NMR study of the interaction of T4 Endonuclease V with DNA

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(GCGCATGGCG)·d(CGCTACCACC), DNA II : d(GCGGTTGGCC)·d(CGCCAACCACC), DNA III : d(GCGGT^TGGCC)·d(CGCCAACCACC), DNA IV : d(GCGGGCGGCCG)·d(CGCCCGCCGCC) and DNA V : d(GCGGCCGCGCG)·d(CGCCCGCCGCC) 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^TGGCC)·d(CGCCAACCACC) 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.