CIDNP Evidence for Reversibility of the Photosensitized Splitting of Pyrimidine Dimers

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The primary effect of UV radiation on DNA is a photocycloaddition reaction between adjacent pyrimidine bases, resulting in a cyclobutane pyrimidine dimer.[1] This type of DNA damage can be repaired by photolyases, *i.e.*, enzymes that bind to the DNA in a light-independent step, and split the cyclobutane ring upon irradiation with near-UV or visible light. This light-induced repair proceeds *via* electron transfer between the pyrimidine dimer and dihydroflavin adenine dinucleotide, $FADH_2$, one of the two chromophores of photolyase.[2] Experiments with isolated pyrimidine dimers and several photosensitizers have shown that the dimer can dissociate *via* both radical cation and radical anion intermediates.[3] Because radical intermediates are involved, the CIDNP technique (chemically induced dynamic nuclear polarization) is extremely suitable for studying these photochemical reactions. The presence of CIDNP signals depends, among other things, on the lifetime of the radical intermediate and on the electron–proton hyperfine interaction. Because the hyperfine interaction patterns in radical anions and cations of pyrimidines are different, a good distinction between the two mechanisms can be made.[4] In particular, recent experiments have demonstrated that the thymine radical anion is involved in the $FADH_2$-sensitized dissociation of a dinucleotide thymine dimer.[5] In this communication we focus on pyrimidine monomers and dimers in which the pyrimidine moieties are linked through the N1-nitrogens with a trimethylene bridge. These bridged pyrimidines serve as a model for adjacent pyrimidine bases that are connected to deoxyriboses of the DNA backbone. The sensitivity of the CIDNP technique allowed the detection of a reaction pathway that thus far escaped attention. The experiments demonstrate that the photosensitized splitting of thymine dimers is reversible. Dimerization occurs for pathways involving both radical anion and radical cation intermediates.

Pseudo-steady-state CIDNP experiments were performed on several thymine monomers and dimers, using the electron-donor N_α-acetyltryptophan (AcTrp) or the electron-acceptor anthraquinone-2-sulfonate (AQS) as a sensitizer.[6] In Figure 1a, the CIDNP spectrum of a simple thymine monomer (T) recorded with AcTrp as a sensitizer is displayed, showing emission signals at 7.40 and 1.91 ppm for the thymine C6-H and C5-CH_3 protons,

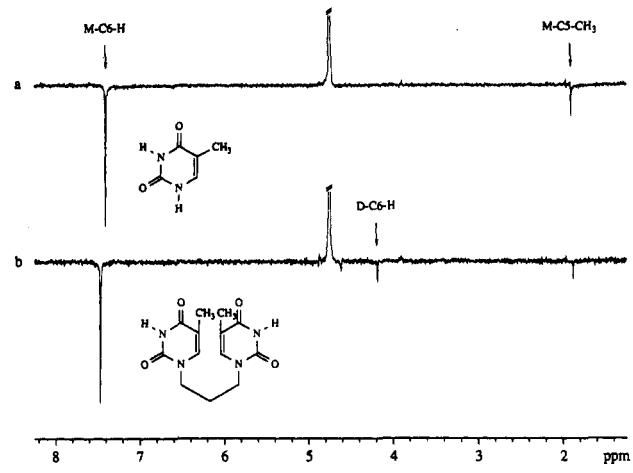

Figure 1. CIDNP spectra of (a) thymine (T) and (b) 1,1′-trimethylenebis(thymine) (T-C_3-T) in D_2O with N_α-acetyltryptophan as a sensitizer. M and D indicate protons belonging to the monomer and the dimer, respectively.

respectively. The emissions are in agreement with a negative value for Γ, as determined with the CIDNP sign rule $\Gamma = \mu\epsilon\Delta g a$:[7] thymine is a recombination product ($\epsilon > 0$) formed by back electron transfer within the geminate radical pair $AcTrp^{\cdot+}T^{\cdot-}$, which is initially formed in the triplet state ($\mu > 0$);[8] further, $T^{\cdot-}$ has a higher g-value than $AcTrp^{\cdot+}$ ($\Delta g > 0$),[9] and in the thymine radical anion $T^{\cdot-}$, the unpaired electron is delocalized between the C4 and the C6 position, which implies that the polarized C6-protons are in α-position with respect to the unpaired electron and consequently have a negative hyperfine coupling constant ($a = -1.18$ mT).[9b] No CIDNP signals of the sensitizer AcTrp are observed, which is due to cancellation of opposite recombination and escape polarization, as a result of very fast exchange between $AcTrp^{\cdot+}$ and ground-state AcTrp at pH 3.2. (ref. 4f, 10).

The CIDNP spectrum of 1,1′-trimethylenebis(thymine) (T-C_3-T) is shown in Figure 1b and contains, in addition to the signals of Figure 1a, an emission at 4.19 ppm. Comparison with the NMR spectrum of the corresponding cyclobutane thymine dimer (T-C_3-T)D shows that this signal belongs to the cyclobutyl C6-H protons of this dimer. Apparently, a ring-closure reaction in the monomer radical anion T-C_3-$T^{\cdot-}$ can occur, followed by back electron transfer within the dimer radical pair $AcTrp^{\cdot+}$-(T-C_3-T)$D^{\cdot-}$. No CIDNP can be generated in this dimer radical pair, not even if it would have a relatively long lifetime, because there are no protons with appreciable hyperfine interaction in the dimer radical anion (T-C_3-T)$D^{\cdot-}$. This can be concluded from

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(6) A pseudo-steady-state CIDNP spectrum is the difference spectrum of the light spectrum (recorded after a laser pulse train consisting of 20 flashes during 1 s) and a dark spectrum. The concentration of thymine was about 3 mM, while the concentration of photosensitizer was adjusted to an optical density of 0.5, which corresponds to a solution of 2 mM AcTrp and 0.2 mM AQS in D_2O. The pH was not adjusted, resulting in pH 3.2 and 7.6 for the AcTrp and the AQS samples, respectively. The NMR experiments were performed at 360 MHz, using the XeCl emission of an excimer laser as the light source (λ = 308 nm, 10–15-ns duration, 20 mJ/pulse at the position of the sample).

(7) Kaptein, R. *Chem. Commun.* **1971**, 732. The parameters have the following meanings: $\Gamma > 0$ for enhanced absorption and $\Gamma < 0$ for emission; initial spin multiplicity $\mu < 0$ for a singlet-born and $\mu > 0$ for a triplet-born radical pair; $\epsilon > 0$ for a recombination product and $\epsilon < 0$ for an escape product; Δg is the difference between the g-value of the radical that contains the polarized proton and that of the other radical; and a denotes the sign of the hyperfine coupling constant.

(8) From a large number of CIDNP spectra of different pyrimidines, it could be concluded that AcTrp reacts from the excited triplet state (ref 4f).

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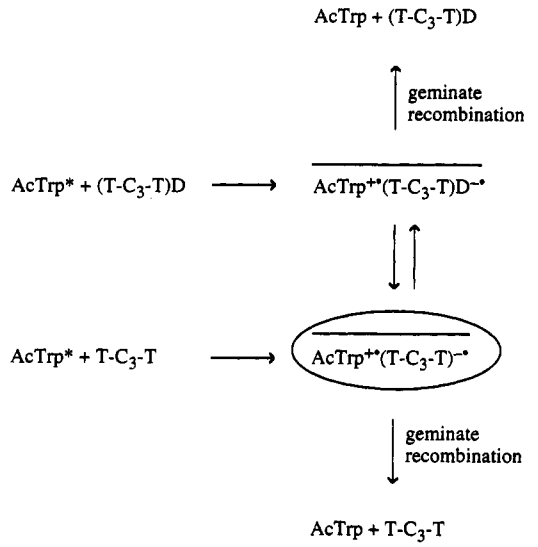

Figure 2. Polarization pathway for the reaction of AcTrp* with monomer T-C_3-T and dimer (T-C_3-T)D. Polarization is generated only in the encircled monomer radical pair AcTrp+T-C_3-T−.

ESR and ENDOR spectra on the 5,6-dihydro-6-methyluracil radical anion, which has an electronic structure similar to that of (T-C_3-T)D−. These spectra show no hyperfine coupling with the proton attached to the sp^3-hybridized C6-carbon.[11] As a consequence, the polarization must originate from the monomer radical pair and be transferred *via* the dimer radical pair to the recombination product of that pair, the dimer (T-C_3-T)D. This phenomenon, that polarization generated in a certain radical pair arises in the recombination product of a subsequent radical pair, is called "memory effect".[12] The identical sign of the monomer and dimer C6-H polarization provides support for this pathway. Although, because of dissociation of the dimer in the photostationary state, the concentration of dimer may be low, due to the sensitivity of the CIDNP method, the existence of the dimerization pathway can be demonstrated. For the nonbridged thymines, the probability of dimerization appears to be much smaller, since no dimer polarization can be observed in the CIDNP spectrum of the simple thymine (Figure 1a). Apparently, the concentration of pyrimidine is then too low for reaction with a pyrimidine anion during its lifetime.

The AcTrp-sensitized CIDNP spectra of the cyclobutane dimers, *cis,syn*-thymine dimer (*cs*TD) and 1,1′-trimethylenebis-(thymine)dimer ((T-C_3-T)D), are identical to those of the corresponding monomers, which demonstrates that in both cases the polarization originates from the monomer radical pair. Again, no polarization is generated in a dimer radical pair, because of a lack of hyperfine coupling of the protons in the thymine dimer radical anion. The presence of dimer polarization in the spectrum of the bridged thymine dimer thus indicates that there is a forward reaction from dimer *via* dimer radical pair to monomer radical pair, in which polarization is generated, followed by the reverse route ending in polarized dimer. The polarization pathway is displayed in Figure 2, in which the reaction of AcTrp* with both monomer T-C_3-T and dimer (T-C_3-T)D is summarized.

The dimerization pathway can also be observed if the electron-acceptor AQS is used as a photosensitizer, as illustrated in Figure 3 for N-methylated thymines. The CIDNP spectrum of 1,3-dimethylthymine (DMT), Figure 3a, contains strong emissions for the monomer C5-CH_3, N1-CH_3, and very weak emission for the monomer C6-H protons (1.94, 3.42, and 7.49 ppm, respectively). These signals are in agreement with spin-sorting in the monomer radical pair AQS−DMT+, in which these protons have a positive hyperfine coupling constant.[13] In Figure 3b, the CIDNP

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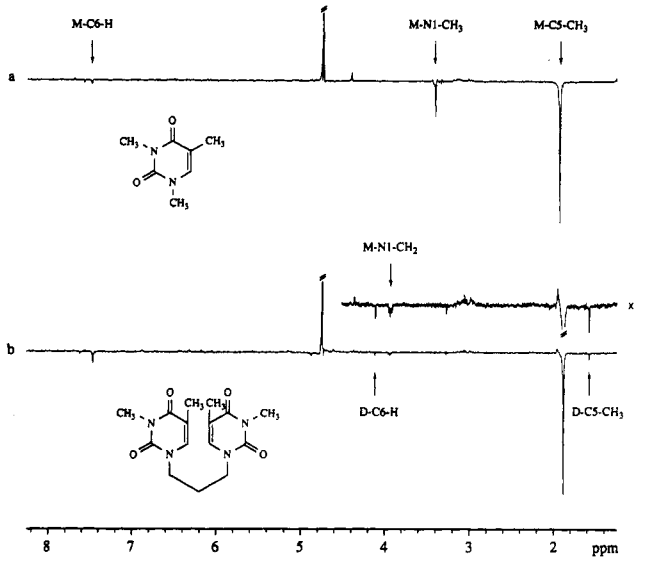

Figure 3. CIDNP spectra of (a) 1,3-dimethylthymine (DMT) and (b) 3,3′-dimethyl-1,1′-trimethylenebis(thymine) (MT-C_3-MT) in D_2O with anthraquinone-2-sulfonate as a sensitizer. M and D indicate protons belonging to the monomer and the dimer, respectively.

spectrum of the bridged analogue 3,3′-dimethyl-1,1′-trimethylenebis(thymine), MT-C_3-MT, is shown. It can be recognized that again protons of the corresponding dimer (MT-C_3-MT)D are polarized: emission for both the C5-CH_3 and the C6-H protons, at 1.58 and 4.12 ppm, respectively. Because there is no hyperfine interaction with the C5-CH_3 protons in the dimer radical cation, the polarization of this methyl group originates from the monomer radical pair and arises in the dimer as a result of the memory effect. The dimer C6-H emission might originate from the monomer radical pair, because the polarization has the same sign as the monomer C6-H signal, but also from the dimer radical pair. This cyclobutyl proton has a very large positive hyperfine coupling in the dimer radical cation (for the electronically similar 5,6-dihydro-6-methyluracil radical cation, a value of 6.92 mT has been reported),[11] and polarization generated in the dimer radical pair would therefore result in an emission peak as well. Furthermore, the ratio of the dimer to monomer C6-H signals at 4.12 and 7.46 ppm is relatively large with respect to the corresponding ratio of the C5-CH_3 protons, which also suggests that both mechanisms contribute to the polarization of the cyclobutyl C6-H proton.

These experiments show that the photosensitized dissociation of pyrimidine dimers, involving radical anion or radical cation intermediates, is reversible if the two pyrimidine moieties are close together. The reversibility may reduce the efficiency of the splitting of thymine dimers in DNA by photolyase, although the enzymatic repair is an efficient process overall (quantum yields between 0.5 and 1).[2b] This reaction may also play a role in the UV-induced formation of cyclobutane thymine dimers, a process that heretofore has been believed to proceed solely *via* a concerted photocycloaddition involving an excited, neutral pyrimidine monomer.

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(13) AQS reacts from the excited triplet state, and the *g*-value of DMT+ is smaller than that of AQS−. The absorption signal at 4.40 ppm is due to an addition product, formed between an escaped DMT+ radical cation and AQS (ref 4f).