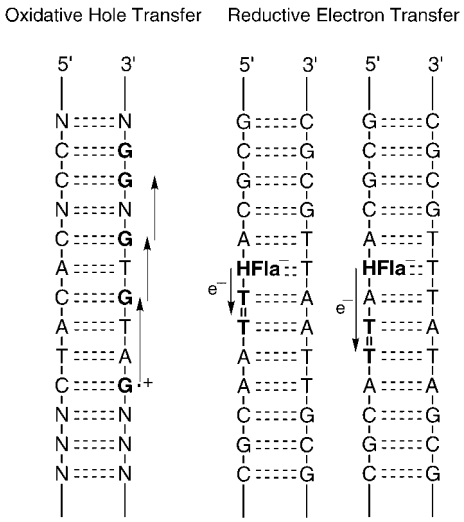
Self-Repairing DNA Based on a Reductive Electron Transfer through the Base Stack**

Anja Schwögler, Lars T. Burgdorf, and Thomas Carell*

DNA photolyases utilize light energy to initiate the repair of highly mutagenic UV-induced cyclobutane pyrimidine dimers that form the major photolesions in DNA.[1, 2] The basis of the repair reaction, which rescues many insects, fish, amphibians, and plants from UV-induced cell death and mutagenesis,[3] is a light-induced electron transfer from a reduced and deprotonated flavin coenzyme to the DNA lesion.[4] The lesion undergoes a spontaneous cycloreversion as its radical anion to the corresponding monomers.[5] Although the general mechanism of the light-driven repair process is known, no information is currently available about the critical electron-donation process from the flavin donor to the dimer acceptor in the DNA strand.[6] In particular, the question as to what extent the DNA double strand is able to mediate the transport of the electron in the base stack is still under debate (Scheme 1).[7] This question is directly linked to

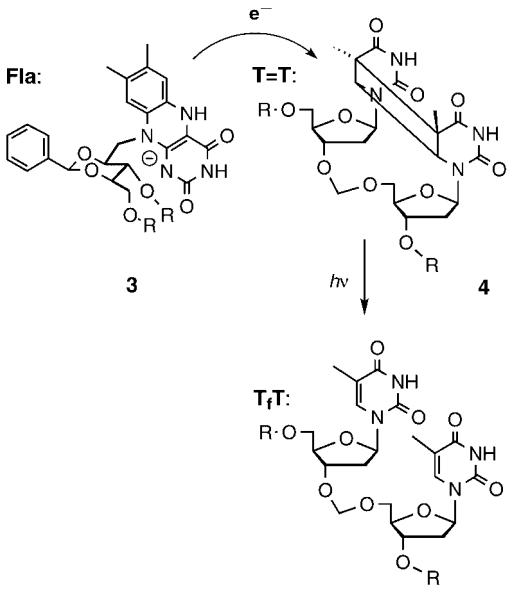


Scheme 1. Schematic depiction of the hole-transfer process (oxidative electron transfer) via guanosine bases and of the proposed reductive electron-transfer event leading to the self-repair of the DNA.

investigations of the electron hole transport properties of DNA.[8] Hole transfer was recently shown to proceed over relatively large distances in an undisturbed DNA double strand.[9, 10] Experiments carried out recently provided compelling evidence that a hopping process in which guanosine bases (which react to form guanosine radical cations) act as stepping stones in the DNA double helix (Scheme 1) could be one basis for the seemingly "distance independent" hole transfer.[11–15]

A deeper understanding of oxidative damage to DNA and the design of DNA-based bioanalytical devices is crucially dependent upon the elucidation of the electron- and hole-transfer properties of double-stranded DNA.[16, 17] Herein we report the preparation of DNA strands containing a flavin building block and a cyclobutane thymidine dimer lesion.[18, 19] These doubly modified DNA strands show light-induced self-repairing properties and allowed insight to be gained into the ability of DNA to mediate a reductive ("surplus") electron-transfer reaction.[20–22]

The oligonucleotides **1** and **2** were synthesized for studying the reductive self-repairing electron transfer and the repair process (Scheme 2). All the DNA double strands investigated contain the riboflavin coenzyme nucleobase **3** and the thymidine dimer DNA lesion **4** as central units. The synthesis and the incorporation of both building blocks into DNA was performed as recently described.[18, 19, 23] The melting temperatures and fluorescence spectra of the double strands were measured to investigate whether the flavin and dimer



1T	5'-d(GCGCA-**Fla**-**T=T**AACGC)-3' 3'-d(CGCGT-T — A-ATTGCG)-5'
1C	5'-d(GCGCA-**Fla**-**T=T**AACGC)-3' 3'-d(CGCGT-C-A-ATTGCG)-5'
1G	5'-d(GCGCA-**Fla**-**T=T**AACGC)-3' 3'-d(CGCGT-G-A-ATTGCG)-5'
1A	5'-d(GCGCA-**Fla**-**T=T**AACGC)-3' 3'-d(CGCGT-A-A-ATTGCG)-5'
2T	5'-d(GCGCA-**Fla**-A**T=T**ACGC)-3' 3'-d(CGCGT-T-TA-ATGCG)-5'
2C	5'-d(GCGCA-**Fla**-A**T=T**ACGC)-3' 3'-d(CGCGT-C-TA-ATGCG)-5'

Scheme 2. Electron-transfer initiated cycloreversion of an analogue of the thymidine dimer lesion in DNA. The six flavin- and dimer-modified DNA double strands are also indicated. R shows the continuation of the DNA strands.

[*] T. Carell, A. Schwögler, L. T. Burgdorf
Fachbereich Chemie
Philipps-Universität Marburg
Hans-Meerwein-Strasse, 35032 Marburg (Germany)
Fax: (+49) 6421-2822189
E-mail: carell@mailer.uni-marburg.de

[**] We thank the University of Marburg and the Volkswagenstiftung for financial support. The Boehringer Ingelheim Fonds is gratefully acknowledged for a scholarship to L.T.B.

  1433-7851/00/3921-3918 $ 17.50+.50/0

containing oligonucleotides[24] form stable double-stranded DNA, which was found to be crucial for the transfer of a hole through DNA. Figure 1 A shows as an example the melting curves determined at 275 nm for the flavin- and dimer-containing double strand **2T**. The normal melting behavior

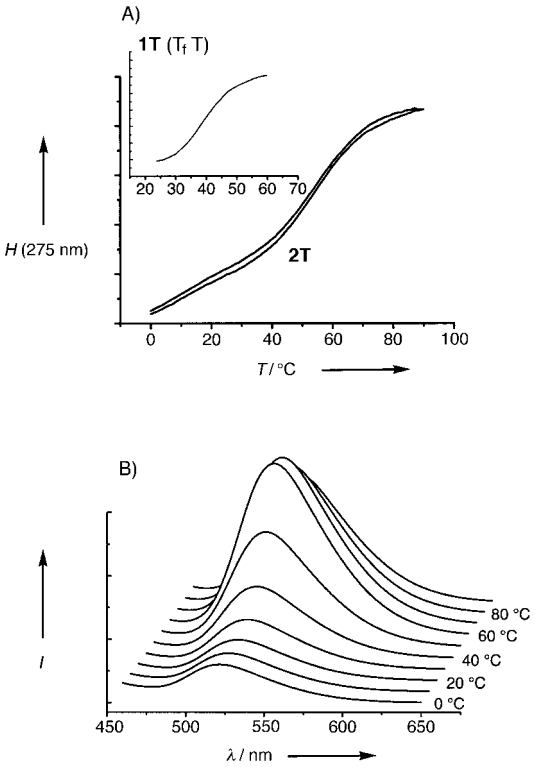


Figure 1. A) Melting curve of **2T** showing both the heating and the cooling curve measured at 275 nm ($c_{DNA} = 3$ μM, 150 mM NaCl, 0.01M Tris pH 7.5). The inset depicts the melting curve of **1T** at 460 nm. This double strand has a Fla-T "base pair" and a formacetal-linked thymidyl and thymidine bases (T_fT; $c_{DNA} = 30$ μM, 0.01 M Tris, pH 7.5, 150 mM NaCl). B) Dependence of the fluorescence of strand **1T** on temperature

of the doubly modified DNA double strands is clearly evident; no hysteresis between the heating and the cooling curve is observed. Double-strand melting was also detectable at 460 nm, where only the flavin coenzyme absorbs light (Figure 1 A inset). This hypochromicity effect of the flavin base supports our assumption that the coenzyme is positioned inside the DNA double helix.[18] The melting points of all flavin-containing DNA strands were found to depend on the flavin counterbase chosen; for example, the following melting points were measured for strand **1** with the various counterbases: **1T**: 58 °C, **1C**: 56 °C, **1G**: 51 °C, and **1A**: 53 °C ($c_{DNA} =$ 3 μM, 150 mM NaCl, 0.01M Tris pH 7.5). The highest melting points were measured when thymidine and cytidine were used, and hence were consequently chosen as the flavin counterbases in this study.

Further evidence for the presence of the flavin in the DNA-base stack was obtained from fluorescence studies. The fluorescence of the flavin in a double strand is strongly quenched if a guanosine acts as the counterbase (**1G**). The reason for the decreased fluorescence is that a light-induced electron transfer occurs from the guanosine to the flavin, which yields a transient $G^{\bullet+} \cdots F1a^{\bullet-}$ zwitterion. The reduced fluorescence is consistent with the hypothesis that both heterocycles are in contact, which is only possible if the flavin is positioned in the DNA stack. Furthermore, studies of the temperature dependence of the fluorescence of the DNA double strand **1G** show a strong increase in the flavin fluorescence upon melting the double strand (Figure 1 B). The hypochromicity observed in the UV-melting experiments and the temperature-dependence of the fluorescence data provide evidence for our assumption that the flavin coenzyme is positioned inside the DNA double helix.

In order to investigate the reductive electron transfer from the flavin to the dimer in the DNA base stack the DNA strands **1T**, **1C**, **2T**, and **2C** were dissolved in Tris buffer (pH 7.5, 150 mM NaCl) and transferred into fluorescence cuvettes and stoppered with a rubber septum. After purging the cuvettes with nitrogen for 10 min, sodium dithionite solution was added to reduce and deprotonate ($pK_a = 6.8$) the flavin coenzyme. The fluorescence arising from the flavin disappeared after reduction. This was monitored during irradiation to ensure complete reduction of the flavin during the whole experiment.[25] The fluorescence cuvettes were finally exposed to monochromatic light in a fluorimeter at 360 nm or to daylight. Nitrogen was slowly bubbled through the DNA solution during its exposure to light to ensure the irradiation conditions were homogenous. Small aliquots were removed from the assay solution with a microsyringe and immediately reoxidized by shaking the samples in the presence of air. Analysis of the samples by reversed-phase HPLC (Figure 2) showed that the dimer lesions in the DNA

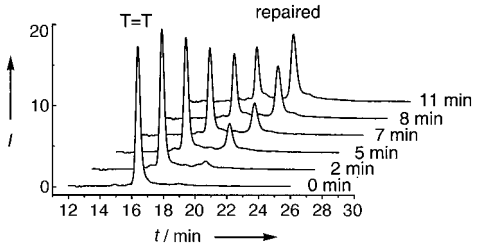


Figure 2. HPLC traces of the aliquots taken during an irradiation experiment with strand **2C** showing the clean conversion of the damage-containing DNA strand into the repaired strand.

double strands were cleanly repaired. The appearance of only one new oligonucleotide was detected, which was proved to be the repaired DNA strand by co-injection. It is clear from Figure 2 that the photo reaction induces a self-repair of the lesion-containing DNA strand into the repaired strand. No other oligonucleotides are formed, which means that side reactions causing DNA degradation do not occur. In fact, complete self-repair of the lesion-containing oligonucleotides under our conditions was accomplished after just 30 min of irradiation. No repair was observable in the absence of light.[26–28] Further experiments, in which a flavin-containing DNA strand was reduced and irradiated in the presence of a DNA strand containing a noncomplementary thymine dimer showed no repair either. Both control experiments prove that the self-repair process is a light-driven reaction that proceeds intramolecularly inside the DNA double strand.

  1433-7851/00/3921-3919 $ 17.50+.50/0

To obtain insight into the ability of DNA to mediate the reductive electron-transfer process all four DNA double strands **1T**, **1C**, **2T**, and **2C**, which contain either a Fla-T or a Fla-C base pair and two different flavin–dimer distances, were investigated for their self-repairing capabilities. The data (Table 1) show that the double strands **1T** and **2T** having a Fla-T base pair (highest melting points) show a faster self-repair than **1C** and **2C**, possibly because they form the most undisturbed double helices.

Table 1. Repair yield data (±10%) and quantum yields (±50%) of the photo process in the DNA strands **1T**, **1C**, **2T**, and **2C**.[a]

DNA double strand	Repair yield (5 min) [%]	Quantum Yield Φ
1T 5′-d(GCGCA-Fla-T=TAACGCG)-3′ 3′-d(CGCGT-T-A ATTGCGC)-5′	35	0.004
1C 5′-d(GCGCA-Fla-T=TAACGCG)-3′ 3′-d(CGCGT-C-A ATTGCGC)-5′	35	0.004
2T 5′-d(GCGCA-Fla-AT=TACGCG)-3′ 3′-d(CGCGT-T-TA ATGCGC)-5′	20	0.003
2C 5′-d(GCGCA-Fla-AT=TACGCG)-3′ 3′-d(CGCGT-C-TA ATGCGC)-5′	15	0.002

[a] $c_{DNA} = 40\ \mu M$, 360 nm, 150 mM NaCl, 0.01M Tris, pH 7.5, the photon flux was measured with a United Detector Technology Dual Channel Optometer, $\varepsilon_{flavinH^-} = 4900\ L\,M^{-1}\,cm^{-1}$.

In addition, the repair of the double strands **1T** and **1C** were found to proceed to about 35% completion after 5 min. This is just a factor of two more efficient than **2T** and **2C** (20 and 15%, respectively), in which an additional A-T base pair separates the electron-transfer partners. This small difference is a very surprising result to us, because the distance between the flavin and the dimer unit increases from 11 (in **1T** and **1C**) to 17 (in **2T** and **2C**) single bonds. In the case of the oxidative hole transfer in DNA such an additional A-T base pair between the electron hole and the next donating guanosine base would result in an approximately tenfold decrease in the reaction yield.[11]

The data show that a reductive electron transfer through the base stack from a flavin electron donor to a UV dimer lesion, as performed by the enzyme DNA photolyase, is possible.[22, 29] The light-driven electron transfer leads exclusively to the cycloreversion of the dimer lesion. This process is effective even when the DNA lesion and the flavin donor are separated by an intervening base pair. The results open a new avenue for the development of the therapeutic repair of thymine dimers and shows that the arming of DNA with flavin coenzymes allows functional oligonucleotides with, in our case, self repairing properties to be created.

Received: July 31, 2000 [Z15558]

[1] A. Sancar, *Biochemistry* **1994**, *33*, 2–9.
[2] T. Carell, *Angew. Chem.* **1995**, *107*, 2697–2700; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2491–2494.
[3] J.-S. Taylor, *Acc. Chem. Res.* **1994**, *27*, 76–82.
[4] P. F. Heelis, R. F. Hartman, S. D. Rose, *Chem. Soc. Rev.* **1995**, 289–297.
[5] T. Carell, R. Epple, *Eur. J. Org. Chem.* **1998**, 1245–1258.
[6] F. D. Lewis, X. Liu, Y. Wu, S. E. Miller, M. R. Wasielewski, R. L. Letsinger, R. Sanishvili, A. Joachimiak, V. Tereshko, M. Egli, *J. Am. Chem. Soc.* **1999**, *121*, 9905–9906.
[7] J. Jortner, M. Bixon, T. Langenbacher, M. E. Michel-Beyerle, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 12759–12765.
[8] F. D. Lewis, T. Wu, Y. Zhang, R. L. Letsinger, S. R. Greenfield, M. R. Wasielewski, *Science* **1997**, *277*, 673–676.
[9] D. B. Hall, R. E. Holmlin, J. K. Barton, *Nature* **1996**, *382*, 731–735.
[10] R. E. Holmlin, P. J. Dandliker, J. K. Barton, *Angew. Chem.* **1997**, *109*, 2830–2848; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2715–2730.
[11] E. Meggers, M. E. Michel-Beyerle, B. Giese, *J. Am. Chem. Soc.* **1998**, *120*, 12950–12955.
[12] B. Giese, S. Wessely, M. Sporman, U. Lindemann, E. Meggers, M. E. Michel-Beyerle, *Angew. Chem.* **1999**, *111*, 1050–1052; *Angew. Chem. Int. Ed.* **1999**, *38*, 996–998. Since the nucleobases T and C possess a similar reduction potential, which is more positive than A and G, these authors postulate a sequence-independent electron-hopping process from a donor to an acceptor attached to the DNA strand. For such a system see ref. [20].
[13] A phonon-assisted polaron-hopping model to explain charge transport through DNA has been forwarded by: D. Ly, L. Sanii, G. B. Schuster, *J. Am. Chem. Soc.* **1999**, *121*, 9400–9410; P. T. Henderson, D. Jones, G. Hampikian, Y. Kan, G. B. Schuster, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 8353–8358; see also G. B. Schuster, *Acc. Chem. Res.* **2000**, *33*, 253–260.
[14] F. D. Lewis, X. Liu, J. Liu, S. E. Miller, R. T. Hayes, M. R. Wasielewski, *Nature* **2000**, *406*, 51–53.
[15] S. O. Kelley, J. K. Barton, *Science* **1999**, *283*, 375–381.
[16] W. K. Pogozelski, T. D. Tullius, *Chem. Rev.* **1998**, *98*, 1089–1107.
[17] D. J. Caruana, A. Heller, *J. Am. Chem. Soc.* **1999**, *121*, 769–774.
[18] A. Schwögler, T. Carell, *Org. Lett.* **2000**, *2*, 1415–1418.
[19] J. Butenandt, A. P. M. Eker, T. Carell, *Chem. Eur. J.* **1998**, *4*, 642–653.
[20] T. J. Meade, J. F. Kayyem, *Angew. Chem.* **1995**, *107*, 358–360; *Angew. Chem. Int. Ed.* **1999**, *34*, 352–354.
[21] M. G. Debije, M. T. Milano, W. A. Bernhard, *Angew. Chem.* **1999**, *111*, 2926–1231; *Angew. Chem. Int. Ed.* **1999**, *38*, 2752–2755.
[22] S. Steenken, *Biol. Chem.* **1997**, *378*, 1293–1297.
[23] J. Butenandt, L. T. Burgdorf, T. Carell, *Synthesis* **1999**, 1085–1105.
[24] Several detailed NMR studies prove that the dimer lesion is positioned inside the DNA double helix. See for example: J. Kemmick, R. Boelens, T. Koning, G. A. van der Marel, G. A. van Boom, R. Kaptein, *Nucleic Acids Res.* **1987**, *15*, 4645–4653; K. McAteer, Y. Jing, J. Koa, J.-S. Taylor, M. A. Kennedy, *J. Mol. Biol.* **1998**, *282*, 1013–1032; J.-K. Kim, D. Patel, B.-S. Choi, *Photochem. Photobiol.* **1995**, 62, 44–50.
[25] Reduction of the flavin in the DNA environment is fully reversible. Shaking the assay solution in the presence of air caused complete reoxidation accompanied by the reoccurrence of the flavin fluorescence.
[26] The repair of a thymine dimer as its radical cations with a rhodium intercalator or a naphthalinediimide, which both remove light-induced an electron from the DNA strand has been reported by P. J. Dandliker, R. E. Holmlin, J. K. Barton, *Science* **1997**, *275*, 1465–1468; D. A. Vicic, D. T. Odom, M. E. Núñez, D. A. Gianolio, L. W. McMaughlin, J. K. Barton, *J. Am. Chem. Soc.* **2000**, *122*, 8603–8611, and ref. [27]. Most recent studies question oxidative dimer repair in DNA.[28] Our own results show that irradiation of DNA strands containing the nonreduced flavin, which also abstracts an electron from the DNA, gives no detectable dimer repair.
[27] P. J. Dandliker, M. E. Núñez, J. K. Barton, *Biochemistry* **1998**, *37*, 6491–6502.
[28] A. K. Dotse, E. K. Boone, G. B. Schuster, *J. Am. Chem. Soc.* **2000**, *122*, 6825–6833.
[29] Radiolysis studies show that thymidine and cytidine radical anions are most likely formed after the addition of an electron since they are the nucleobases with the most positive reduction potentials. Rapid protonation of the cytidine radical anion ($pK_a > 13$) is a highly favorable reaction and should strongly compete with a potential electron-hopping process. See for example ref. [22] and S. Steenken, J. P. Telo, H. M. Novais, L. P. Candeias, *J. Am. Chem. Soc.* **1992**, *114*, 4701–4709. Electron transfer from a thymidine or cytidine radical anion to the dimer should be an endergonic reaction because of the more negative reduction potential of the dimer unit: S.-R. Yeh, D. E. Falvey, *J. Am. Chem. Soc.* **1992**, *114*, 7313–7314. Further studies are now needed to explain the basis for the self-repair process.

  1433-7851/00/3921-3920 $ 17.50+.50/0