Electron Transfer in DNA

Excess Electron Transfer Driven DNA Does Not Depend on the Transfer Direction**

*Clemens Haas, Katja Kräling, Michaela Cichon, Nicole Rahe, and Thomas Carell**

The transport of electronic charge through DNA continues to surprise the scientific community. A wealth of scientific data has established the ability of DNA to transport a positive charge over large distances.[1] The charge movement is now believed to occur by a hopping mechanism in which the hole jumps between purine bases that act as temporary charge carriers.[2–8] Although guanine, the easiest base to oxidize, functions as the most efficient charge transporter, it was recently established that adenine residues, particularly in homo A:T sequences, may also function as stepping stones during the hole-hopping process.[5] Data collected by the Giese research group suggest that this hopping of a positive charge between purine bases is possible because the competing reaction of the G and A radical cations with water is very slow ($k_{H_2O} = 10^4\ s^{-1}$). Nevertheless, the reaction with water induces oxidative DNA lesions. It is not yet completely clear how the hole transfer process is influenced by the direction of electron transfer. Some researchers have observed differences in the efficiency of transfer in opposite directions and have exploited these differences for the development of DNA-based analytical devices.[9] Others see no directional dependence of the electron-transfer efficiency.[10]

We and others have recently started to develop donor–acceptor-modified DNA duplexes to investigate the transfer of an extra electron, a negative charge, through DNA.[11–15] It has been established that long-range processes involving excess electron transfer also proceed by hopping.[15,16] In the reductive mode, however, the pyrimidines T and C function as temporary charge carriers.[17] No efficient chemical reactions leading to degradation of the pyrimidine radical anions are known, therefore, transfer of a negative charge could proceed without concomitant DNA degradation. In principle, this fact makes such a mode of transfer perfectly suited for the design of DNA-based analytical and electronic devices.[18,19] Analysis of how the DNA structure and donor/acceptor positions in the duplex influence electron transfer through DNA is consequently of great importance.

Herein we describe experiments performed with DNA:PNA hybrid duplexes **1**–**8** (Table 1) designed to address how excess electron transfer in DNA is influenced by the direction of electron-transfer-driven of a thymine dimer DNA lesion (5′→3′ versus 3′→5′). The electron-transfer experiments are based on the fact that a reduced, deprotonated flavin such as **10**, when embedded in a double helix, is capable of light-induced injection of an electron into the base stack (Scheme 1). This electron travels through the duplex until it reaches the thymine dimer **11**, which has an open backbone.[20] This dimer cleaves upon reduction (rate of cleavage, $k_{split} \approx 10^6\ s^{-1}$),[21] which induces a readily detectable strand break.

The eight DNA:PNA hybrid duplexes (**1**–**8**) contain the thymidine dimer acceptor molecule **11** in the DNA strand and the flavin electron donor **10** in the PNA strand. In the first four DNA:PNA hybrids (**1**–**4**), the electron travels from the 5′ to the 3′ end over distances of about 3.4 (**1**), 10.2 (**2**), 17.0 (**3**), and 23.8 Å (**4**). In the other four DNA:PNA hybrids (**5**–**8**),

Table 1: DNA:PNA hybrid double-strands **1**–**8** used to study the directional and distance dependence of the excess electron transfer process.[a]

Compound	Name	Sequence
1	DNA	5′-GCA-AAA-AAA-A**TT**-CGC-3′
	PNA	*KK-CGT-TTT-TTT-**F**AA-GCG-KK-NH$_2$*
2	DNA	5′-GCA-AAA-AAA-A**TT**-CGC-3′
	PNA	*KK-CGT-TTT-T**F**T-TAA-GCG-KK-NH$_2$*
3	DNA	5′-GCA-AAA-AAA-A**TT**-CGC-3′
	PNA	*KK-CGT-TT**F**-TTT-TAA-GCG-KK-NH$_2$*
4	DNA	5′-GCA-AAA-AAA-A**TT**-CGC-3′
	PNA	*KK-CGT-**F**TT-TTT-TAA-GCG-KK-NH$_2$*
5	DNA	5′-CGC-**TT**A-AAA-AAA-ACG-3′
	PNA	*KK-GCG-AA**F**-TTT-TTT-TGC-KK-NH$_2$*
6	DNA	5′-CGC-**TT**A-AAA-AAA-ACG-3′
	PNA	*KK-GCG-AAT-T**F**T-TTT-TGC-KK-NH$_2$*
7	DNA	5′-CGC-**TT**A-AAA-AAA-ACG-3′
	PNA	*KK-GCG-AAT-TTT-**F**TT-TGC-KK-NH$_2$*
8	DNA	5′-CGC-**TT**A-AAA-AAA-ACG-3′
	PNA	*KK-GCG-AAT-TTT-TT**F**-TGC-KK-NH$_2$*
9[b]	DNA	5′-CGC-G**TT**-TTT-TTT-TGC-GCC-GC-3′
	PNA	*KK-CGT-TTT-T**F**T-TAA-GCG-KK-NH$_2$*

[a] PNA bases are shown in italics. *K* = lysine; needed for solubility. F = flavin. [b] A mixture of a DNA and a PNA strand that are unable to pair.

[*] Dipl.-Chem. C. Haas, K. Kräling, Dipl.-Chem. M. Cichon, Dipl.-Chem. N. Rahe, Prof. Dr. T. Carell[+]
Philipps University Marburg
Department of Chemistry
Hans-Meerwein Strasse, 35032 Marburg (Germany)
Fax: (+49) 6421-282-2189
E-mail: Thomas.Carell@cup.uni.muenchen.de

[+] New Address:
Ludwig-Maximilians University Munich
Department of Chemistry
Butenandtstrasse 5–13, 81377 München (Germany)

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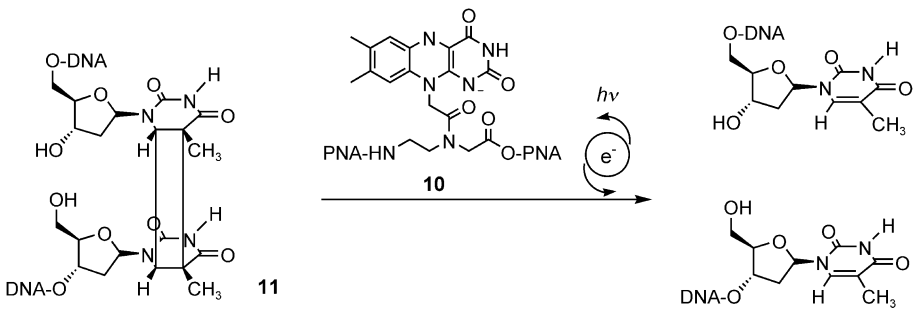

Scheme 1. Depiction of the dimer electron acceptor **11** and the flavin electron donor **10**, which induces cleavage of the DNA strand into two halves upon single-electron reduction.

electron transfer has to proceed in the opposite direction (3′→5′) over the same distances. The base sequences between the flavin donor **10** and the dimer acceptor **11** were kept the same in **5**–**8** as in **1**–**4**, respectively. Previous experiments showed that the efficiency of the electron-transfer-driven dimer opening is lower in double strands with a less-ordered structure. We therefore used G:C-rich sequences at the ends of the DNA:PNA duplexes to achieve the highest possible duplex stability. Thermal denaturing studies with all eight duplexes proved the high thermal stability of the duplex structures; despite the presence of two perturbing unnatural bases, all the DNA:PNA double strands were found to melt between 60 °C and 80 °C, namely, well above room temperature.

The electron-transfer measurements were made on individual solutions of the DNA:PNA hybrid double strands **1**–**8** ($c_{DNA} = 20$ μM, 0.01M H_3PO_4/Na_2HPO_4, pH 7.0, 0.01M NaCl) in fluorescence spectroscopy cuvettes. The solutions were purged with nitrogen to establish anaerobic conditions. A standard dithionite solution was subsequently added to reduce the flavin. The cuvettes containing the so-prepared solutions were irradiated with a 1000-W Hg(Xe) lamp equipped with a cooled 360-nm cut-off filter. The dimer cleavage yield was analyzed as described previously.[13] In short: The solutions were irradiated for a fixed time at 10 °C, well below the individual melting temperatures of the duplexes. The solutions were then poured into Eppendorff vials and shaken whilst being exposed to air for about 2 h to reoxidize the flavin. The resulting solutions were analyzed by ion-exchange chromatography. All data reported herein are average values calculated from the results of at least three fully independent experiments. The error in the measurements made in these independent experiments was determined to be less than ±20%.

Before we analyzed the directional dependence of the electron transfer, we investigated whether the electron travels from the flavin donor to the dimer acceptor intramolecularly or whether an intermolecular electron transfer between different duplexes can take place. In the first experiment, we prepared the strand mixture **9**, which contained a flavin–PNA strand and a dimer-containing DNA strand. These strands are unable to form a duplex, as was proven by addition of the cyanine dye 3,3′-diethylthiadicarbocyanine idodide ($DiSC_2(5)$).[22] The dye binds exclusively to PNA-containing duplex structures, and results in the absorption maximum shifting from 650 to 540 nm. Figure 1 shows the UV spectrum of mixture **9** after addition of the dye (blue line). The absorption maximum at 650 nm proves the absence of double strands. Irradiation of this solution after reduction of the flavin with dithionite (no dye present) and analysis of the irradiated solution by ion-exchange chromatography provided a chromatogram with a sharp signal at 47 min that corresponds to the DNA strand present in mixture **9**. No other DNA strand was detected, which shows that dimer splitting did not take place in this solution (data not shown). In a second experiment, we paired the PNA strand of mixture **9** with a matching dimer-containing DNA strand. A clear absorption shift to 540 nm was detected after addition of the dye $DISC_2(5)$ (Figure 1, red line), which

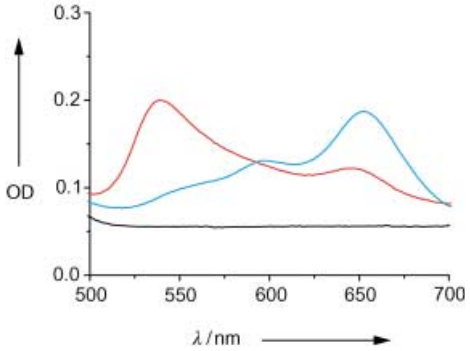

Figure 1. UV spectra of PNA:DNA solutions (PNA, 3 μM; DNA, 3 μM; NaCl, 10 mM; H_3PO_4/Na_2HPO_4, 10 mM, pH 7.0; 10 °C). Red line: PNA:DNA solution of a flavin-containing PNA strand and a dimer-containing DNA strand that form a stable duplex (entry 2 in Table 1) after addition of the dye $DiSC_2(5)$; blue line: PNA:DNA solution of a flavin-containing PNA strand and a dimer-containing DNA strand that are unable to form a stable duplex (entry 9 in Table 1) after addition of the dye $DiSC_2(5)$; black line: PNA:DNA solutions before addition of the dye $DiSC_2(5)$. OD, optical density.

indicates efficient formation of a duplex. Irradiation and analysis of the solution by HPLC gave a chromatogram with a sharp peak at 30 min resulting from the DNA strand. In this experiment we clearly observed two sharp new signals at 21 min and 4 min which were caused by the two DNA fragments expected to result from cleavage of the duplex. These results show that cleavage of the dimer takes place in this PNA:DNA double strand (data not shown). We concluded that, under our conditions, duplex formation is a prerequisite for efficient excess electron transfer from the flavin donor to the dimer acceptor.

We studied the directional dependence of the excess electron-transfer-driven repair reaction by irradiation of the PNA:DNA duplexes **1**–**8**. The yields for the cleavage measured after irradiation for 20 min are listed in Table 2, together with the melting points of the duplexes. The yields

Table 2: Dimer cleavage yields after irradiation of the PNA:DNA double strands **1**–**8** and mixture **9** for 20 min.[a]

Compound	M.p. [°C][b]	Distance [Å][c]	Transfer direction	Yield [%]
1	82	3.4	5′→3′	22 ± 5
2	67	10.2	5′→3′	24 ± 5
3	69	17.0	5′→3′	23 ± 5
4	70	23.8	5′→3′	25 ± 5
5	77	3.4	3′→5′	30 ± 6
6	[d]	10.2	3′→5′	15 ± 3
7	68	17.0	3′→5′	36 ± 7
8	69	23.8	3′→5′	46 ± 9
9	not detectable	not defined		0

[a] Irradiation conditions: 1000-W Hg(Xe) lamp, 360-nm cut-off filter, 10 °C.[b] 3 μM DNA, 3 μM PNA, 10 mM NaCl, 10 mM H_3PO_4/Na_2HPO_4 (pH 7.0). [c] Separation of donor and acceptor assuming an ideal B conformation. [d] Not measured.

show that dimer cleavage proceeds efficiently in all investigated DNA:PNA double strands **1**–**8**, even when the electron transfer occurs over a distance of about 24 Å. We observed that the cleavage of the dimer is not very distance-dependent, an outcome in agreement with the results of earlier studies. The DNA:PNA hybrids with the largest flavin···thymine dimer separation consistently undergo slightly faster dimer cleavage. In accordance with an earlier report of ours, we believe that a larger separation of the two unnatural bases allows the duplex to adopt a more-ordered duplex structure between the two potentially disruptive elements.[12]

If we compare the cleavage data obtained with the PNA:DNA series **1**–**4** (electron transfer in the 5′→3′ direction) with that obtained for the series **5**–**8** (electron transfer 3′→5′), we observe no large difference in repair yield. This result shows that the repair of a thymine dimer by a reduced and deprotonated flavin is independent of the direction of electron transfer, even for transfer over rather large distances of around 24 Å.

In summary, we have investigated how the direction of excess electron transfer influences the transfer efficiency. We first established that duplex formation is a prerequisite for intramolecular interstrand electron transfer. Yields for the cleavage were recorded for electron transfer in the 5′→3′ and 3′→5′ directions over four different distances, with the same sequence composition between the flavin donor and the dimer acceptor for both transfer directions. Analysis of these results showed that the yields of the cleavage are, within the error limits of our analysis, the same for both transfer directions. This result must be interpreted in the light of data reported by others about the directional dependence of the hole-transfer process, in which the reaction of the intermediate G and A radical cations with water ($k_{water} = 6 \times 10^4\ s^{-1}$) is rate determining. The charge equilibrates along the DNA strand until the slow reaction with water finally eliminates the positive charge to give an oxidative DNA lesion. The fact that we observed no distance or directional dependence on the yield of dimer cleavage must be analyzed with consideration of the rate of dimer cleavage. This cleavage reaction is not well defined but data from Yeh and Falvey point to a reaction rate of $k_{split} = 10^6\ s^{-1}$.[21] If the rates of electron transfer are faster than this value then dimer splitting would become the rate-determining step.[23] We can therefore conclude that the rate of excess electron transfer through DNA over distances of up to 24 Å is similar in the two directions within the time frame of our system, which is defined by the rate of dimer cleavage ($< 10^6\ s^{-1}$).

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