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Excess Electron-Transfer-Based Repair of a Cis-Syn Thymine Dimer in DNA Is Not Sequence Dependent

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Oxidative damage to DNA is initiated by the formation of guanine radical cations because guanine has the lowest oxidation potential among all nucleobases.[1] For more than a decade, it has been clear that the positive charge, deposited on a guanine, can move through DNA over large distances, giving final DNA damage far away from the initial oxidation site.[2] Recent detailed investigations[3,4] have shown that the positive charge hops through DNA in a thermally activated process using guanines as intermediate charge carriers (guanine hopping).[5] Hopping of the positive charge through A:T sequences is also fast, but initial oxidation of A by a G radical cation is slow due to the higher oxidation potential of this base.[6] The result is a strong sequence dependence of the DNA damaging process.

The transfer of a negative charge through DNA, in contrast, has been studied only recently.[7−10] This process is, however, important for DNA repair because DNA-photolyases inject electrons into UV-induced thymine dimer lesions to trigger a cycloreversion reaction leading to DNA repair.[11] To investigate this excess electron driven repair reaction, we reported recently about the site specific incorporation of a flavin electron donor and a thymine dimer acceptor into DNA double strands[12] and DNA hairpins.[13] Investigation of the excess electron-transfer driven repair of the thymine dimer in these systems supported a suggestion by Giese that excess electrons hop through DNA using pyrimidines as stepping stones.[14] We could show that excess electron hopping in DNA is only weakly distance dependent.[12,13,15] This result was recently supported by S. E. Rokita using a tetramethyl-diaminonaphthalene donor and a BrdU as the electron acceptor.[10]

Herein, we provide the first information of how the sequence between the dimer and the flavin influences the electron-transfer driven repair process. We observe that the repair of a thymine dimer by an excess electron transfer over distances between 13.6 and 17.0 Å is independent of the intervening base sequence.

For the investigation, we prepared the five DNA hairpins **1**−**5** depicted in Scheme 1. They contain as the head of the hairpin the flavin **6**,[15] which in its reduced, deprotonated, and light excited state functions as a strong electron donor ($E_{red}* = -2.6$ V against NHE)[16] able to reduce all nucleobases. This electron donor is also used by DNA-photolyases to repair thymine dimers. The acceptor molecule is the cyclobutane thymine dimer **7**,[17] containing an opened backbone. **7** translates the single electron reduction into a readily detectable strand break. DNA hairpins were chosen for the investigation because they allow analysis of an electron-transfer event in well-defined structures, even over short distances due to their high and concentration-independent melting behavior.[15,18] All melting points of the used hairpins are listed in Table 1.

The DNA hairpins **1**−**5** (Scheme 1) possess a different base sequence. Hairpins **1** and **4** contain a homo-A:T stretch between the flavin donor and the dimer acceptor. In hairpins **2** and **3**, one of the A:T base pairs is replaced by a G:C base pair at different positions. Finally, in hairpin **5**, the flavin donor and the dimer acceptor are separated by a homo-G:C stretch.

The distance between the donor and the acceptor in the hairpins **1**−**3** is about 13.6 Å. This distance increased in hairpins **4** and **5** to about 17.0 Å, which ensures that the electron transfer proceeds by charge hopping, where the intermediate base pairs function as charge carriers.[19,20]

For the dimer cleavage measurements, all DNA hairpins **1**−**5** were irradiated separately ($c_{DNA} = 20$ μM, 0.01 M Tris, pH = 7.4, 0.15 M NaCl) in fluorescence cuvettes with a 1000 W Xe-lamp, equipped with a cooled 360 nm cutoff filter. Analysis of the dimer cleavage yield was performed as recently described.[12,15] In short,

Scheme 1. Depiction of the Flavin Electron Donor **6** and of the Dimer Acceptor **7**, Together with the Prepared Flavin (Green)- and TT (Red)-Dimer-Containing DNA Hairpins **1**−**5**

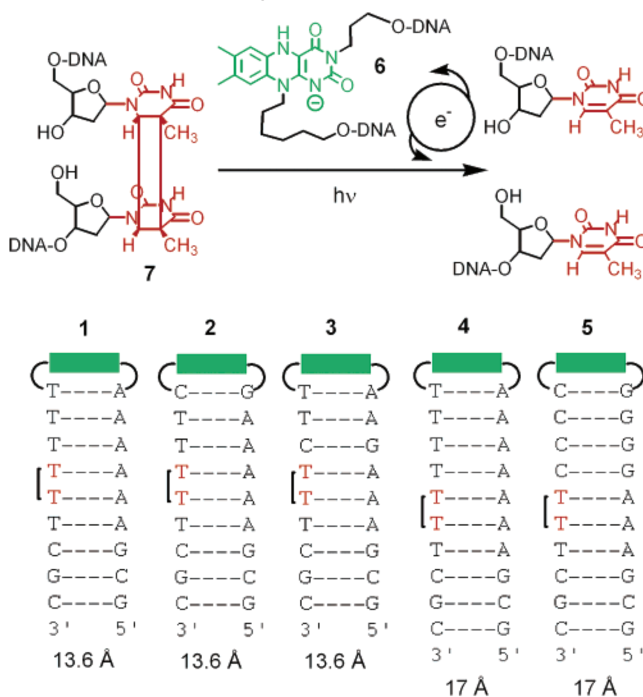

Table 1. Melting Points of the Hairpins **1**−**5** ($c_{DNA} = 3$ μM, 0.01 M Tris, pH = 7.4, 0.15 M NaCl)[a]

	hairpin				
	1	2	3	4	5
mp [°C]	45	48	46	40	50
yield [%/min][b]	4.2	4.3	3.9	2.5	3.0

[a] The distance between the flavin and the dimer was estimated assuming ideal B-conformation of the hairpin stems. The calculated yields of dimer cleavage are given as % cleavage per minute. [b] ±20% as determined from three independent measurements. The data were determined from the first 5 min of irradiation after linear approximation of the data.

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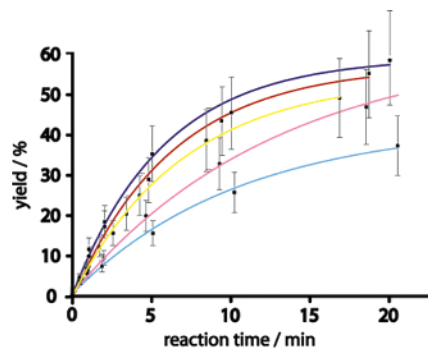

Figure 1. Depiction of the time-dependent formation of the cleaved DNA strands cut at the dimer site. Light blue = **4**, pink = **5**, yellow = **3**, red = **2**, blue = **1**.

10 μL samples were removed from the assay solution after defined time intervals, aerated for 2 h, and analyzed by ion-exchange chromatography. The time-dependent formation of the cleaved product strands is shown in Figure 1. The obtained yield data listed in Table 1 represent averaged values obtained from at least three independent experiments. All irradiations were performed well below the individual melting temperatures at 4 °C. From these independent experiments, we could determine the errors of the measurements to be ≤20%.

The data show that dimer "repair" proceeds efficiently in all investigated hairpins **1**−**5**. In agreement with earlier studies, dimer cleavage is only about 30% slower in hairpins **4** and **5** (light blue and pink curves), showing again the weak distance dependence of the repair reaction. Important for this study, however, is the observation that the electron is able to travel efficiently over a distance of even 17 Å, which is only possible by hopping.[19]

The data obtained from hairpins **1**−**3** (flavin dimer distance = 13.6 Å) indicate that the sequence between the flavin and the dimer does not influence the dimer splitting yield. All three hairpins give very similar repair yields in the experiment. Also interesting is the direct comparison of the repair efficiency through the homo-A:T sequence in **4** and the homo-G:C sequence in **5**. We clearly observe no strong sequence effect here too. In fact, repair through a homo-A:T stretch gives reproducibly slightly slower yields. The reason for this effect could be a more flexible hairpin structure, which would be in agreement with the lower melting point measured for hairpin **4**. This argument would be in line with data from experiments performed with flavin- and dimer-containing DNA: PNA heteroduplexes, which also showed that decreasing the duplex integrity reduces the repair yield.[21]

The surprising result reported in this study is that the reductive cleavage of a thymine dimer in DNA hairpins is not sequence dependent! In hairpins **1**−**3**, replacement of an A:T base pair by a G:C base pair does not influence the repair yield. However, to draw any conclusions about the electron-transfer event from the reported repair data, we have to define the kinetic regime, observable with our system.

In the case of oxidative hole transfer, it was estimated that the positive charge hops between a G and a GG separated by one A:T base pair with a rate constant between $k_{hop} = 10^6-10^8\ s^{-1}$.[22] Hopping between adjacent adenines as charge carriers is believed to be faster with $k_{hop} > 10^8\ s^{-1}$ over a distance range of 7−22 Å.[23] The competing charge eliminating reaction of the guanine radical cation with water is significantly slower ($k_{water} = 6 \times 10^4\ s^{-1}$).[5]

In our system, the rate of the dimer splitting reaction is not well defined. The dimer splitting rate is, however, likely to be between $k_{split} = 10^6-10^9\ s^{-1}$.[16,24,25] If we assume that electrons hop as fast over pyrimidines as holes over purines, than dimer cleavage is comparable to excess electron hopping. If we assume that the excess electron transfer through A:T and G:C base pairs is likely faster than $10^8\ s^{-1}$, we can explain our result with a partially rate-determining dimer opening reaction. We can conclude that excess electron transfer through DNA is a surprisingly fast process, faster than the opening of the cyclobutane ring of UV-induced DNA lesions.

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