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제 목	NMR study of the interaction of T ₄ Endonuclease V with DNA
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내 용	

In order to obtain insight into the mechanism by which DNA containing a thymine photo-dimer is recognized by the excision repair enzyme, T₄ endonuclease V, we have taken NMR study of this protein and its complex with oligonucleotides. The conformations of five different DNA duplexes DNA I : d(GCGGATGGCG)·d(CGCCTACCGC), DNA II : d(GCGGTTGGCG)·d(CGCCAACCGC), DNA III : d(GCGGT[^]TGGCG)·d(CGCCAACCGC), DNA IV : d(GCGGGCGGCG)·d(CGCCCGCCGC) and DNA V : d(GCGGCCGGCG)·d(CGCCGGCCGC) were studied by ¹H NMR. The NMR spectra of these five DNA duplexes in the absence of the enzyme clearly show that the formation of a thymine dimer within the DNA induces only a minor distortion in the structure, and that the overall structure of B type DNA is retained. The photo-dimer formation is found to cause a large change in chemical shifts at the GC7 base pair, which is located at the 3'-side of the thymine dimer, accompanied by the major conformational change at the thymine dimer site. The binding of a mutant T₄ endonuclease V (E23Q), which is unable to digest DNA containing a thymine dimer, to the DNA duplex d(GCGGT[^]TGGCG)·d(CGCCAACCGC) causes a large down-field shift in the imino proton resonance of GC7. Therefore, this position is thought to be either the crucial point of the interaction with T₄ endonuclease V, or the site of a conformational change in the DNA caused by the binding of T₄ endonuclease V.

Usually, it is very difficult to assign NMR peaks in DNA * protein complex because of severe peak overlaps. In order to overcome these peak overlaps, we used a method of deuterium incorporation.