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DNA Modification |Hot Paper|

Modulation of Excess Electron Transfer through LUMO Gradients in DNA Containing Phenanthrenyl Base Surrogates

Pascal Roethlisberger, Veerabhadrarao Kaliginediand, and Christian J. Leumann*[a]

Abstract: The modulation of excess electron transfer (EET) within DNA containing a dimethylaminopyrene (*C*-AP) as an electron donor and 5-bromouracil (^{Br}dU) as an electron acceptor through phenanthrenyl pairs (phen-R) could be achieved by modifying the phenanthrenyl base surrogates with electron withdrawing and donating groups. Arranging the phenanthrenyl units to form a descending LUMO gradient increased the EET efficiency compared to the electron transfer through uniform LUMOs or an ascending LUMO gradient.

The well-defined double helical structure of DNA with linearly arranged base pairs is a suitable scaffold for charge transfer and is therefore subject to intense studies in DNA damage,[1] sensors,[2] and applications in molecular electronics.[3] Reductive electron transfer, also called excess electron transfer (EET), in DNA is a process that is directed through the lowest unoccupied molecular orbital (LUMO) of the DNA bases. Investigations elucidated that the charge transfer over longer distances occurs by electron hopping, mostly through thymine bases ($k = 10^{10}$ s^{-1}).[4] In earlier studies it was shown that the replacement of the natural base pairs by non-hydrogen-bonding base surrogates with extended aromatic surfaces such as phenanthrene could have beneficial conducting properties and could overcome the physicochemical limitations of the natural nucleobases.[5] Regarding the reduction of such base surrogates the choice of the electron injector is crucial for the success of the experiments. Investigations by Grigorenko et al. revealed that pyrene (^{Py}dU, $E_{red}^* = -2.2$ V vs. NHE)[6] only enables a superexchange mechanism, whereas phenothiazine (PTZ, $E_{red}^* = -2.7$ V vs. SCE)[7] allowed to trigger the system into an electron hopping mechanism with a transport that spreads over longer distances.[5] A photoexcitable dimethylaminopyrenyl donor attached to a deoxyuracil (^{AP}dU, $E_{red}^* = -2.2$ V vs. NHE)[8] that exhibits suitable redox properties for long range charge transfer experiments was successfully used by Bätzner et al. to inject an electron in hydroquinoline base surrogates.[9]

Herein, we investigated the EET through DNA containing phenanthrenyl base surrogates with different reduction potentials and LUMO energy levels (Figure 1). It is believed that the electron transfer within DNA can be modulated by the installation of a potential energy gradient.[10] The predicted advantage of such a redox/LUMO gradient was envisioned to be the unidirectionality of the electron transfer and therefore a gain in efficiency. The installation of the different reduction potentials was deemed to be possible by the introduction of electron-withdrawing (CN) and -donating (NH_2) groups at the 7-position of the phenanthrene (phen). The synthesis of the ^{NH2}phen and phen phosphoramidites applicable for automated DNA synthesis was performed according to published procedures.[11] The

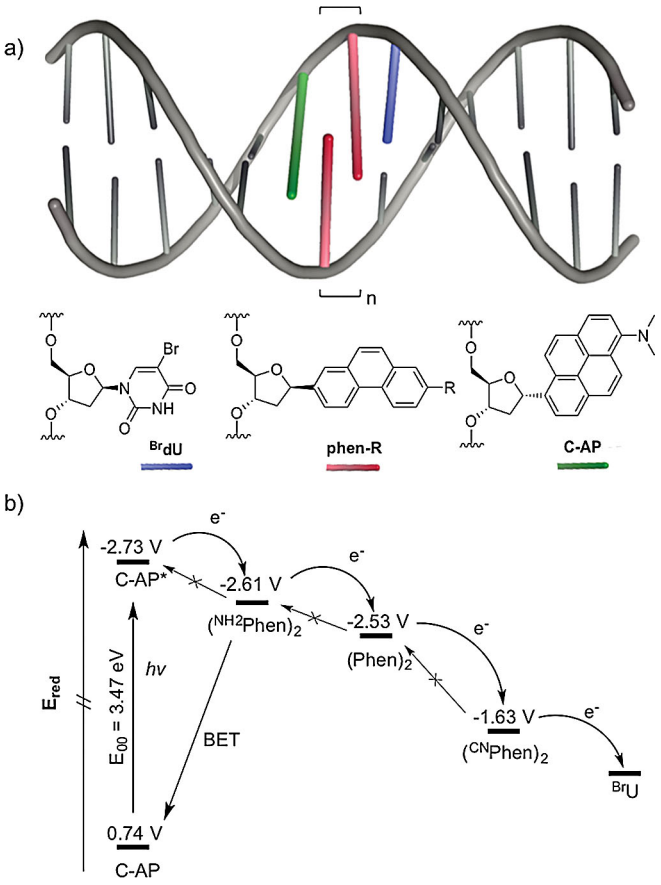

Figure 1. a) Representation of the EET system, consisting of a photoexcitable *C*-AP donor opposite an abasic site, zipper-like stacked phenanthrenyls and a 5-bromouracil (^{Br}dU) as electron acceptor. b) Representation of the energetics in the electron-transfer process of the system consisting of a *C*-AP donor, the modified phenanthrenyls and the ^{Br}dU acceptor.

[a] *Dr. P. Roethlisberger, Dr. V. Kaliginediand, Prof. Dr. C. J. Leumann*
Department of Chemistry and Biochemistry
University of Bern
Freiestrasse 3, 3012 Bern (Switzerland)
E-mail: christian.leumann@dcb.unibe.ch

Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under:
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introduction of a cyano group required a palladium-catalyzed substitution of the intermediate **9** with copper(I) cyanide. Tritylation and phosphitilation of the ^{CN}phen *C*-nucleoside occurred under standard conditions and yielded the corresponding phosphoramidite **12** (Supporting Information, Scheme S3). The redox properties of the building blocks were analyzed by cyclic voltammetry at the level of the free nucleosides **10** (^{CN}pen, $E_{red} = -1.63$ V vs. Ag/AgCl), **13** (^{NH2}phen, $E_{red} = -2.60$ V vs. Ag/AgCl) and **14** (phen, $E_{red} = -2.52$ V vs. Ag/AgCl). Furthermore, the density functional theory calculations (B3LYP/6-31G*) were found to correlate with the experimentally determined reduction potentials (Supporting Information, Figure S1).

To study the EET properties through phenanthrenyl base surrogates, a α-*C*-nucleosidic dimethylamino-pyrene (*C*-AP) was synthesized that could intercalate well against an abasic site, which enables an efficient photoinduced electron injection owing to the close proximity to the phenanthrenyl stack.[12] The synthesis involved a nucleosidation of the chloro Hofer sugar and a Gilman cuprate[13] of the 6-bromo-*N*,*N*-dimethylpyren-1-amine, which was received from bromination, nitrification, reduction, and dimethylation of the amine function of the pyrene (Supporting Information, Schemes S1, S2). According to cyclic voltammetry, this donor (**6**) was found to have suitable reduction potential in the excited state ($E_{red}^* = -2.7$ V vs. Ag/AgCl) to reduce all the phenanthrenyl units.

As an electron acceptor, 5-bromouracil (^{Br}dU) was used, which releases a bromine anion (Br^-) after encounter and capture a migrating electron that is injected into the DNA upon excitation of the *C*-AP at 420 nm. The formed 5-uracyl radical abstracts a hydrogen form the 5′ adjacent deoxyribose, which eventually affords alkali label products under aqueous conditions.[14] The EET efficiency can then be evaluated by fragment analysis using polyacrylamide gel electrophoresis (PAGE) and control sequences as markers for the specific fragments (see Figure 2).

With the phosphoramidites of *C*-AP, phen, ^{CN}phen, ^{NH2}phen, and ^{Br}dU, a series of oligonucleotides were synthesized containing either a single (**D1**–**D16**) or triple (**D17**–**D29**) phenanthrenyl modifications between the pyrenyl donor and the electron acceptor. Thermal denaturing studies revealed that single phen modifications in general lead to a destabilization. On the other hand, multiple phen modifications stabilized the duplex compared to the natural base pairs. This effect was observed in earlier studies with non-hydrogen bonding base surrogates and was found to be an enthalpy-driven process induced by the increased hydrophobic interactions of such base surrogates.[11,15] An expected decrease in stability was observed for duplexes containing electron-donating groups, and vice versa a stabilization of duplexes containing electron-withdrawing groups.

Circular dichroism (CD) spectroscopy revealed that the secondary structure of natural DNA is not disturbed by the phenanthrenyl or pyrenyl modifications.

Initial EET experiments were performed with duplexes **D1**, **D2**, **D3** lacking the donor or **D4**, **D5**, **D6** without an acceptor. Both series did not show any fragment formation upon irradiation. In general, a non-specific cleavage after piperidine treatment without irradiation occurred owing to the applied heat.[16] Along with the specific short fragments a low mobility band occurred in sequences with ^{NH2}phen and phen pairs. According to mass spectrometry, the reaction product correlates to an intrastrand crosslink, as already observed in earlier EET studies with phen pairs (Supporting Information, Figure S5, S6).[5]

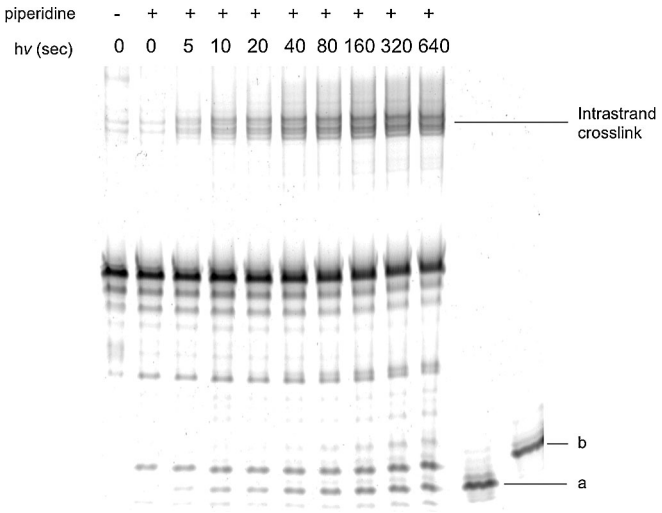

Figure 2. Representative denaturing PAGE showing the fragmentation of the duplex **D8** after 640 s of irradiation at 420 nm. Conditions: 4 μM duplex, 10 mM NaH_2PO_4, 0.15 M NaCl, pH 7.0. The duplex was exposed to the UV light for the indicated amount of time and analyzed after subsequent piperidine treatment at 90 °C for 30 min. Lane 1 contains the control under light exclusion and without piperidine treatment. The additional lanes show the specific fragments; a) PO_4-ACGC-FAM; b) PO_4-TACGC-FAM.

Electron transfer through single phenanthrenyl (**D8**, **D11**, **D14**) pairs is less effective than EET through A/T (**D7**) base pairs owing to the fact that thymine (−0.95 eV)[17] exhibits a lower reduction potential compared to all the phenanthrenyl base surrogates. Additionally, a suppression of hole transfer processes was found for duplexes with base mismatches[18] and bulge positions,[19] suggesting that a slight perturbation of the base stack in the case of single phenanthrenyl pairs is accompanied with suppressing effects as well (see Figure 3).[20]

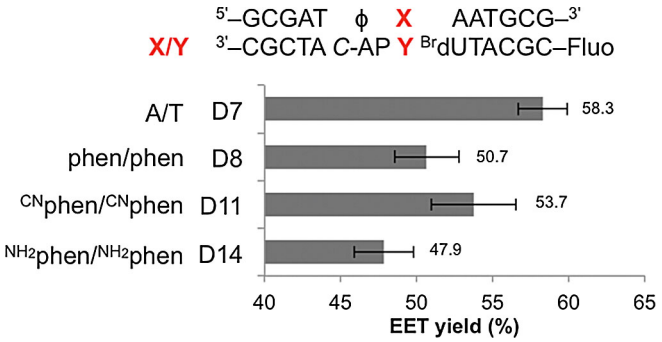

Figure 3. DNA cleavage yields for duplexes **D7-D16** after 640 s irradiation at 420 nm.

Comparing the EET yield of the different phenanthrenyl pairs, it was found that the efficiency of the electron transfer processes correlates with the reducibility of the phenanthrenyl base surrogates (^{CN}phen > phen > ^{NH2}phen). The differences in yield, however, were found to be within a 6% range. This could be explained by the fact that electron transfer over short distances can also occur via hole transfer and therefore lowers the influence of the reducibility in such processes. Analysis of the permutated phenanthrenyl sequences (**D9**, **D10**, **D12**, **D13**) show no statistically significant difference in EET yield and are therefore not further discussed (Supporting Information, Table S4).

Extending the phenanthrenyl units from one to three consecutive incorporations for each strand allows the installation of an electron gradient over longer distance. In this context, gel electrophoretic analysis of irradiated duplexes **D17**, **D18**, **D19**, **D20**, **D21**, lacking an electron donor, revealed two different features: 1) in general no major irradiation dependent strand cleavage occurred through three consecutive phen (**D17**) and ^{CN}phen (**D19**) base pairs; 2) installation of a LUMO gradient in an ascending manner produced an irradiation dependent fragmentation with a yield of up to 33.7% over 640 seconds without the use of an electron injector. It is assumed that the consecutive phenanthrenyl units can form an exciplex and absorb light at higher wavelength. An unexpected dominant strand cleavage product, with a lower mobility than the 5mer, was observed for the ^{NH2}phen containing duplex **D18**. It occurs in the dark as well as in a time dependent fashion upon irradiation. The same fragmentation pattern, but in much lower extent, was observed for duplex **D21** having an ascending LUMO gradient with ^{NH2}phen at the 5′ end of the phen stack. It is not evident from these studies why this fragment is produced in the absence of irradiation at low temperatures (4 °C storage). However, a possible explanation for the fragmentation is that a ground-state reaction is enabled by an enlarged π-stack of ^{NH2}phen units, which could stabilize the resulting cationic species on the 5′ ^{NH2}phen in a similar way to what has been observed for guanine rich sequences in hole transfer (HT) studies.[21]

The EET efficiency through three consecutive A–T base pairs decreased by 13.3% (Figure 4, compare **D7** to **D22**), while the efficiency through three phen pairs remained the same (compare **D8** and **D23**). On the other hand, the extension of the π-stack of ^{CN}phen enhanced the EET yield by +6.3% (compare **D24** to **D11**). Comparing the cleavage product yield of homologous duplexes, it was observed that the electron transfer through ^{CN}phen (**D24**) is 10.7% more efficient than through unsubstituted phen (**D23**) and 15.1% higher than through A–T base pairs (**D22**). Interestingly the increased electron-transfer yield through phen units coincides with an increased stability of the DNA duplexes (see the Supporting Information). It is believed that a favorable conformation of a stabilized duplex allows more efficient electron transfer, as described in hole transfer studies by Wasielewski and co-workers.[22] The fact that the electron transfer efficiency is higher for phen stacks than for neutral A–T base pairs that have an intrinsic lower reduction potential is implying that the transfer process is not solely dependent on the LUMO energy of the participants.

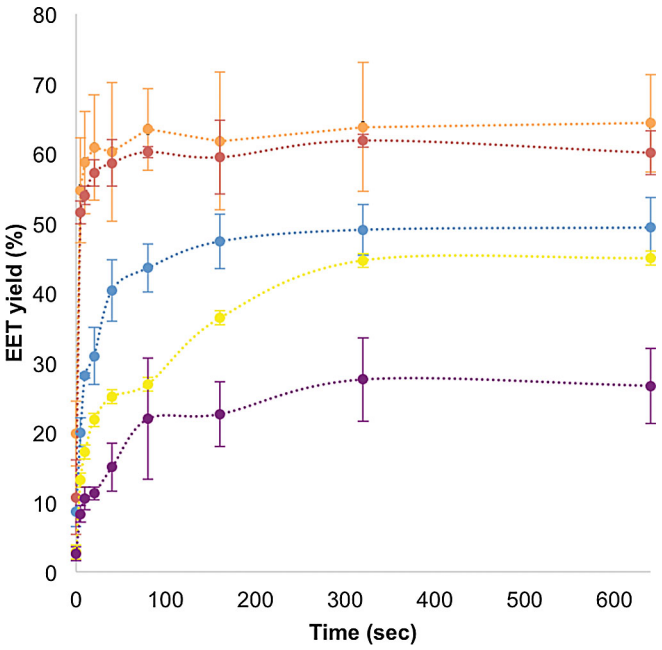

Figure 4. EET from excited *C*-AP to ^{Br}dU through multiple base surrogates as a function of time. (**D27**; purple C^{N}phenphen^{NH2}phen), (**D22**; yellow TTT), (**D23**; blue phenphenphen), (**D24**; red C^{N}phen^{CN}phen^{CN}phen), (**D26**; orange N^{H2}phenphen^{CN}phen). Conditions are given in the legend of Figure 2.

Installation of a descending LUMO gradient as in **D26** resulted in an EET yield that is higher by 4.2% compared to three ^{CN}phen (**D24**) or 15.0% higher compared to three consecutive phenanthrene residues (**D23**; Figure 5). A suppression of back electron transfer and charge recombination could be used to explain the increase in EET.[23] Furthermore, an over two-fold lower transfer performance was observed for the ascending LUMO gradient (**D27**, 26.7%) compared to the descending LUMO gradient (**D26**, 64.3%), highlighting the importance of an exergonic process. Interestingly, the EET yield through duplexes with mixed ascending and descending strands (**D28** and **D29**) a relative high electron transfer yield (54.7% to

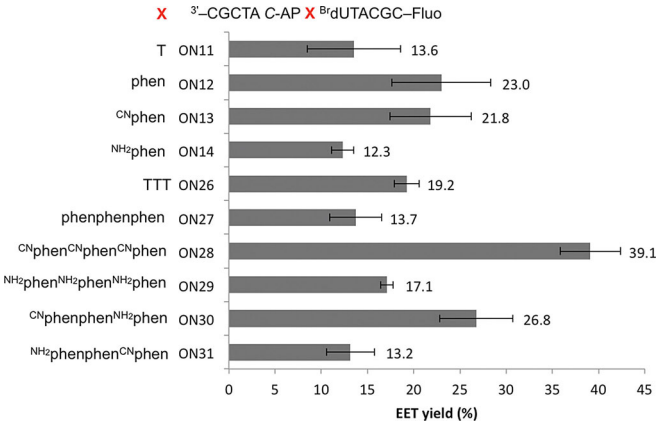

Figure 5. Comparison of DNA cleavage yields of single strands after 640 seconds irradiation at 420 nm.

53.5%) was observed, assuming that the electron transfer occurs not solely by electron hopping but also via electron tunneling to overcome the endergonic migrating steps.

When the electron transfer through single strands was tested, a circa 50% loss of EET efficiency was observed in strands with single phen (**ON12**) and ^{CN}phen (**ON13**) incorporations, while a loss of about 75% of EET was determined for thymine- (**ON11**) and ^{NH2}phen- (**ON14**) containing strands compared to their duplexes. Prolonging the distance between the donor and acceptor in single strands only led to a positive effect on the EET yield in the case of ^{CN}phen (**ON28**-**ON13**, 19.3%) but showed little effect in all other single strands. The observation that phenanthrene with an intrinsic higher reduction potential than thymine (−1.05 vs. Ag/$AgClO_4$ or −1.86 vs. NHE)[17,24] shows a higher electron transfer yield for long-range migrations, indicate that the electron transfer efficiency cannot be explained by the driving force solely, even though the transfer yields increased with decreasing LUMO energies of the phen units. Okamoto et al. introduced the concept of expanded aromatic systems to increase HT efficiencies by taking the advantage of the enhanced π-stacking properties. Experimental findings showed that the expanded aromatic hole mediator enhance the charge transfer over long distances (20 bp).[25] Thus, it is believed that inter alia a high driving force, obtained by the installation of a descending LUMO gradient as well as the intrinsic large aromatic π-surfaces play a crucial role for the EET efficiency.

In summary, the *C*-nucleosidic *C*-AP donor was found to be powerful and stable electron donor for EET experiments with an absorption band around 400 nm allowing for a selective excitation. Introducing modified phen base surrogates allowed the study of electron transfer through LUMO energy gradients. Although the interaction of the phenanthrenyl pairs within DNA could alter their LUMO levels to some extent, it is still possible to estimate the efficiency of the EET based on the LUMO levels of the isolated polyaromatic nucleosides. Indeed, an enhancement of electron transfer was found through a descending gradient compared to flat or ascending LUMO energy levels. The control of the electron transfer directionality widens the potential application of devices based on artificial DNA.

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Keywords: electron transfer · oligonucleotides · phenanthrene · stacking interactions

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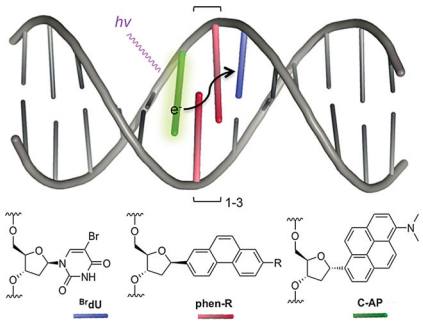
COMMUNICATION

Excess electron transfer (EET) can be modulated within DNA by introducing a dimethylaminopyrene (C-AP) base surrogate as an electron donor, 5-bromouracil (^{Br}dU) as an electron acceptor, and by changing the electronic nature of the phenanthrenyl pair.



DNA Modification

*P. Roethlisberger, V. Kaliginediand, C. J. Leumann**

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