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Letter to the Editor: Backbone assignments for endonuclease V from bacteriophage T4 with deuterium labeling

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Biological context

Endonuclease V from bacteriophage T4 (T4 endo V) is a DNA repair enzyme, which catalyzes the first step of the UV-induced pyrimidine dimer-specific excision repair pathway. Although T4 endo V is a relatively small protein consisting of 138 residues, this enzyme is responsible for two catalytic activities as a glycosylase and an endonuclease in damaged DNA repair pathway. Crystal structure revealed that T4 endo V is composed of a single compact domain of 3 α-helices (Morikawa et al., 1992). The structure of the mutant enzyme, T4 endo V (E23Q), which retains DNA binding specificity but lacks catalytic activity, complexed with a DNA duplex containing a thymine dimer, was also determined by X-ray crystallography and the concave surface covered with many positively charged amino acids implied the interface for DNA binding (Vassylyev et al., 1995). Here we report the ^{1}H, ^{13}Cα, ^{13}Cβ, ^{13}C and ^{15}N assignments for T4 endo V from the deuterated sample. It provides the basic information for further research to get an insight into DNA repair mechanism.

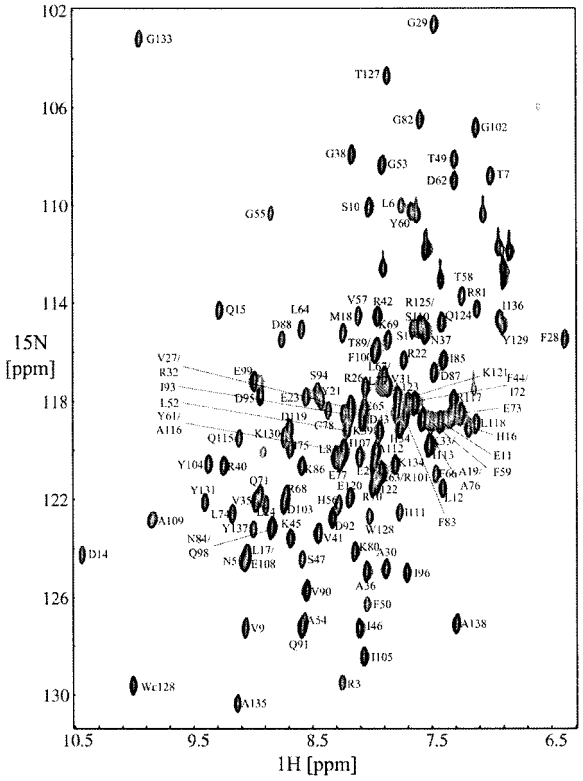

Figure 1. 2D [^{15}N, ^{1}H]-HSQC spectrum of [*U*-70% ^{2}H; U-^{15}N]-T4 endo V. The Backbone resonances were completely assigned and indicated by the one-letter amino acid code and the sequence number. The backbone resonances from Ile 4, Ile 51, and Tyr 132 are located at outside of the figure range and the resonance of Ala 13 is not shown because of its low signal intensity.

Methods and experiments

Recombinant T4 endo V was expressed in *E. coli* BL21 (DE3) under T7 promotor. T4 endo V was purified and prepared for NMR measurements as described (Lee et al, 1994). Three NMR samples, [*U*−^{15}N]-, [*U*-70% ^{2}H; U-^{15}N]- and [*U*-90% ^{2}H;

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$U-^{13}C/^{15}N$]-T4 endo V were prepared and dissolved in 50 mM potassium phosphate buffer (90% H_2O/10% D_2O, pH 6.5) with 2 mM NaN_3. All NMR spectra were obtained with 1.5 mM protein on a Bruker DRX 500 and 600 spectrometer. NMR measurements were performed at low temperature, 20 °C, since the sample gelled above 20 °C. Because the large contents of α-helix (about 47%, calculated from crystal structure) and the low measuring temperature hindered the sequence-specific assignments, deuterium labeling technique was introduced. For identification of spin systems, we also prepared the residue-selectively ^{15}N-labeled T4 endo V for Ala, Arg, Asp, Gly, Lys, Thr, Tyr, Val, and [Ile, Leu, and Val], respectively. Sequence-specific assignments of backbone resonances for ^{1}H, $^{13}C\alpha$, $^{13}C\beta$, ^{13}C, and ^{15}N were obtained using 2D [^{15}N, ^{1}H]-HSQC spectrum, and the combination of triple resonance experiments, 3D ct-HNCA, 3D ct-HN(CO)CA, 3D ct-HNCACB, 3D ct-HN(CO)CACB, and 3D ct-HNCO spectra (Yamazaki et al., 1994; Shan et al., 1996) on deuterated T4 endo V. Using $^{15}N/^{1}H$ correlations from residue-selectively labeled HSQC spectra as starting points of sequence-specific assignments, the complete assignments for backbone and Cβ resonances were allowed from inter- and intra-residue correlations of triple resonance experiments. Hα resonances were identified from 3D [^{15}N, ^{1}H]-TOCSY- and NOESY-HSQC spectra and 3D HNHA spectra on 70% deuterated T4 endo V (Palmer et al., 1991). Sequence-specific assignments were also confirmed using sequential NOEs measured in 3D [^{15}N, ^{1}H]-NOESY-HSQC spectra, especially for helical regions. Proton chemical shifts were referenced to the methyl signal of 2, 2-dimethylsilapentane-5-sulfonic acid (DSS) externally. ^{13}C and ^{15}N chemical shifts were referenced indirectly to DSS. Deuterium labeling effect was considered to correct Cα and Cβ chemical shifts (Venters et al., 1996). The NMR spectra were processed using the program NMRPipe/NMRDraw (Delaglio et al., 1995) and analyzed using the program NMRView (Johnson and Blevins, 1994).

Extent of assignments and data deposition

Backbone $^{1}H/^{15}N$ resonances were assigned except for N-terminal two amino acids, Met 1 and Thr 2, and for Pro 25, 48, 97, 106, and 126. ^{13}C resonances for Cα and Cβ were completely assigned from Thr 2 to Ala 138 except for Cα of Met 1 and for Cβ of Ser 10, Ser 110, and Trp 128. Carbonyl carbon resonances were also completely assigned except for Met 1, Ala 138, and the preceding residues of Pro. 113 Hα resonances out of 138 residues were unambiguously assigned and the ambiguities from the signal degeneracy or from Pro were excluded. The backbone and Cβ chemical shifts have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under the BMRB accession number 5244.

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