Electron Transfer in DNA

Excess Electron Transport Through DNA: A Single Electron Repairs More than One UV-Induced Lesion**

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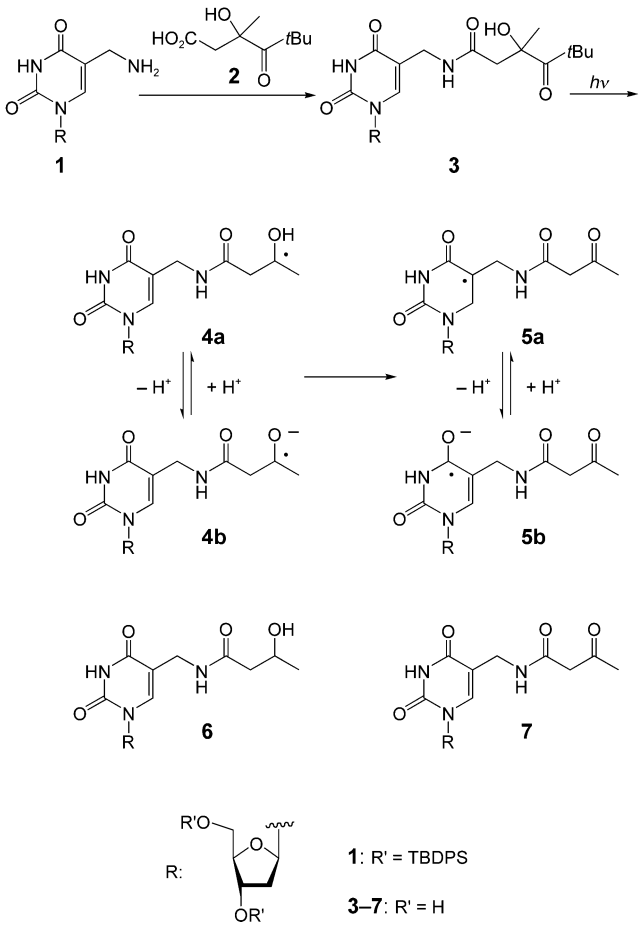
The investigation of long-distance charge transport through DNA is complicated because experimental results depend upon the charge injection and charge detection systems used. The rate of charge injection into a DNA base depends on the redox potential of the injection system and also on the structure of the DNA base since distortions of the base may change its orbital overlap pattern and solvation energy.[1] The nature of the charge detection system used to analyze the arriving charge after its multistep transport through DNA is also of crucial importance in studies on long-distance charge transport. The influence of factors such as sequence on the charge-transport rate can be measured experimentally only if the charge detection is the fastest step of the whole process. In assays where detection is approximately as fast as, or slower than the charge transfer, the experimental results also reflect the equilibration of the charge over the DNA bases according to the Curtin–Hammett principle.[2] This situation has been discussed in detail for long-distance hole transport through DNA.[3]

Although hole transport through DNA is now rather well understood, little is known about the transport of negative charge (an extra electron) through the DNA double helix. Recent observations have show that electrons also travel over significant distances through DNA by a hopping process in which pyrimidine bases act as temporary charge carriers.[4] Experiments to investigate the sequence-dependence of such extra electron transfer through DNA give conflicting results. Although almost no sequence effect was observed in one study,[5] another study[6] showed that an extra electron moves more efficiently through A:T than through G:C base pairs. It is clear that experiments on long-distance electron transport through DNA lead to different results if different assay systems are used. Herein, we try to explain the different results with the help of the new injection system **3**. In contrast to the electron injectors used in the previous studies,[4–6] the injector **3** transfers only one extra electron into the DNA double strand upon irradiation. The basis for the design of the new injector is the less-negative redox potential of thymine[7] compared to those of dialkyl ketones.[8] Thus, a ketyl radical anion should reduce an adjacent thymine base. We therefore synthesized the thymidine derivative **3** by attaching the ketyl radical precursor **2** to a thymine base (Scheme 1).[9]



Scheme 1. Synthesis and reaction of the modified thymine **3**. TBDPS = *tert*-butyldiphenylsilyl.

Photolysis of ketone **3** at 75 K gave a product that exhibited the ESR spectrum of the thymine-based radical **5a** (Figure 1).[10] The identity of the compound could be deduced by comparing our measurements to a simulated spectrum with hyperfine coupling constants of 39.2 and 10.5 G for the two adjacent CH_2 groups. These coupling constants are similar to those observed for the unsubstituted thymine radical in frozen solution.[11]

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[**] We thank the Swiss National Science Foundation (NCCR), the German Science Foundation, the Volkswagen Foundation, and the Fonds der Chemischen Industrie. T.C. is grateful for a Kékulé stipend.

  DOI: 10.1002/ange.200353264

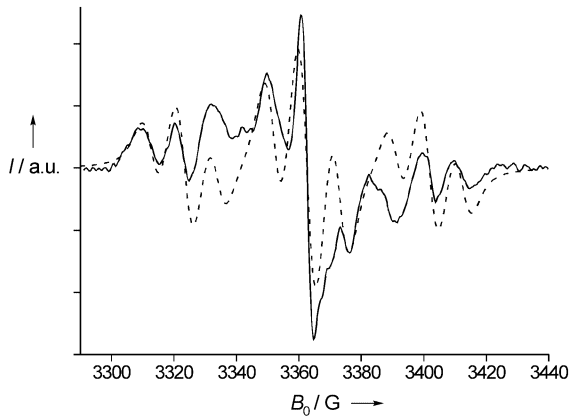
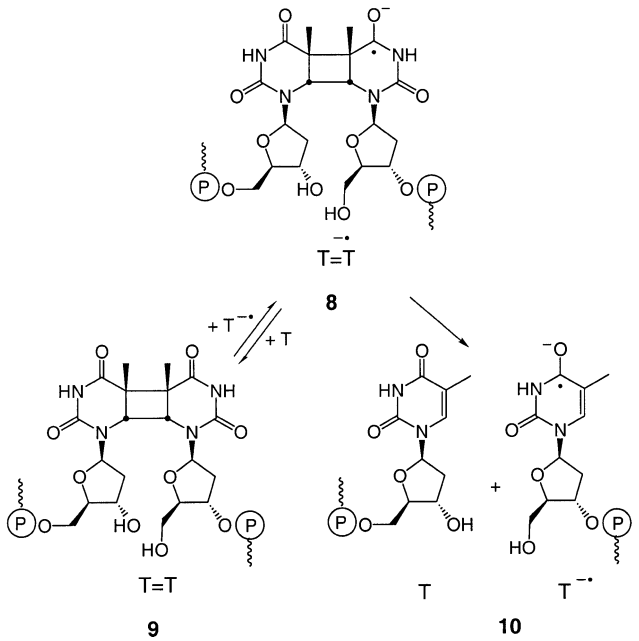


Figure 1. Continuous wave X-band ESR spectrum of **5a** in CH_3CN at 77 K. The dashed line represents the simulation.

The formation of **5a** can be explained as the result of a Norrish cleavage[12] of the *tert*-butyl ketone **3** and subsequent electron transfer to the thymine system (**4**→**5**).[13] The intermediate radicals **4a**,**b** were detected by addition of the H-donor glutathione, which resulted in formation of alcohol **6** in up to 30% yield.[14] In the absence of glutathione, ketone **7** was formed as the main product (90%).

We used the open-backboned thymine dimer **9** (T=T; Scheme 2) as the electron detection system in our experiments.[4] Single-electron capture by the dimer induces a cycloreversion leading to a strand break (**9**→**10**).[4] The intermediate in this process is the dimer radical anion **8**.[15] To measure the influence of distance on the transport of an electron through DNA we incorporated the electron injector **3** (T_X) and the dimer **9** (T=T) into DNA single strands at various sites and hybridized these strands with slightly longer complementary strands. In this way, we synthesized the modified double strands **11a–c** (Figure 2).[16] Norrish photolysis of the new electron injector **3** results in donation of a single electron to the DNA duplex. The electron travels through the base stack and eventually cleaves the cyclobutane ring of the thymine dimer. This process leads to the formation of the shorter DNA strands **12** and **13** in a 1:1 ratio.[17] The cleavage yield decreased from 14 to 7 to 5% when the number (n) of adenine:thymine $(A:T)_n$ base pairs between the electron injection and detection systems was increased from one to three. This decrease in yield is typical of a multistep reaction in which the electron hops between adjacent thymine bases,[18] and is in full accord with the data reported previously.[4]



Scheme 2. Competition between cleavage and electron transfer for thymine radical anion **8**.

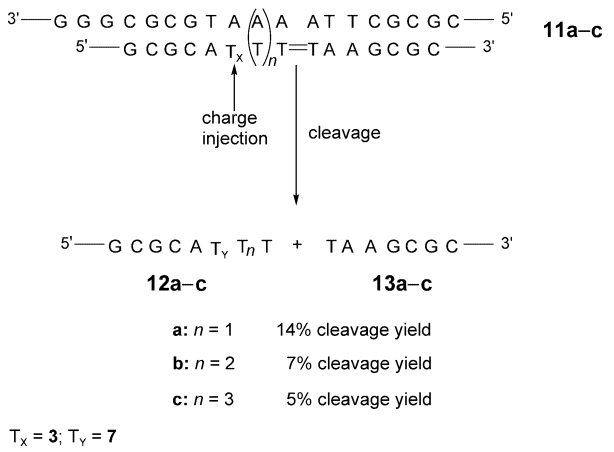


Figure 2. The efficiency of the cleavage of the thymine dimer in DNA double strands **11a–c**. Electron injection occurs through photolysis of the modified nucleotide **3**.

To investigate how the dimer cleavage process competes with the charge movement, we prepared the double strand **14**, which contains two thymine dimers separated by a single A:T base pair (Figure 3). According to the suggested cleavage mechanism (Scheme 2), the negative charge is not annihilated after the first cycloreversion and should, therefore, be able to cleave another thymine dimer (T=T). Irradiation of double

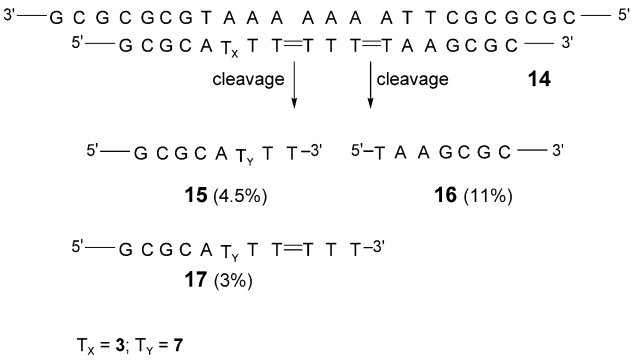


Figure 3. Cleavage of proximal and distal thymine dimers after photolysis of DNA double strand **14**.

strand **14** indeed resulted in cleavage at both the proximal and the distal thymine dimer site and formation of the shorter strands **15** and **16**.[17] Surprisingly, the cleavage yield at the distal site (**16** = 11 %) was more than twice that at the proximal site (**15** = 4.5 %).[19]

If an electron migrates to the distal thymine dimer only after the proximal dimer has been cleaved (Scheme 2), then the yield ratio **16**/**15** cannot be larger than 1.0:1. The observed **16**/**15** ratio of 2.4:1 indicates that a second scenario for electron transport to the distal thymine dimer must exist in which the proximal thymine dimer is not cleaved. This observation was confirmed by detection of the cleavage product **17** (3 ± 1 %), in which the proximal thymine dimer is intact but the distal dimer has been cleaved. These important observations demonstrate that the cleavage rate of the thymine dimer radical anion (**8**→**10**) is comparable to the electron-transfer process (**8**→**9**).[20] Thus, the transition-state energy of the charge detection process at the thymine dimer is as high as that of the electron-transfer steps.[21] As a result, possible effects of the DNA sequence on the rate of electron transport through DNA are detected as weakened signals by the thymine dimer assay. Ito and Rokita[6] used bromouracil for charge detection. This compound has a less negative redox potential than thymine or the thymine dimer.[22] Therefore, the bromouracil charge-detection system might be faster than the dimer clock used in our experiments. This difference could explain why the assay used by Ito and Rokita detects an influence of the base-pair sequence (A:T versus G:C) and of the charge-transport direction (3′ versus 5′) on electron transport,[6] but this effect is not observable with the thymine dimer detection system.[5]

Received: November 5, 2003 [Z53264]
Published Online: March 9, 2004

Keywords: DNA damage · electron transfer · ESR spectroscopy · nucleotides · radical ions

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[9] The details of the synthesis will be described in a full paper.
[10] A solution of **3** (7 mM) in CH_3CN was deoxygenated and frozen in liquid N_2 (77 K). The sample was photolyzed for 10 min at 77 K by using a high-pressure mercury lamp (500 W) in conjunction with a 320-nm cut-off filter. ESR spectra were recorded on a Bruker ESR 300E spectrometer. The spectra were acquired at a microwave power of 0.1 mW, a modulation amplitude of 2.5 G, a receiver gain of 1×10^5, a conversion time of 40.96 ms, and with a time constant of 40.96 ms. The ESR spectrum obtained immediately after irradiation is a superposition of the spectra from the *tert*-butyl radical, the thymine-based radical, and the ketyl radical. After one week, only the thymine-based radical was detectable; the two other radicals had decayed. The formation of the three constituents and the analysis of the spectra are similar to the processes described in O. Schiemann, E. Feresin, T. Carl, B. Giese, *ChemPhysChem* **2004**, *5*, 270.
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[13] Mixtures of **3** and glutathione (1:5) in H_2O/CH_3OH (4:1) were irradiated for 10 min with a 500-W mercury arc lamp (320-nm cut-off filter) and the products **6** and **7** were analyzed by HPLC (co-injection and MS). Deprotonation of the hydroxyalkyl radical has to occur for efficient electron transfer from the electron-injecting radical to the thymine. In DNA experiments, the surrounding water or phosphate ions might accept the proton. Even the neighboring carbonyl group could assist the deprotonation of the hydroxyalkyl radical. Thus, electron transfer is presumably coupled with proton transfer.
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[16] The modified strands were synthesized according to the recently described method (see ref. [4]). For the incorporation of the modified thymine **3**, CH_2Cl_2 was used as a solvent. Complementary strands slightly longer than the modified strands were used to improve the separation achievable by HPLC. The melting points of the double strands were up to 4 °C higher than those of the unmodified strands. All melting points measured under the conditions of the photolysis experiments were above 60 °C.
[17] Photolysis (ORIEL 68810 mercury arc lamp, 500 W; 320-nm cut-off filter) of DNA double strands **11a–c** and **14** ($c_{DNA} = 5$ μM, 20 mM NaH_2PO_4 buffer solution, pH 7.0, 150 mM NaCl) was carried out in the absence of O_2 at 15 °C. After 10 min, the solutions were quantitatively analyzed by reversed-phase HPLC (Merck RP-18e; LiChrospher, 5 μm; linear gradient of 0.1M triethylammonium acetate buffer/acetonitrile from 94:6 to 80:20 over 40 min) and by MALDI TOF mass spectrometry. The relative errors in the reported yields are ± 5 % for **12**, **13**, **15**, and **16** and ± 25 % for **17**.
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[19] Our assay injects only one electron per strand. The cleavage of the distal site is not the result of a second charge injection step, as occurs in experiments with recoverable photoinjectors. Our cleavage yields should therefore be compared with quantum yields.
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