Electron transfer through a stable phenanthrenyl pair in DNA†

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Within an oligonucleotide duplex, excess electron transfer from a excited 5-(pyren-1-yl)uridine through an internal stacked phenanthrenyl pair to 5-bromouridine as an electron acceptor was observed.

The phenomenon of charge transport through the DNA duplex is of great interest in both, fundamental and applied research[1] and makes DNA an attractive candidate for bottom-up assembly of molecular electronic devices and circuits. However, the conductive properties of canonical DNA is constrained to the electronic properties of the two natural base pairs. Therefore, attempts to create DNA-based, supramolecular assemblies with tuned electronic properties were recently undertaken.[2] In this context, the substitution of the natural DNA bases by designer aromatic residues could be a promising alternative.

We recently found that up to seven consecutive biphenyl pairs can be inserted into the DNA base stack with gradual increase of thermal stability.[3a–c] A solution NMR structure of a duplex containing one substituted biphenyl pair showed the hydrophobic residues to be stacked into the helix, forming a stable interstrand intercalative recognition motif.[3d] With the intention of probing electron transfer through this novel DNA architecture we recently redesigned the aromatic units and investigated the thermal stabilities and the fluorescence properties of duplexes containing phenanthrenyl-*C*-nucleosides (dPhen, Fig. 1).[4]

In the context outlined above, it became of interest to study the charge transfer properties through this novel DNA architecture. We decided to investigate excess electron transfer (EET) as this is less damaging compared to hole transfer, and thus shows more potential for future applications in nanotechnology. EET in DNA has been shown in the past to proceed *via* a thermally activated hopping mechanism with the pyrimidine bases acting as electron relay stations.[5]

To investigate EET the oligonucleotide duplexes **1–6** (Table 1) were prepared. 5-(Pyren-1-yl)uridine (Py-dU) was employed as a photoinducible ($\lambda_{exc} \approx$ 340–360 nm) electron donor and 5-bromouridine as an electron acceptor and reporter group.[6] Upon photoexcitation of Py-dU, an electron is injected into the DNA stack. When the electron encounters a Br-dU unit the latter undergoes elimination of a bromide ion, leaving behind a reactive deoxyuridine-5-yl radical which leads to strand cleavage after base treatment.[7] This methodology was used before to monitor EET in DNA.[6,8] To follow and quantify strand cleavage by polyacrylamide gel electrophoresis (PAGE), Br-dU-containing oligonucleotides and corresponding control strands were 5′-labeled with fluorescein (Fluo).

The thermal stability profiles of duplexes **1–6** (Table 1) were consistent with previously published data.[4,9] Duplex **4**, lacking the Py-dU nucleoside, was the most stable one. Incorporation

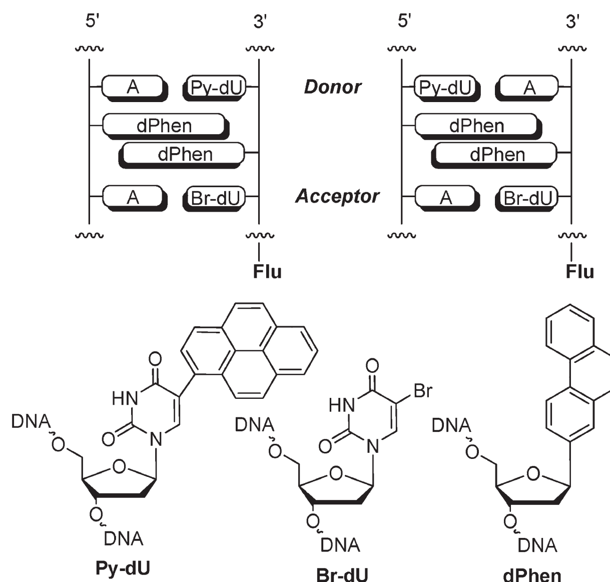

Fig. 1 Schematic representation of the phenanthrenyl duplexes **3** and **5** and chemical structures of electron donating (Py-dU), electron accepting (Br-dU) as well as phenanthrenyl (dPhen) nucleosides.

Table 1 Sequence and thermal stability data for duplexes **1–6**

No	Duplex sequence	T_m/°C[a]
1	5′-d(GCGAT A *A* A ATGCG)	
	3′-d(CGCTA ^{Py}U *T* ^{Br}U TACGC)-Fluo	46.9
2	5′-d(GCGAT A *A* A ATGCG)	
	3′-d(CGCTA ^{Py}U *T* T TACGC)-Fluo	46.1
3	5′-d(GCGAT A *Phen* A ATGCG)	
	3′-d(CGCTA ^{Py}U *Phen* ^{Br}U TACGC)-Fluo	46.5
4	5′-d(GCGAT A *Phen* A ATGCG)	
	3′-d(CGCTA T *Phen* ^{Br}U TACGC)-Fluo	51.5
5	5′-d(GCGAT ^{Py}U *Phen* A ATGCG)	
	3′-d(CGCTA A *Phen* ^{Br}U TACGC)-Fluo	40.7
6	5′-d(GCGAT ^{Py}U *Phen* A ATGCG)	
	3′-d(CGCTA A *Phen* T TACGC)-Fluo	40.8

[a] c = 1.2 μM duplex in 10 mM NaH_2PO_4, 0.15 M NaCl, pH 7.0. Estimated error in T_m = ±0.5 °C.

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of Py-dU was found to be destabilizing, the degree of destabilization being nearest-neighbor dependent as reported before.[9] Replacement of an A–T pair by a dPhen pair essentially did not alter the stability of the duplex (**1** *vs.* **3**). Thus, thermal stability data are in agreement with normal Watson–Crick pairing of Py-dU also in dPhen containing duplexes.

Duplexes (4 μM duplex, 10 mM Na-P_i-buffer, 150 mM NaCl, pH 7.0) were first annealed at 70 °C for 10 min in the dark and then cooled to room temperature and deoxygenated by bubbling Ar through the solution for 20 min. Samples were placed in quartz cuvettes and irradiated with a tungsten lamp through a 3 mm window glass with a cut-off <300 nm at +14–16 °C. Aliquots were taken in given time intervals, treated with piperidine (2.2 μL) heated to 90 °C for 30 min to effect strand cleavage and applied to PAGE. Fluorescence images of gels for duplexes **3** and **5** as examples are depicted in Fig. 2 (for duplexes **1**, **2**, **4** and **6** see Fig. S1, ESI†).

Clearly four major bands representing reaction products **a–d** appeared upon irradiation of all Br-dU containing duplexes. Products **b** and **c**, arising from cleavage at the Br-dU unit (pentamer) and at the 3′-side of the neighboring thymine or phen residue (hexamer) are either already present before irradiation and/or show no or only a weak time dependent increase in intensity. Due to the location of the strand break their formation cannot directly be explained as the consequence of a deoxyuridin-5-yl radical which is the primary product after EET. Products **a** and **d** (Fig. 2, lanes 2–10 and 12–20) clearly accumulated in a time dependent manner. Product **a** corresponds to the fluorescein labeled, 3′-phosphorylated tetramer fragment arising from selective strand scission after abstraction of the C2′ hydrogen atom from the 5′-adjacent nucleotide by the deoxyuridine-5-yl radical.[7b,10] This fragment is formed exclusively in duplexes **1**, **3** and **5** and not or only in trace amounts in **2**, **4** and **6**. The structure of fragment **a** was independently confirmed by isolation from the gel and ESI^- mass analysis (*m*/*z* found: 1776.0; calcd 1776.3). Thus, its formation clearly requires simultaneously the presence of Py-dU and Br-dU and can therefore be considered as a direct consequence of EET. The low-mobility band **d** appeared upon irradiation of all Br-dU containing duplexes, regardless of the presence of Py-dU or dPhen, and can most likely be attributed to a cross-linked duplex arising from follow-up reactions of a deoxyuridine radical intermediate.[11]

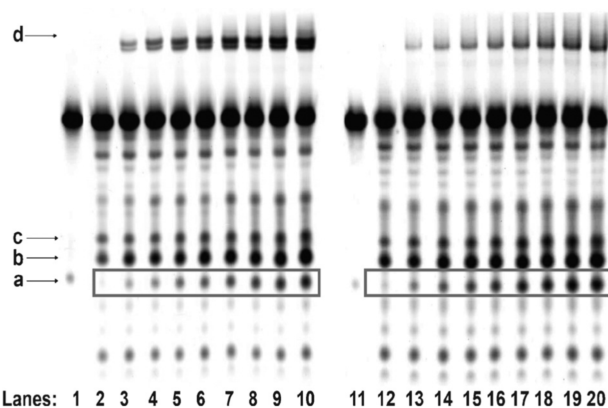

Fig. 2 Fluorescence images of 20% denaturing PAGEs showing strand scission after UV irradiation (>300 nm) of duplexes **5** (left) and **3** (right). Conditions: 4 μM duplex, 10 mM Na-Pi, 150 mM NaCl, pH 7.0, 15 °C. Lanes: 1; duplex **5** without irradiation and piperidine treatment; 2–10; after 0, 5, 10, 15, 20, 30, 40, 60 and 90 min of irradiation and piperidine treatment. 11; duplex **3** without irradiation and piperidine treatment; 12–20; after 0, 5, 10, 15, 20, 30, 40, 60 and 90 min of irradiation and piperidine treatment.

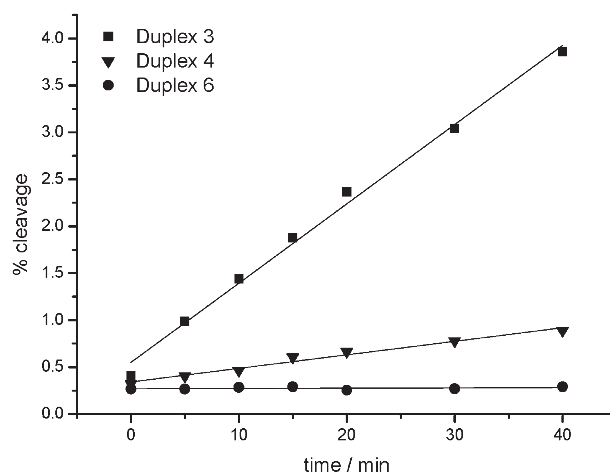

Fig. 3 Time course of the accumulation of product **a** (in % *vs.* intact duplex) as a function of time from PAGE. Conditions as for Fig. 2.

Quantification of cleavage product **a** *vs.* time for the dphen containing duplex **3** and the controls **4** and **6** is presented in Fig. 3. No time dependent strand cleavage was observed upon irradiation of duplex **6**, containing no Br-dU, and only a weak cleavage upon irradiation of duplex **4**, containing no Py-dU unit. This confirms that efficient strand cleavage requires both, Py-dU and Br-dU. When injector and acceptor units are located in different strands (duplex **5**), again, a time dependent cleavage is observed although slightly less efficient compared to duplex **3**, where injector and donor units reside in the same strand (see ESI†). This is comparable to the behavior observed for natural DNA duplexes with injector and acceptor residues on different strands.[12]

The reduction potential of phenanthrene E_{red}(Phen) in dioxane/water was measured to be −2.22 V (*vs.* NHE)[13] and is thus substantially higher than that of thymine, which varies between −1.8 V and −1.1 V *vs.* NHE, depending on the solvent (DMF and water, resp.).[14,15] The reduction potential of pyrene in DMF was reported to be −1.8 V *vs.* NHE.[16] Although these potentials are considerably solvent dependent it seems that E_{red} of phenanthrene is consistently more negative than that of pyrene, rendering charge transfer from the excited pyrene to the phenanthrene an endergonic and thus unlikely process. We therefore assume that electron transfer in duplexes **3** and **5** follows a superexchange mechanism. In contrast to this, EET in duplex **1**, where charge transfer from excited pyrene to thymine is exergonic, follows the known hopping mechanism. Thus the roughly seven-fold enhanced efficiency of EET in duplex **1** compared to **3** (27% *vs.* 4% formation of product **a** in 40 min) is due to two different mechanisms, namely the more efficient electron hopping/molecular wire mechanism over a distance of 6.8 Å in duplex **1** and superexchange/tunneling at

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the distance limits[1] (*ca.* 10.2 Å, assuming an ideal interstrand stacked structure) in the latter duplex.

In summary, this study provides first evidence for electron migration through a stable hydrophobic pair in a DNA duplex most likely *via* a superexchange/tunneling mechanism. This hydrophobic pair is built on interstrand stacking interactions of the aromatic units and not on hydrogen bonds or coordinative bonds to a metal ion. Substituting the dPhen residues with electron accepting or donating functional groups may therefore be a viable way of tuning their reduction potentials and concomitantly improve their charge transfer properties without interfering with the local duplex architecture.[4]

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