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Efficient Interstrand Excess Electron Transfer in PNA:DNA Hybrids

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The observation that radical cations in DNA double strands move over large distances fueled over the past decade an enormous interest to decipher the mechanism behind this long distance process.[1] Studies by Barton,[2,3] Giese,[4,5] Lewis/Wasielewski,[6,7] and Schuster[8] now provide compelling evidence that radical cations migrate in DNA by successive hopping between guanines. Hopping via adenines is possible in pure A:T sequences.[5] This imposes a strong sequence dependence onto the transfer reaction. In contrast to the transport of radical cations, very little is known about the transfer of excess electrons through the base stack.[9] This process, however, is important for the development of DNA derived charge-transfer media in nanoelectronic devices.[10,11] First insights into the excess electron mobility in DNA double strands were gained from EPR experiments, which revealed that the transfer proceeds below −80 °C dominantly by superexchange with a β-value of 0.9 Å^{-1}.[12−15] This limits the excess electron transfer to small distances of <10 Å. Above −80 °C, a thermally activated long-range excess electron hopping via thymine and cytosine charge carriers may become possible.[16]

Recently, we prepared DNA double strands containing a photo triggered flavin electron donor and a thymine dimer electron acceptor **T=T**, spaced by an increasing number of $(A:T)_n$ base pairs.[17] The thymine dimer **T=T** (Scheme 1) undergoes a rapid [2 + 2] cycloreversion upon single electron reduction, which induces a strand break. This allows rapid analysis of the excess electron transfer from the flavin to the **T=T** acceptor.

Herein, we report the first data about an interstrand excess electron transfer in mixed sequences. The investigation was performed with PNA[18]:DNA hybrid double strands, because (1) these hybrids form very stable double strands, even after incorporation of a flavin donor and a **T=T** acceptor, (2) the flavin is situated inside the double strand, independent of the kind of counterbase, and (3) the positively charged PNA strands allow one to set up of a precise HPLC-based electron-transfer assay.

The investigated PNA:DNA duplexes are depicted in Figure 1. They contain one of the flavin donors **F1** or **F2**[19] (Scheme 1) in the PNA strand and the **T=T** in the DNA counterstrand.

The flavin-containing PNA strands were prepared using standard Fmoc-based PNA synthesis. The **T=T**-containing DNA strands were synthesized using standard phosphoramidite DNA chemistry and a silyldiether protected **T=T** building block as described recently.[20] The **F1/F2**-PNA:**T=T**-DNA double strands were finally annealed. All double strands (except **3**, see below) exhibit melting points well above room temperature (Figure 1). Fluorescence melting studies show a strong increase of the flavin fluorescence intensity at the melting temperature, which indicates that the flavin is situated inside the duplex. The CD spectra of all depicted strands show maxima at 220 and 275 nm and a minimum at 250 nm indicative for PNA:DNA double strands.[18] Addition of the cyanine dye $DiSC_2(5)$ to the double strand solutions gives the required color change from blue to purple, which also supports double strand formation.[21] Excess electron-transfer studies in the duplexes (c_{DNA} = 20 μM, 0.01 M Tris, pH = 7.5) were performed in small glass vials. After purging of the corresponding double strand solution with nitrogen for 5 min, a basic sodium dithionite solution was added to reduce the flavin, thereby converting it into a strong electron donor. Complete reduction was monitored by fluorescence spectroscopy. The solutions were irradiated at 10 °C (white light), and small samples were removed from the assay after defined time intervals. These samples were reoxidized by shaking them exposed to air. The samples were finally analyzed by ion exchange

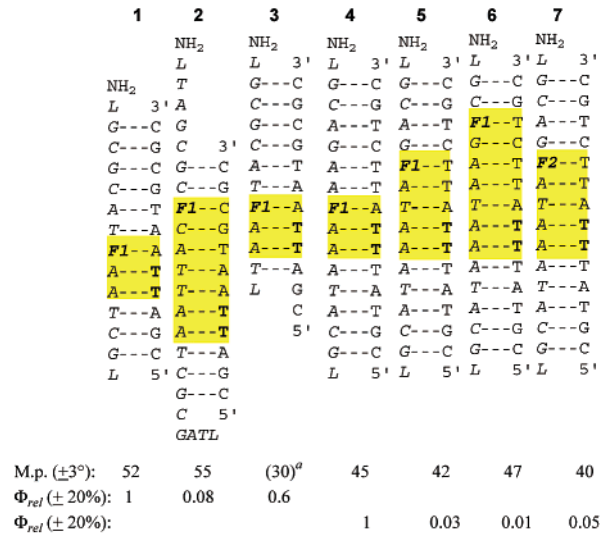
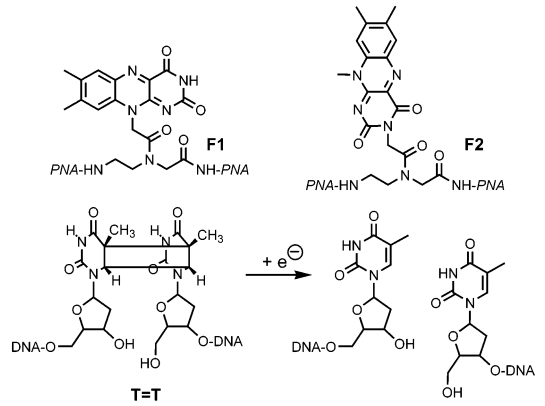


Figure 1. PNA:DNA hybrids prepared, for excess electron-transfer studies from a flavin (**F1** or **F2**) to a **T=T**. PNA bases are shown in italics. L = Lysine, NH_2 = N-terminus. Strand 2 contains overhangs to stabilize both ends. [a]Very flat and unstructured melting curve, indicating significant 5′ end-fraying.

Scheme 1. The Flavin PNA Building Blocks and the Cleavable **T=T**



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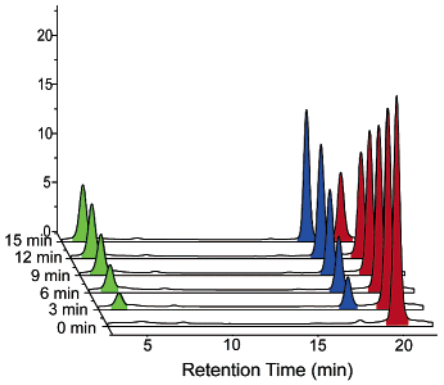

Figure 2. Analysis of the light-induced cycloreversion of **T**=**T** in duplex **1**. The irradiated samples were analyzed by ion-exchange HPLC (Nucleogel SAX1000-8 column, 50 × 4.6 mm, linear gradient: 100% 0.1 M NaCl to 100% 1 M NaCl over 40 min, pH = 13, room temperature, Det.: 260 nm). DNA strands formed out of **1**: 20 min, 5′-CGA**T**=**T**AATGCGC-3′; 18 min, 5′-TAATGCGC-3′; 5 min, 5′-CGAT-3′.

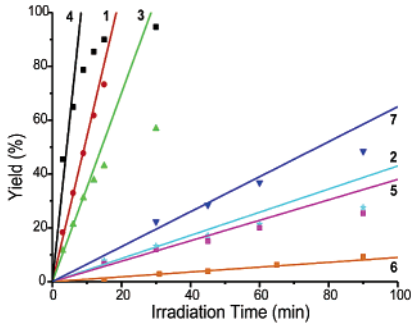

Figure 3. Plot of the photoirradiation **T**=**T** splitting yields against the irradiation time. The initial rates were obtained from a linear approximation of the first data points.

chromatography. A typical set of HPL-chromatograms obtained during the experiment with double strand **1** is depicted in Figure 2.

The PNA strands were modified with lysines to provide sufficient solubility and to reduce the elution time below 3 min under denaturing basic ion exchange chromatography conditions. The negatively charged DNA single strands of **1**−**7** all elute with the highest retention times. The two fragments, obtained after reductive **T**=**T** cleavage, elute much earlier, which allowed baseline separation of all three oligonucleotides in the assay. From Figure 2 it is clearly evident that upon irradiation of the PNA:DNA double strands, only the expected two new DNA strands are formed. No other DNA products were detected, underlining that the reductive ring opening is a clean process. This is different as compared to oxidative hole transfer, where the guanine radical cations react with water to give oxidative lesions. Plots of the reductive **T**=**T** cleavage yields against the irradiation time for all PNA:DNA hybrids **1**−**7** are shown in Figure 3.

The reductive electron transfer in the PNA:DNA hybrids proceeds with a rather high quantum yield of $\Phi_{abs}(366\ nm) = 0.001 \pm 0.001$ in the double strands **1** and **4**. This is comparable to quantum yields obtained for the intrastrand cleavage of the **T**=**T** in a DNA:DNA duplex with the flavin and the **T**=**T** located next to each other. The comparable quantum yields show that the electron can jump efficiently from **F1** in the PNA strand to the **T**=**T** in the DNA counterstrand. Because of the relative high efficiency of this interstrand excess electron transfer, we could next study the distance dependence of the process. In duplex **2**, the distance between **F1** and the **T**=**T** is increased from 3.4 Å (**1**) to about 17 Å (**2**). In **2**, the **T**=**T** cleavage is by a factor of about 12 less efficient, showing that every additional base pair between the two redox partners on averages reduces the cleavage efficiency by a factor of about 2. This is again in good agreement with earlier results.[17] We next explored the sequence dependence of the electron-transfer process with the two double strands **4** and **6**. Here again the distance is increased from 3.4 Å (**4**) to 17 Å (**6**), and the two chromophors are separated by the same number of A:T and G:C base pairs. In **4** and **6**, the distance effect is, however, much more pronounced. The splitting efficiency is reduced in **6** by a factor of about 100 and is in consequence only slightly above background. The much stronger distance dependence was confirmed with the double strand **5**, in which the cleavage efficiency is reduced already by a factor of 30, although the distance is just increased to about 10 Å.

The fact that also the stacking situation influences the excess electron transfer was finally established with the double strands **3** and **7**. Double strand **3** exhibits increased fraying of the **T**=**T**-containing double strand ends due to a lack of proper base pairing. Strand **7** contains instead of **F1** the flavin donor **F2**, which is located more deeply inside the duplex. The transfer efficiency responds to both changes. The excess electron transfer is decreased in **3** as compared to **1** and increased in **7** relative to **5**.

Our studies show that an interstrand excess electron transfer can efficiently proceed in PNA:DNA double strands. The results indicate that the excess electron transfer is somewhat sequence dependent and influenced by the precise stacking situation.

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Supporting Information Available: HPL-chromatograms and MALDI-Tof mass spectra of all PNA and DNA strands (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Kelley, S. O.; Jackson, N. M.; Hill, M. G.; Barton, J. K. *Angew. Chem.* **1999**, *111*, 991−996.
(2) Williams, T. T.; Barton, J. K. *J. Am. Chem. Soc.* **2002**, *124*, 1840−1841.
(3) Bhattacharya, P. K.; Barton, J. K. *J. Am. Chem. Soc.* **2001**, *123*, 8649−8656.
(4) Giese, B. *Acc. Chem. Res.* **2000**, *33*, 631−636.
(5) Giese, B.; Amaudrut, J.; Köhler, A.-K.; Spormann, M.; Wessely, S. *Nature* **2001**, *4112*, 318−320.
(6) Lewis, F. D.; Letsinger, R. L.; Wasielewski, M. R. *Acc. Chem. Res.* **2001**, *34*, 159−170.
(7) Lewis, F. D.; Liu, X.; Liu, J.; Miller, S. E.; Hayes, R. T.; Wasielewski, M. R. *Nature* **2000**, *406*, 51−53.
(8) Schuster, G. B. *Acc. Chem. Res.* **2000**, *33*, 253−260.
(9) Lewis, F. D.; Liu, X.; Wu, Y.; Miller, S. E.; Wasielewski, M. R.; Letsinger, R. L.; Sanishvili, R.; Joachimiak, A.; Tereshko, V.; Egli, M. *J. Am. Chem. Soc.* **1999**, *121*, 9905−9906.
(10) Porath, D.; Bezryadin, A.; De Vries, S.; Dekker, C. *Nature* **2000**, *403*, 635−638.
(11) Fink, H.-W.; Schönenberger, C. *Nature* **1999**, *398*, 407−410.
(12) Debije, M. G.; Milano, M. T.; Bernhard, W. A. *Angew. Chem., Int. Ed.* **1999**, *38*, 2752−2755.
(13) Pezeshk, A.; Symons, M. C. R.; McClymont, J. D. *J. Phys. Chem.* **1996**, *100*, 18562−18566.
(14) Messer, A.; Carpenter, K.; Forzley, K.; Buchanan, J.; Yang, S.; Razskazovskii, Y.; Cai, Z.; Sevilla, M. D. *J. Phys. Chem. B* **2000**, *104*, 1128−1136.
(15) Cai, Z.; Gu, Z.; Sevilla, M. D. *J. Phys. Chem. B* **2000**, *104*, 10406−10411.
(16) Giese, B.; Wessely, S.; Sporman, M.; Lindemann, U.; Meggers, E.; Michel-Beyerle, M. E. *Angew. Chem., Int. Ed.* **1999**, *38*, 996−998.
(17) Behrens, C.; Burgdorf, L. T.; Schwögler, A.; Carell, T. *Angew. Chem., Int. Ed.* **2002**, *114*, 1841−1844.
(18) *Peptide Nucleic Acids*; Nielsen, P. E., Egholm, M., Eds.; Horizon Scientific Press: Norfolk, 1999.
(19) Ikeda, H.; Yoshida, K.; Ozeki, M.; Saito, I. *Tetrahedron Lett.* **2001**, *42*, 2529−2531.
(20) Kundu, L. M.; Burgdorf, L. T.; Kleiner, O.; Batschauer, A.; Carell, T. *ChemBioChem* **2002**, accepted.
(21) Smith, J. O.; Olson, D. A.; Armitage, B. A. *J. Am. Chem. Soc.* **1999**, *121*, 2686−2695.

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