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Excess electron transfer in flavin-capped, thymine dimer-containing DNA hairpins

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The transfer of an excess electron through DNA was investigated with DNA hairpins, which contain a flavin cap functioning as an electron donor. A thymine dimer with an open backbone acts as the electron acceptor. The dimer translates the electron capture into a strand break, which is readily detectable by HPLC. Analysis of four hairpins, in which the distance between the flavin donor and the dimer acceptor was systematically increased, revealed a flat distance dependence of the repair efficiency supporting the view that excess electrons hop through DNA using intermediate A–T base pairs as temporary charge carriers.

Radical cations, or so called electron holes, can travel in DNA over large distances. They induce base damage at positions in DNA far distant from the initial oxidation site. This links hole transfer to the processes of mutagenesis and DNA-repair.[1] Recent studies by Barton and co-workers[2–4] Giese *et al.*[5,6] Lewis and Wasielewski and their co-workers,[7,8] and Schuster[9] clarified that radical cations migrate in DNA by successive hopping using guanines and to a much lesser extend also adenines as temporary charge carriers (Fig. 1).[5,6] Efficient charge transfer is consequently strongly sequence dependent. Mismatches, bulges or other factors that interrupt the base stack were found to have a profound influence on the charge transfer efficiency,[10,11] underlining that proper intercalation of the involved redox partners is a prerequisite for efficient charge transfer.

In contrast to hole hopping, very little is known about the transfer of excess electrons through the base stack.[12] This process, however, is important for the development of DNA based nanoelectronic devices.[13,14] Insights into the excess electron transfer capabilities of DNA come today mainly from EPR experiments, which showed that the transfer proceeds below −80 °C predominantly by superexchange,[15–18] limiting the transfer distance to about 10 Å. Above that temperature electrons seem to hop through DNA, using thymines and cytosines as temporary charge carriers as shown in Fig. 1B. We recently studied excess electron transfer in flavin-donor/dimer-acceptor modified DNA double strands and concluded that electrons hop through DNA using A–T base pairs as stepping stones.[19] The analysis, however, was limited to studies over intermediate distances because DNA double strands containing the flavin and the dimer close together had very low melting points. In order to analyze excess electron transfer over shorter distances, we now prepared the pure A–T DNA hairpins **1–4**. They contain the flavin-cap **5**, which in the reduced and deprotonated state functions as a strong light triggered electron donor (E_{red}* = −2.6 V against NHE).[20] The hairpins contain next to the flavin the backbone opened thymine dimer **6**,[21] which upon single electron reduction, undergoes a cycloreversion reaction, translating electron capture into a HPLC-detectable strand break as shown in Scheme 1.

The DNA hairpins **1–4** (Scheme 1) feature a systematically increased distance (6.8 Å–17 Å) between the flavin donor **5** and the dimer acceptor **6**. All DNA hairpins show concentration independent melting points (c_{DNA} varied between 10 and 100 μM) which is typical for DNA hairpins (Table 1). Due to the presence of the thymine dimer in the hairpin stems, the melting points are rather low, but still high enough to allow the intended measurements. We have noticed recently that the repair rates drop if the dimer and the flavin are not properly stacked in the duplex and therefore extended the stem length after the thymine dimer in the hairpins **3** and **4** to 5 or 4 A–T base pairs, respectively. For all hairpins we were able to measure the melting transitions using UV-spectroscopy not only at 260 nm

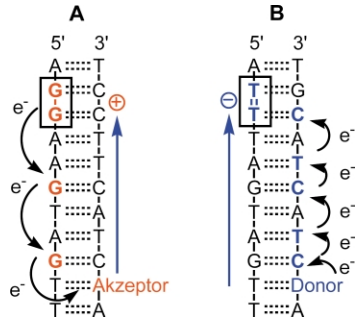
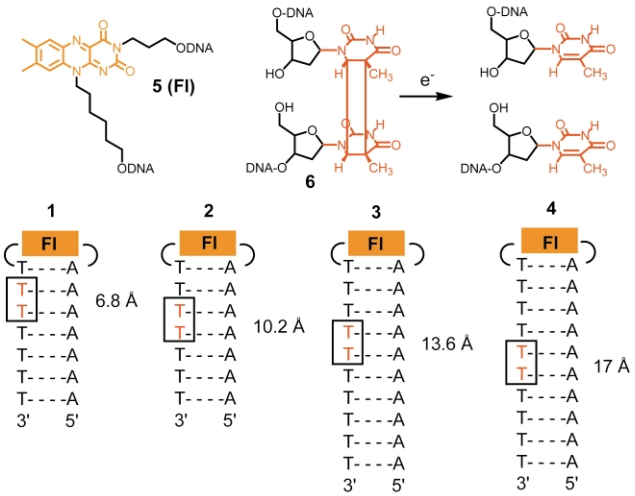


Fig. 1 Depiction of the hole hopping process (A) *via* guanines and of the proposed excess electron hopping (B) *via* thymines and cytosines in double helical DNA.



Scheme 1 Depiction of the flavin electron donor **5** and of the dimer acceptor **6** together with the hairpins **1–4** used to study the distance dependence of the excess electron transfer process.

Table 1 Melting points (°C) of the DNA hairpins **1–4** and determined repair yield (% min^{-1}) after 1 minute of irradiation[a]

Hairpin	1	2	3	4
Melting point	28	12	36	30
Repair yield (5 °C)[b]	2.0	0.5	0.5	0.1
Repair yield (0 °C)[b]	2.9	0.7	1.2	0.1

[a] c_{DNA} = 20 μM, 0.01 M Tris, pH = 7.4, 150 mM NaCl.[b] Ion exchange chromatography: Nucleogel SAX 1000-8 (VA 50/4.6), pH = 12. Linear gradient of 0.2 M NaCl to 1 M NaCl over 35 min.

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but also at 460 nm. In addition, melting was also observable by fluorescence spectroscopy (λ_{ex} = 360 nm, λ_{em} = 520 nm). Because the flavin is the only fluorescent chromophore with an absorption above 300 nm, the data show that the flavin is stacked on top of the hairpins below the melting temperature. CD-spectra reveal for all hairpins a minimum at 250 nm and two maxima at 280 nm and 220 nm indicative of a B-type DNA double helix structure of the hairpin stem.[22]

For the electron transfer measurements, small aliquots of DNA hairpin solutions (c_{DNA} = 20 μM, 0.01 M Tris, pH = 7.4, 150 mM NaCl) were irradiated in cuvettes, stoppered with a rubber septum. After purging of the hairpin solutions with nitrogen for about 10 min to establish anaerobic conditions, a basic sodium dithionite solution was added to reduce the flavin, which is thus converted into the required strong electron donor. The solutions were subsequently irradiated at 5 °C or 0 °C well below their melting temperatures, at 366 nm for about 1 h. After a defined time, a sample was removed from the assay solution and shaken for 1 h in the dark exposed to air, to reoxidise the flavin. The samples were finally analyzed by ion exchange HPLC and MALDI-TOF mass spectrometry. The listed repair data are averaged values from three independent studies.

The data in Table 1 show that dimer cleavage proceeds efficiently in all hairpins **1–4**. Excess electron transfer decreases with increasing distance from 2% repair per minute at 5 °C in **1** to about 0.1% repair per minute in hairpin **4**. If we consider that the distance between the two redox partners increases in these hairpins from 6.8 Å to about 17 Å, we can conclude that the distance dependence is shallow and hence not in agreement with a Marcus type behavior. Overall, however, the distance dependence of the excess electron transfer is more pronounced than hole transfer, which is in good agreement with a recent short time spectroscopic study.[12]

In the Marcus model, one would expect a decrease of the repair yield by a factor of about 8 with every additional base pair introduced between the dimer and the flavin. This would predict for hairpin **4** a repair yield of about 0.004% min^{-1}, which is one to two orders of magnitude lower than observed. Marcus type electron transfer is exponentially distance dependent with $k_{ET} \sim A \exp(-\beta' \Delta r)$. For DNA, β'-values between 0.7 $Å^{-1}$ and 1.2 $Å^{-1}$were determined.[23] A plot of our yield data (ln *y*) against the distance Δr (Fig. 2) provided very low β′-value of about 0.3 $Å^{-1}$ at 5 °C and at 0 °C showing that a direct Marcus-type electron transfer from the flavin to the dimer is unlikely. In the hopping model, the electron is not directly transferred but uses intermediate charge carriers to hop from the donor to the acceptor. In our hairpins, the A–T base pairs could function as temporary charge carriers. In the hopping scenario, the transfer efficiency is much less distance dependent. With $\ln(k_{ET}) \sim -\eta \ln(N)$, the transfer rate is proportional to the number of hopping steps (*N*).[23,24] The proportionality factor η should be around 2 if the electron moves in a random walk like process.[23,24] A plot of our measured cleavage yields per minute ln(*y*) against ln(*N*) (Fig. 2) provided an η-value close to 2 in very good agreement with the hopping model and with data obtained by us recently with DNA double strands.

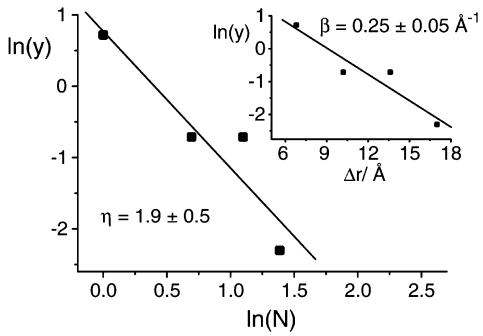

Fig. 2 Analysis of the obtained T=T repair yield in the Marcus (inset) and in the hopping model for excess electron transfer. 5 °C data.

In summary, we analyzed for the first time excess electron transfer in DNA hairpins over distances between 6.8 Å–17 Å. Analysis of the data using the Marcus model furnished a β'-value of 0.3 $Å^{-1}$, which is not consistent with a direct electron transfer. Interpretation of the data using the hopping model gave a good fit between experiment and theory.

The idea that excess electrons hop through DNA in a random walk like process is also plausible from a thermodynamic standpoint, because thymine (−2.1 V) and the T=T dimer (−2.2 V)[20] possess very similar reduction portentials.

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