Photo-CIDNP Study of the Splitting of the Dinucleotide

cis,syn-Thymine Dimer with Reduced Flavin as a Sensitizer:
Evidence for a Thymine Radical Anion Intermediate

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Abstract. Photochemically induced dynamic nuclear polarization (photo-CIDNP) studies were performed on the splitting of cis,syn 2'-deoxythymidylyl-(3'→5')-2'-deoxythymidine cyclobutane dimer (cis-dTpdT), using reduced flavin as a sensitizer. This system serves as a model for the light-induced repair mechanism of thymine dimers in DNA by the enzyme photolyase. The CIDNP spectrum shows enhanced absorption of the two C6-H protons of the corresponding monomer dTpdT, which demonstrates that a thymine radical anion is involved in the splitting of the dimer. This is supported by a similar CIDNP spectrum that is obtained with the electron-donor N-acetyl tryptophan as a sensitizer. This result suggests that the light-induced splitting of thymine dimers in DNA by photolyase also proceeds via the thymine radical anion. The small difference in intensity of the two CIDNP signals belonging to the C6-H protons shows that the unpaired electron in the monomer radical has a slight preference for the thymine moiety at the 5' terminus.

1. Introduction

The cis,syn thymine dimer is the major photoproduct that arises in DNA exposed to UV light [1]. It is formed by an intrastrand photocycloaddition reaction between adjacent thymine bases. This form of UV damage can be repaired by photolyases, i.e. enzymes that bind to thymine dimers in DNA and split the cyclobutane ring upon irradiation with near-UV or visible light [2, 3]. Photolyases contain two chromophores, one of which is reduced flavin adenine dinucleotide, FADH₂. It has been demonstrated that this is the chromophore involved in the electron transfer reaction with the thymine dimer [4, 5]. Two mechanisms have been suggested for the enzymatic reaction: either electron transfer of the excited chromophore FADH₂⁺ to the thymine dimer or electron transfer from dimer to chromophore. The first mechanism involves a reduction of the dimer resulting in a dimer radical anion that then dissociates into monomer and monomer radical anion. In the second mechanism a dimer radical cation is
formed that also dissociates into monomeric fragments. Electron transfer between monomer radical and chromophore radical produces diamagnetic thymine monomers, at which point the repair process is completed. To date, the direction of the electron transfer is unknown, although several arguments are in favor of electron donation from reduced flavin to thymine dimer [3, 6].

As a model system for the DNA repair mechanism the photo-sensitized splitting of isolated pyrimidine dimers has been studied, using electron-acceptors like quinones and oxidized flavins, and electron-donors like indoles and reduced flavins. Photochemically induced dynamic nuclear polarization (photo-CIDNP) has proven a powerful method for studying the splitting of pyrimidine dimers [7–12]. With anthraquinone-2-sulfonate [7–10] or oxidized flavin [11] as a sensitizer the existence of pyrimidine radical cations has been demonstrated, and pyrimidine radical anions have been observed during indole-sensitized pyrimidine dimer splitting [10, 12]. The two splitting mechanisms give rise to completely different CIDNP spectra, because the electron spin density distribution in pyrimidine radical anions is different from that in radical cations.

In this paper CIDNP experiments are presented that were performed on a dinucleotide thymine dimer, the cis,syn cyclobutane dimer of 2'-deoxythymidylyl-(3'→5')-2'-deoxythymidine, cs-dTp[dT] (Fig. 1). To study the mechanism of splitting with a photolyase, its active chromophore, a reduced flavin, is used as a sensitizer. It will appear that the resulting CIDNP spectra resemble those that are obtained with the electron-donor N6-acetyl tryptophan, which indicates that the splitting proceeds via a thymine radical anion.

Fig. 1. Structure of the cis,syn cyclobutane dimer of 2'-deoxythymidylyl-(3'→5')-2'-deoxythymidine (cs-dTp[dT]), with numbering of the atoms. The C5-methyl groups and the C6-protons reside on the same side of the cyclobutane ring. A and B refer to the 5' and 3' terminal fragments, respectively.