytic core of γδ resolvase

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Biological context

γδ resolvase is a 20 kDa site-specific recombinase that catalyzes site-specific DNA recombination in the final stage of transposition in *E. coli* by resolving the cointegrate plasmid into two separate plasmids (Grindley, 1994). The resolution reaction is initiated by the formation of a synaptosome, where dimers of resolvase interact with two different res DNA sequences. Each res sequence contains three separate dimer binding sites designated as sites I, II and III. Following the formation of the synaptosome, resolvase catalyzes the cleavage at the crossover points in res site I and mediates the DNA strand exchange. Crystal structures of the resolvase dimer/DNA binding site complex are available; however, the molecular mechanism enabling crossover site cleavage and subsequent strand exchange are not known (Yang and Steitz, 1995). In the sub-unit rotation mechanism for strand exchange, two resolvase dimers would dissociate and an exchanged pair of dimers would reform. A possible intermediate in the strand exchange process is the monomeric form of resolvase in a subunit rotation model (Pan et al., 1997).

The resolvase protein consists of 183 amino acids which can be organized into three regions. The DNA binding domain is composed of residues 149–183 and is formed by three helices including a helix-turn-helix motif that interacts with the DNA binding site (Liu et al., 1994). The catalytic core domain of resolvase consists of residues 1–100 and contains the active residue Ser10. Residues 101–148 can be characterized as a linker region forming the dimerization helices and facilitating additional interactions with the DNA binding site.

NMR solution structural studies on resolvase have been initiated to investigate the mechanisms of strand cleavage and exchange. Initial investigations on the monomeric I110R mutant resulted in broadened resonances. However, tryptic cleavage of the monomeric form resulted in the production of a stable 12 kDa catalytic core domain consisting of residues 1–105. The cloned catalytic core domain represents the catalytic domain of the full length resolvase that is decoupled from interactions with the dimerization helices and, as such, is likely to represent an intermediate structure during catalysis. Nearly complete backbone and side chain assignments have been obtained for the ^{1}H, ^{15}N and ^{13}C resonances of the cloned catalytic core of resolvase.

Methods and results

Production of the 12 kDa fragment (Res12K) was achieved by mutation of Met 106 to a stop codon in a pET23 overexpression plasmid. The mutated plasmid (pDJ1.3) was isolated and transformed into *E. coli* strain BL21/DE3 with the accessory plasmid pLysS. ^{15}N labeled and ^{15}N, ^{13}C double labeled Res12K was produced by IPTG induction in a minimal media supplemented with vitamins and $^{15}NH_4Cl$ or U^{13}C glucose (Weber et al., 1992). The cell culture was grown at 37 °C and IPTG was added after the optical density (OD) at 600 nm reached 0.9–1.0. Further growth continued for an additional 3–4 h before harvesting. The ^{15}N/^{13}C labeled protein was expressed at levels greater than 100 mg/L of cell culture. The expressed protein remained in the soluble fraction of the cell extract and was subsequently purified with

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ammonium sulfate precipitation and cation exchange chromatography. Samples of ∼2 mM protein were prepared in 1M sodium chloride solution with 50 mM sodium phosphate buffer, pH 6.5, 0.01% sodium azide and 10% D_2O. Lower salt concentrations resulted in broadened linewidths.

Experiments were performed at 25 °C on Varian INOVA spectrometers equipped with triple resonance probes and pulse field gradients. All experiments were performed on a 500 MHz spectrometer except for the HCCH-TOCSY experiment which was performed on a 600 MHz spectrometer. The data were processed using Felix95 (MSI, San Diego, CA, U.S.A.) and analyzed using XEASY (Bartels et al., 1995). In the acquisition dimension, a convolution filter was applied to suppress the solvent signal, followed by apodization with sine-squared window functions with shifts of 70-80°, zero-filling and Fourier transformation. Linear prediction was also used in the indirect dimensions.

The backbone assignments were obtained on the recombinant catalytic core domain using 3D triple resonance data experiments that included HNCA, HN(CO)CA, HNCACB and CBCA(CO)NH (Muhandiram and Kay, 1994). Sequential through-bond connectivities were made for the backbone HN, CA and CB resonances (Figure 1). Carbonyl carbon resonances were assigned using the HNCO experiments. Side chain chemical shift assignments were completed by initially establishing partial connectivities with HC(CO)NH-TOCSY and C(CO)NH-TOCSY spectra (Grzesiek et al., 1993). Nearly complete ^1H and ^{13}C side chain assignments were obtained by analysis of the HCCH-TOCSY spectra (Kay et al., 1993). The aromatic resonances were assigned using 2D homonuclear NOESY data.

Extent of assignments and data deposition

The backbone ^1H-^{15}N backbone assignments were completed for all residues with the exception of residues 1, 65, 67, 68 and 70–72 which were not observed in the HSQC spectrum. Side chain ^1H and ^{13}C assignments (and ^{15}N assignments for Asn and Gln) are essentially complete with the exceptions of the residues 1, 65, 67, 68 and 70–72, the Arg guanidinium groups, three Met methyl group protons, ^{13}C resonances for the aromatic rings, and the ζ protons for Phe 4, 34 and 92. The $^{13}C_\alpha$ and $^{13}C_\beta$ resonances for residues 65, 68 and 72 have been assigned. Arg ϵ-NH assignments for all but four Arg residues were made. Stereospecific assignments have also been obtained for the β protons of 17 residues, the γ methyl protons of Val 20 and the δ methyl protons of Leu 51. The ^1H, ^{13}C and ^{15}N chemical shifts for the catalytic core domain of resolvase have been deposited at the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 4269.

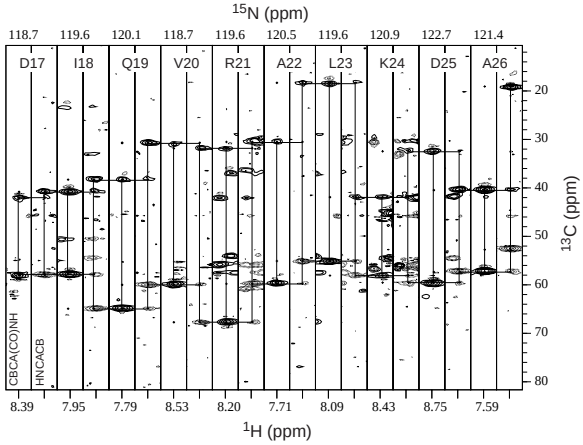

Figure 1. Representative strips from the CBCA(CO)NH and HNCACB spectra showing connectivities for residues 17 to 26 in the catalytic core of resolvase.

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