NMR peak assignment for the elucidation of the solution structure of T4 Endonuclease V

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Bacteriophage T4 endonuclease V initiates the repair of ultraviolet (UV)-induced pyrimidine dimer photoproducts in duplex DNA. The mechanism of DNA strand cleavage involves four sequential steps: linear diffusion along dsDNA, pyrimidine dimer-specific binding, pyrimidine dimer-DNA glycosylase activity, and AP lyase activity. Although crystal structure is known for this enzyme, solution structure has not been yet known. In order to elucidate the solution structure of this enzyme, NMR spectroscopy was used. As a basis for the NMR peak assignment of the protein, HSQC spectrum was obtained on the uniformly ^{15}N-labeled T4 endonuclease V. Each amide peak of the spectrum were classified according to amino acid spin systems by interpreting the spectrum of ^{15}N amino acid-specific labeled T4 endonuclease V. The assignment was mainly obtained from three-dimensional NMR spectra such as 3D NOESY-HMQC, 3D TOCSY-HMQC. These experiments were carried out with uniformly ^{15}N-labeled sample. In order to assign the resonance of backbone atom, triple-resonance three-dimensional NMR experiments were also performed using double labeled($^{15}N/^{13}C$) sample. 3D HNCA, HN(CO)CA, HNCO, HN(CA)HA spectra were recorded for this purpose. The results of assignments were used to interpret the interaction of this enzyme with DNA. HSQC spectrum was obtained for T4 endonuclease V with specific ^{15}N-labeled amino acids that have been known for important residue in catalysis. By comparing the spectrum of enzyme*DNA complex with that of the enzyme, we could confirm the important role of some residues of Thr, Arg, Tyr in activity. The results of assignments were also used to predict the secondary structure by chemical shift index (CSI).