DNA Lesions

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Mechanism of UV-Induced Formation of Dewar Lesions in DNA **

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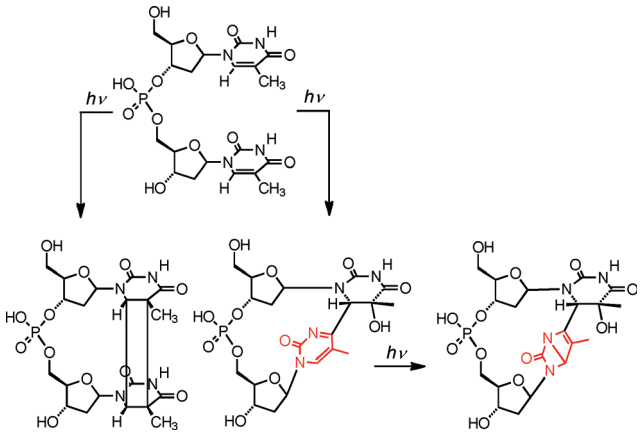
Living organisms exposed to sunlight are constantly challenged by the formation of UV-induced lesions in DNA, which induce cell death and cause mutations.[1] UV irradiation of TpT and TpC sequences leads to the formation of two primary lesions, namely cyclobutane-pyrimidine dimers (CPD)[2] and (6-4) lesions,[3] as depicted in Scheme 1. These (6-4) lesions possess an additional absorption at $\lambda_{max} = 320$ nm and rearrange to give Dewar valence isomers.[4]

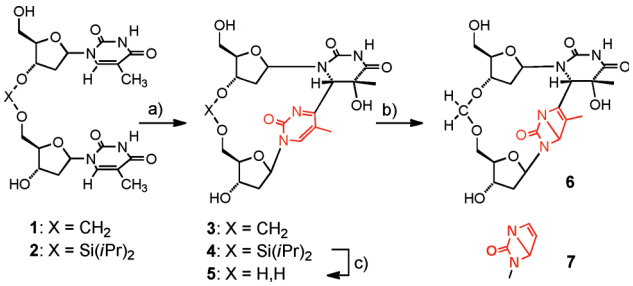
Whereas the mechanisms that lead to the formation of CPD and (6-4) lesions are today rather well understood, very little is known about the (6-4) to Dewar rearrangement. Dewar lesions are major UV-induced reaction products formed in genetic material upon exposure to sunlight, and the mechanism of formation is of paramount importance.[5] The Dewar lesions are formed formally in a photochemically allowed 4π electrocyclization reaction, which generates a β-lactam structure fused to a second four-membered ring system. The double-bond character of the amide bond within the β-lactam structure renders these compounds heterocyclic versions of the bicyclic structure of benzene initially proposed by Dewar in 1867.[6] This complex structure results in these compounds being characterized by poor repairability and strong mutagenicity.[7] As such, they are involved in the formation of a broad range of mutations, especially at TpC sites, which are hotspots of UV-induced carcinogenesis.



Scheme 1. Photchemical reactions at TpT (top) sites in DNA that lead to the formation of CPD (bottom left), (6-4) lesions (bottom middle), and Dewar lesions (bottom right).

To clarify the mechanism of formation of the strained Dewar structure we synthesized (6-4) lesion dinucleotides and studied their photochemical rearrangement to Dewar isomers by time-resolved UV-pump IR-probe spectroscopy.[2a,8] These data together with ab initio calculations of the excited state allowed us to show that the 4π electrocyclization is a rather slow reaction, but with an unusually high quantum yield. Interestingly, we found the reaction to be critically controlled by the backbone structure of the DNA.

For the study we prepared the two T-T dinucleotides **1** and **2** (Scheme 2). Compound **1** contains a bio-isosteric formacetal linker instead of the natural phosphodiester.[9] Compound **2** is a (6-4) lesion, with a silyl spacer so that the backbone can be cleaved at a later date.[10] Both compounds are synthetically accessibly in large quantities (250 mg), as needed for the following studies. Compounds **1** and **2** were irradiated with UV light (254 nm) under anaerobic conditions to give the corresponding T(6-4)T dinucleotides **3** and **4**. We subsequently purified the (6-4) compounds by reversed-phase



Scheme 2. Synthesis of the (6-4) lesions **3** and **5** needed for the study. a) 254 nm; b) 365 nm; c) NH_4OH.

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HPLC and in the case of compound **4** cleaved the silyl spacer to access the backbone-lacking structure **5**.

A small amount of the formacetal T(6-4)T compound **3** was rearranged upon irradiation with white light to give the T(Dew)T lesion **6**. The reaction of **3** to **6** is a quasi spot-to-spot reaction, which proceeded in quantitative yield without the appearance of any side products, thus underlining the efficiency of the formation of the Dewar isomer (see Figure S1a in the Supporting Information). To our surprise we noted that irradiation of the backbone-opened but otherwise identical (6-4) lesion **5** (for the absorption spectra see Figure S2 in the Supporting Information) provided no Dewar isomer, even on extended irradiation of minutes to hours and an increased intensity of the light source (see Figure S1b in the Supporting Information). This is also true for 5-methyl-2-pyrimidone (**7**), which also gives no Dewar isomer upon irradiation. Analysis of the reaction showed only the presence of starting material, then subsequent decomposition. This observation shows that the reaction from the (6-4) lesion to the Dewar isomer is not a simple 4π electrocyclization within the pyrimidone substructure of the (6-4) lesion but a more complex reaction.

To study the reaction in more detail we performed time-resolved studies. We thought that time-resolved IR measurements would be best suited to gain data about the reaction dynamics since strained structures are formed. Indeed, significant differences between the T(6-4)T lesion and the Dewar isomer were detected in the range of the C=O stretching vibrations (Figure 1a). The Dewar lesion T(Dew)T (Figure 1a, solid curve) shows weaker C=O bands than the T(6-4)T lesion (Figure 1a, dashed curve). In addition it shows a strong characteristic IR marker band around 1780 cm^{-1}. The absorption difference spectrum upon formation of the Dewar isomer is shown in Figure 1b (shaded area). In the following we used the pronounced Dewar band at 1780 cm^{-1} to monitor the appearance of the Dewar isomer.[11]

For the time-resolved experiments, femtosecond UV (323 nm) pulses were used to excite the T(6-4)T lesion. The absorption changes ΔA were measured with suitably delayed broadband probing pulses. The difference spectra recorded at certain delay times are plotted in Figure 1c. A decrease in the absorption at the positions of the C=O-stretching modes (around 1660 cm^{-1}) of the T(6-4)T lesion is observed immediately after excitation (e.g. at 11 ps). No absorption change is observed at 1780 cm^{-1}. With increasing delay time, the absorption decrease at 1664 cm^{-1} recovers (a time constant of 130 ps is evident in Figure 1d) and the appearance of new absorptions at the position of the Dewar marker band are observed (see Figure 1c,d). An experiment in which the visible/UV range was probed revealed that the increase in the absorption on the picosecond time scale is caused by excited-state absorptions (see Figure S3 in the Supporting Information). With a time constant of 130 ps, most of the induced absorption disappeared, thus pointing to the decay of the excited electronic state of the T(6-4)T lesion. (A detailed discussion of the transient UV/Vis measurements is provided in the Supporting Information.) At around 250 ps (Figure 1c), the IR difference spectrum shows the definite presence of the Dewar isomer, but the stationary IR difference spectrum is not reached (see Figure 1b,c). The remaining differences, most pronounced in the range of the C=O bands and negligible at 1780 cm^{-1}, relax on the nanosecond time scale. After 10 μs, the transient difference spectrum (points in Figure 1b) finally agrees well with the difference spectrum obtained by illumination of the steady state. These observations allow us to conclude that the Dewar valence isomer is formed directly from the excited electronic state with a time constant of 130 ps.

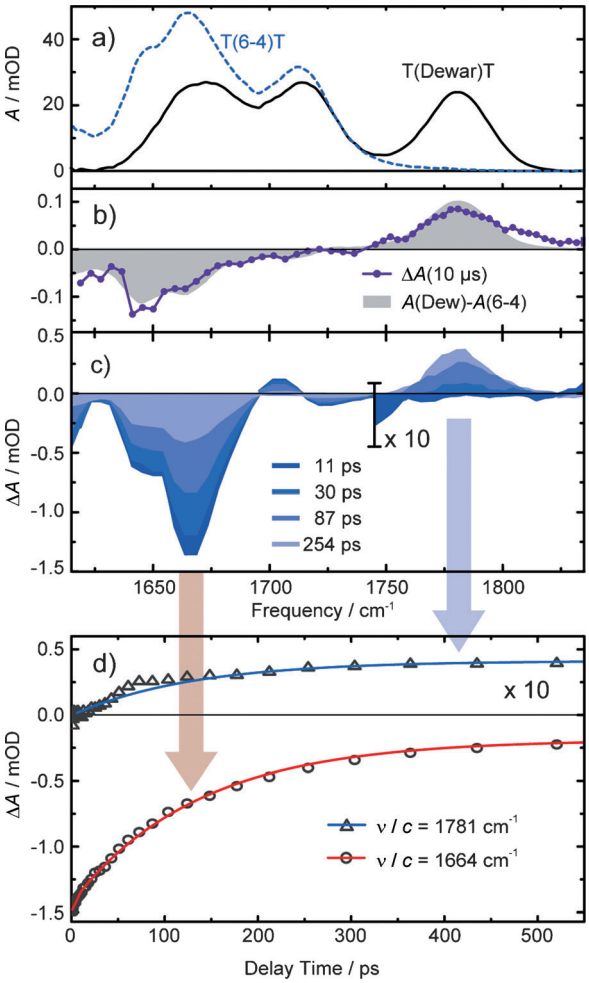

Figure 1. a) Stationary IR absorption spectra of T(6-4)T (dashed curve) and T(Dew)T (solid curve). b) Stationary IR difference spectrum between T(Dew)T and T(6-4)T (shaded area) and transient data from a late probing time of 10 μs (points). c) Transient absorption difference spectra recorded during the first 254 ps. The spectra show the bleaching and partial recovery of the absorption of the C=O stretching bands around 1660 cm^{-1} and the formation of the Dewar marker band at 1780 cm^{-1}. d) The dynamics of the absorption changes are plotted for two probing frequencies $\nu/c = 1664$ cm^{-1} and 1781 cm^{-1}. The transient absorption data were recorded at room temperature and are corrected for the temperature dependency of the solvent D_2O (see the Supporting Information). Different excitation densities are used in the picosecond and nanosecond experiments.

The pronounced IR marker band allowed us furthermore to quantify the photoconversion efficiency η in independent experiments (see the Supporting Information). A remarkably high conversion efficiency of $\eta = (8.2 \pm 2)\,\%$ was measured.[12]

These values show that formation of the Dewar isomer is one of the most efficient light-induced reactions in DNA. For comparison, the quantum yield for the formation of cyclobutane-pyrimidine dimers is only about 1%[12] and the quantum yield for the formation of the T(6-4)T DNA lesions is even lower, at only 0.1% (Scheme 1).[13]

Detailed insight into the mechanism of Dewar formation was obtained by theoretical studies. These studies also enabled us to answer the question of why the opened-backbone (6-4) lesion **5** did not react to form the Dewar lesion. The calculations are based on a hybrid approach,[14] where the properties of the chromophore are investigated by highly correlated ab initio methods, while the forces induced by the confining backbone are treated at a lower quantum mechanical (QM) level. The equations of motion for the nuclei are solved within the on-the-fly framework, including non-adiabatic relaxation pathways, for details see the Supporting Information).[15] We initially performed calculations on T(6-4)T **3** with the methylene linkage and compared the data with the free 5-methyl-2-pyrimidone (**7**, 5M2P), which served as a model for the absorbing chromophore. The chromophore was treated at the MS-CASPT2/CASSCF(12/9) level of theory.[16] The steric effects of the complete dinucleotide **3** were included in the ONIOM method by a QM/QM partitioning approach (see the Supporting Information).[17] The results of the theoretical study are illustrated in Figure 2. After $\pi\pi^*$ photoexcitation, both (6-4) systems **3** and **5** relax towards a minimum in the excited S_1 state. Access to the conical intersections (CoIn), which would allow rapid transfer to the electronic ground state (see Figure 2), is prevented by an energetic barrier,[18] which explains the long lifetime of the excited state of 130 ps. For 5-methyl-2-pyrimidone (**7**), we calculated that a strong out of plane motion of the N3 atom occurs in the excited state of this molecule, while at the same time the C4 atom changes from sp^2 to sp^3 hybridization. This combined process allows the molecule to reach $CoIn_{5M2P}$, from where the compound can reach the ground state (Figure 2).[19] The same motion is possible in the (6-4) dinucleotide **5** with the opened backbone.[20] The C4 rehybridization enabled by the motion of N3 in the excited state allows both compounds **5** and **7** to quickly reach the electronic ground state after photoexcitation. This prevents formation of the Dewar isomer.

We find for the (6-4) lesion **3** with the backbone that this critical motion accompanied by rehybridization can not occur. The backbone clamps the structure so that another conical intersection is reached upon photoexcitation ($CoIn_{Dewar}$). This crossing point to the ground state is located only 0.226 eV below the transition state for the electrocyclic reaction. At $CoIn_{Dewar}$ we find that the (6-4) lesion adopts a strong biradical-like character with strong geometric and electronic similarities to the Dewar isomer. This explains the high quantum yield with which the Dewar lesion is finally formed when the pyrimidone substructure is linked by the backbone to the pyrimidine ring within the (6-4) lesion.

The most important discovery is the observation that the backbone itself controls formation of the Dewar isomer. The ring tension within the rigid macrocycle prevents the $sp^2 \rightarrow sp^3$ rehybridization of the C4 atom, which blocks access to

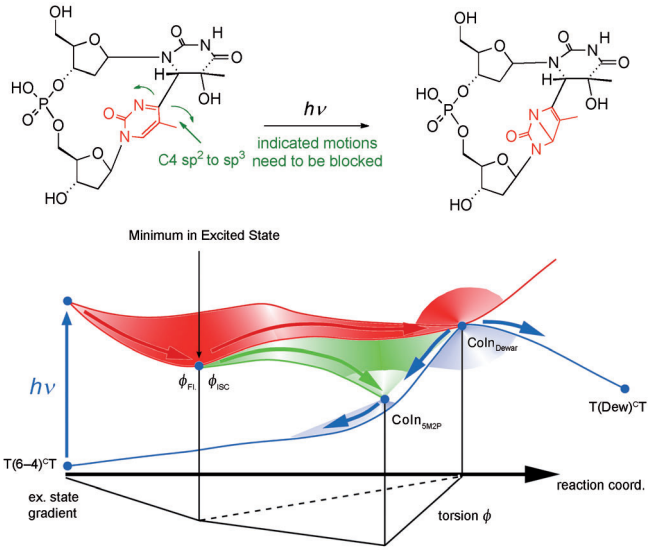

Figure 2. Schematic depiction of the reaction mechanism: The photochemical pathway to the Dewar valence isomer exists only for the (6-4) dinucleotide with an intact backbone structure. The molecules **3**, **5**, and the minimal chromophore **7** initially relax along the same gradient towards the excited-state minimum. The out-of-plane motion of the N3 atom combined with rehybridization at C4 (torsion ϕ) allows the chromophore **7** to reach a conical intersection (green area), which drives the system back to the reactant structure (internal conversion). Actually, this rehybridization is blocked in the presence of an intact phosphodiester or formacetal backbone in **3** (red area). Here, a biradical-like $CoIn_{Dewar}$ formed by an in-phase out-of-plane motion of N3 and C6 serves as the branching point for either internal conversion or photochemical Dewar valence isomerization. This pathway becomes the lowest accessibly conical intersection $CoIn_{Dewar}$ in the (6-4) dinucleotide (molecules **3**) with an intact backbone.

$CoIn_{5M2P}$. Instead, the molecule has to continue on the excited-state surface until it reaches $CoIn_{Dewar}$. At this point, a strong biradical character is obtained, which allows efficient reaction to the Dewar valence isomer. The DNA backbone is the true reason why Dewar lesions can form. Our results show that not only the bases together with the DNA conformation determine lesion formation[13] but that also the DNA backbone itself contributes to UV-induced mutagenesis.

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