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DNA (6-4) Photolyases Reduce Dewar Isomers for Isomerization into (6-4) Lesions

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UV irradiation of DNA causes formation of three different types of DNA lesions, termed CPD, (6-4), and Dewar lesions, depicted in Figure 1.[1] In all cases, two pyrimidines (**1** and **2**) located on top of each other in the DNA duplex react. A [2π+2π] cycloaddition gives rise to cyclobutane pyrimidine dimers (CPD). A Paternó−Büchi reaction yields initially oxetane or azetedine intermediates, which rearrange spontaneously to (6-4) lesions (**3** and **4**). These (6-4) lesions undergo upon irradiation with UV-A/B a 4π sigmatropic rearrangement to give the corresponding Dewar valence isomers T(Dew)T (**5**) and T(Dew)C (**6**).[2] The predominant UV-induced reaction products are the T(CPD)T and T(6-4)T lesions.[3,4] The formed UV induced lesions are in many species the subject of a direct light induced genome repair reaction catalyzed by CPD and (6-4) photolyases, respectively.[5] The mechanism of how these enzymes repair CPD and (6-4) lesions has been investigated over the past years biochemically[1,2,5,6] and with the help of model compounds.[7−11] Co-crystal structures of CPD and (6-4) photolyases in complex with both CPD and (6-4) lesion containing DNA[12−15] reveal the atomic details of the lesion recognition and repair processes. It is known today their repair requires as an initial step a light driven electron transfer from an FADH$^-$ cofactor to the lesion. To date the Dewar repair mechanism is not known. A. Sancar and co-workers revealed that T(Dew)T repair is performed by (6-4) photolyases[16] and also reported that the repair process proceeds with a surprisingly low quantum yield.

Recently we showed that (6-4) photolyases repair T(Dew)C much more efficiently than T(Dew)T lesions.[15] Using DNA substrates with artificial lesions (**8** and **9**) we demonstrate here that Dewar repair requires an electron transfer driven reversal of the 4π sigmatropic rearrangement to give the corresponding (6-4) species. Therefore we irradiated ssDNA oligonucleotides including an internal TpT/C sequence (**1** and **2**) at 254 nm in the glovebox as described previously.[13,15] The oligonucleotides containing T(6-4)T and T(6-4)C were separated by reversed phase HPLC and obtained in purities of >98%. Additionally, this procedure was also carried out with the oligonucleotides (**ODN1**, **2** and **3**) containing a TpC* with a special *N*4-methylcytosine (see Supporting Information (SI), Scheme S1). Again efficient formation of the T(6-4)C* lesion **8** was observed (Figure S1, Table S1). This was followed by subsequent irradiation of the oligonucleotides at 365 nm to convert the (6-4) lesions (**3**, **4**, and **8**) into the corresponding Dewar valence isomers (**5**, **6**, and **9**). In all cases the reaction was quantitative.[15] Full (6-4) to Dewar conversion required 1 h of irradiation in the case of T(6-4)T (**3**), 3 h for T(6-4)C (**4**), and 10 h for the conversion of T(6-4)C* (**8**). This shows that the additional methyl group reduces the (6-4) to Dewar conversion efficiency (see SI, Figure S3). It is important to note that irradiation of all (6-4) lesions provides the corresponding Dewar valence isomers as the stable end products of the irradiation procedure.

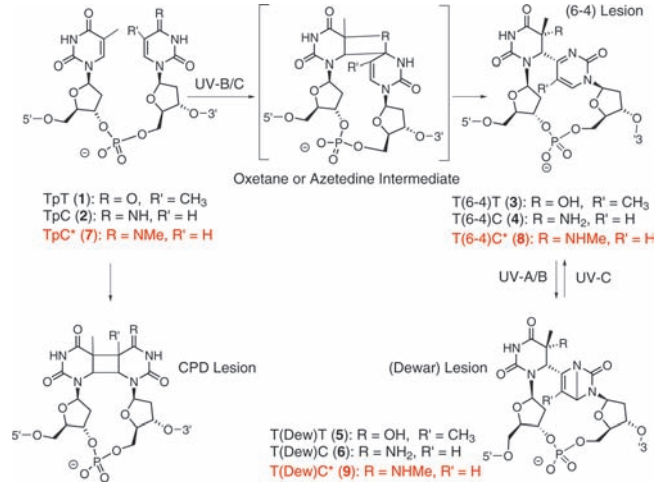

Figure 1. Formation of UV-induced T(6-4)T (**3**), T(6-4)C (**4**), T(6-4)C* (**8**), T(Dew)T (**5**), T(Dew)C (**6**), and T(Dew)C* (**9**) lesions from dipyrimidine sequences T-T (**1**), T-C (**2**), and T-C* (**7**).

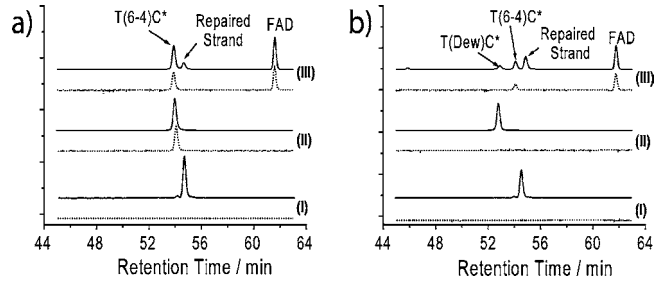

Figure 2. Analysis of the DNA repair reaction by reversed phase HPLC (Solid line detection at 260 nm, brocken line at 325 nm). (a) Repair assay of **ODN 2** containing T(6-4)C* (**8**). (b) Repair assay of **ODN 2D** containing T(Dew)C* (**9**). (I) HPL chromatograms of undamaged DNA. (II) HPL chromatograms of **ODN 2**/**ODN 2D**. (III) HPL chromatograms obtained after addition of (6-4) photolyase followed by irradiation.

To investigate the repair of the different lesions the (6-4) photolyase from *D. melanogaster* was added to the DNA solutions, followed by irradiation with white light. In agreement with literature[15] the lesions T(6-4)T (**3**) and T(6-4)C (**4**) were efficiently repaired by the enzyme.[2,6] Moreover, we observed repair of the T(6-4)C* analogue **8** although higher enzyme concentration and longer reaction times were required (Figure 2a). Next, repair of the Dewar valence isomers **5**, **6**, and **9** was investigated. As expected, addition of the (6-4) photolyase to a solution of T(Dew)C (**6**) containing DNA resulted in the repair of the lesion. Under these conditions, repair of the T(Dew)T DNA could not be observed in agreement with the previously reported low quantum yield.[2] Moreover, the enzyme was also able to repair T(Dew)C* (**9**), and

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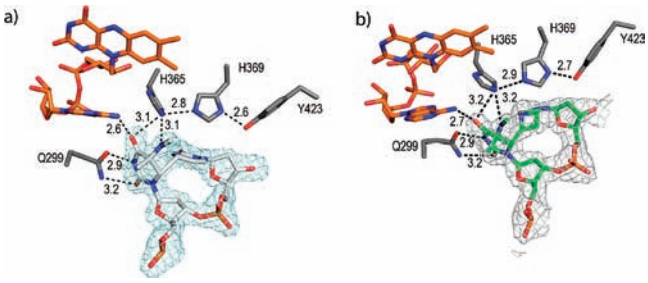

Figure 3. Active site of the (6-4) photolyase in complex with (a) T(6-4)C* and (b) T(Dew)C*. The Fo-*D*Fc omit electron density maps of the lesions are contoured at 3 σ level. Blue N, red O, orange carbon atoms FAD, light gray and green carbon atoms of the T(6-4)C* and (Dew)C* DNA lesions, dark gray carbon atoms of amino acid residues. (See SI, Table S2 for data collection and structure refinement details.)

analysis of the repair assay showed the appearance of a third DNA strand (Figure 2b). Co-injection experiments as well as HPLC-MS studies of the isolated peak after enzymatic digestion of the respected DNA (see SI, Figure S5) confirmed that the signal corresponds to an oligonucleotide containing the T(6-4)C* lesion. Obviously, the slow repair of T(6-4)C* (**8**) by the (6-4) photolyase allows its accumulation as the Dewar repair intermediate.

To further prove that the enzyme converts the Dewar isomers into the respective (6-4) lesions, all three Dewar lesions (**5**, **6**, **9**) containing oligonucleotides were treated with a mutant enzyme (H365N), which is unable to repair (6-4) lesions (see SI, Figure S8).[13] In these experiments we observed the accumulation of the (6-4) lesion intermediate not only for T(Dew)C* but also for T(Dew)C. No T(Dew)T conversion was detected, showing that in this case the Dewar to (6-4) rearrangement is impossible. To investigate if the Dewar to (6-4) conversion requires an electron transfer we repeated the experiments with the wild type (6-4) photolyase, in which the FAD cofactor was kept in the oxidized state. There no Dewar to (6-4) rearrangement was observed (Figure S6). As a control experiment reduced $FADH^-$ was added to the DNA solution and irradiated with white light to rule out that the rearrangement process is triggered by free $FAD/FADH^-$ released by denatured enzyme. Again, no DNA conversion was detected (see SI, Figure S7). The inability of $FADH^-$ alone to induce the rearrangement was confirmed with a model compound study (Figure S8). These experiments show that repair of Dewar isomers requires their conversion into the corresponding (6-4) lesions. The enzyme reverses the 4π sigmatropic rearrangement, leading to formation of the Dewar isomers by electron injection, in agreement with a theoretical prediction by J.-S. Taylor.[17]

To study how the enzyme recognizes the T(6-4)C* and T(Dew)C* lesion analogues in the active site, we crystallized DNA duplexes containing **8** and **9** together with the (6-4) photolyase from *D. melanogaster*. The crystals diffracted X-rays to a 2.0 Å **8** and 2.3 Å **9** spacing, respectively. A view of the active site containing the lesion analogues and the FAD cofactor needed for electron injection is depicted in Figure 3. Both compounds are bound in the active site as previously observed for the natural T(6-4)T and T(6-4)C lesion, with the key amino acid residues involved in the repair reaction and the FAD cofactor correctly positioned for repair (see Figure 3, SI, and Figure S9).

In summary the data presented here show that repair of Dewar valence isomers by (6-4) photolyases involves the rearrangement of the Dewar lesions into the corresponding (6-4) lesions. This reaction requires electron injection. Consequently (6-4) photolyases have two catalytic functions: They not only split (6-4) lesions but also catalyze the formal 4π sigmatropic rearrangement of Dewar isomers to (6-4) lesions. If efficient electron injection into the Dewar isomers requires an electron deficient ring system, which might be established by a protonated amino group in C containing lesions, the slow T(Dew)T repair could be understood as a result of an unfavorable electron transfer step.

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Supporting Information Available: Experimental details, Figures S1−S9, Scheme S1, and Tables S1 and S2. This material is available free of charge via the Internet at http://pubs.acs.org.

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