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$$[PdL_2] + PhI \rightarrow [Pd(I)L_2(Ph)] + \Delta H_r \quad (1)$$

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Weak Distance Dependence of Excess Electron Transfer in DNA**

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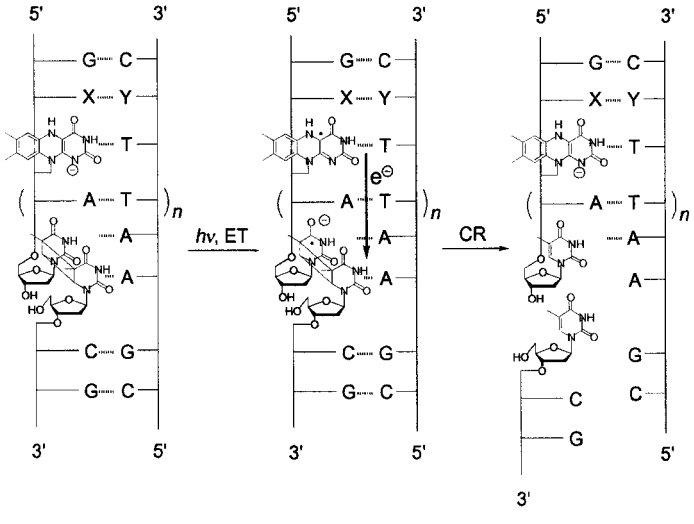
Electron transfer phenomena in DNA are of fundamental importance for DNA damage[1] and DNA repair.[2] In this context, the movement of a positive charge (hole) through DNA has been studied intensively over the past few years.[3–6] After a controversial debate about the "hole conductivity" of double-stranded DNA,[7, 8] it is now accepted that a positive charge can move through DNA over significant distances.[9] Two mechanisms, namely coherent superexchange for small transfer distances[10, 11] and hole[4] or polaron hopping[6] for long-range transfer are evoked to describe this phenomenon. Experimentally, it is becoming clear that long-range hole transfer requires intermediate guanines and to a lesser extent also adenines to function as temporarily oxidizable "stepping stones".[12] Whether long-range hole transfer has biological consequences is unclear, but there are speculations that the process might define the genome sites damaged during oxidative stress.[13]

In contrast to hole transfer, little is known about the transport of excess electrons (negative charges) through a DNA duplex.[14, 15] Such an excess electron transfer, however, is important in biology because DNA photolyase enzymes[16–18] repair UV-induced cyclobutane pyrimidine dimer lesions in the DNA duplex by an electron transfer from a reduced and deprotonated $FADH^-$ cofactor to the dimer lesion.[19] The cytotoxic and mutagenic dimer lesions undergo rapid cycloreversion after one-electron reduction, which rescues cells from UV-induced cell death.[20] Recently γ-radiolysis together with electron paramagnetic resonance (EPR) studies provided initial evidence that excess electrons might also move through the DNA duplex by thermally activated hopping.[21–24] In the reductive regime, however, thymines and cytosines are predicted to function as "stepping stones" because they are the most easily reduced nucleobases.[25, 26]

We report here on the distance dependence of an excess electron transfer in a defined donor–DNA–acceptor system recently introduced by us as a photolyase mimic.[27, 28] In DNA double strands like **1** (Scheme 1), the electron flows from a photoexcited reduced flavin electron donor to a thymine dimer acceptor, separated by adenine:thymine bridges $(A:T)_n$ of various lengths. The electron injection is initiated by irradiation of the DNA double strand at 360 nm which causes excitation of the reduced and deprotonated flavin donor. The injected electron can either recombine with the resulting neutral flavin radical or it can move until it reduces the thymine dimer **1**→**2**. The negative charge captured by the dimer subsequently triggers a cycloreversion **2**→**3**. To allow rapid quantification of this dimer reversal step, we introduced a special dimer into the DNA strand, which lacks the connecting phosphodiester unit between the 3′- and 5′-



Scheme 1. Depiction of the DNA double strands containing the reduced flavin electron injector and the dimer electron acceptor. Photoexcitation (*hν*) initiates charge injection and transfer of the negative charge (ET) to the dimer unit (**1**→**2**). This causes cycloreversion (CR) of the dimer, which is accompanied by a strand break (**2**→**3**). Photolysis was performed in a fluorimeter (JASCO) equipped with a 150-W xenon lamp and a monochromator (10-nm band path) at 360 nm and at pH 9 (Tris buffer). Irradiations were carried out after reduction of the flavin in the DNA duplex by addition of sodium dithionite solution in the absence of oxygen at 10 °C.

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hydroxy groups.[29] Cycloreversion of this "backbone-opened" dimer induces a strand break, which is easily detectable by HPLC.[30]

The distance dependence of the reductive charge transfer was investigated with the DNA double-strand series **1a–e** (see Figure 2a). The double strands were dissolved in buffer (10^{-5} M), and the solutions were transferred to UV cuvettes sealed with rubber septa. The cuvettes were purged with nitrogen for 20 min to establish anaerobic conditions, then a sodium dithionite solution was added to reduce the flavin. These solutions were stirred and irradiated under exactly identical conditions in a fluorimeter with the same light intensity. During the irradiation small samples (10 μL) were removed with a microsyringe, immediatly reoxidized by shaking them exposed to air, and analyzed for the amount of cleaved product by reversed-phase HPLC.[31, 32]

The HPLC analysis of an irradition reaction performed with **1a** is depicted in Figure 1. Clearly evident is the clean photoinduced cleavage of **1a** ($t_{ret} = 13$ min) to give the flavin-containing shorter product strand ($t_{ret} = 11$ min). All DNA

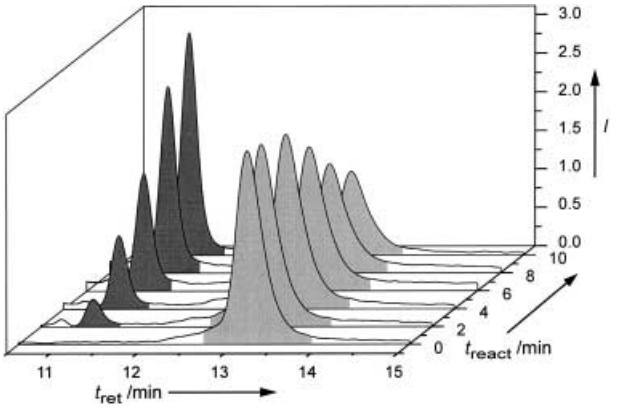

Figure 1. Time-dependent HPLC analysis of the electron transfer driven cycloreversion of **1a**. During the irradiation experiment small aliquots (10 μL) were removed from the assay solution. These samples were shaken rigorously in the presence of air in the dark to reoxidize the flavin. Analysis of these samples by ion-exchange chromatography with a Nucleogen DEAE column (125 mm × 4 mm, 60 Å/7 μm) and a KCl gradient (0 M KCl → 1.0 M KCl, 20 mM sodium acetate buffer pH 6) enabled detection at 360 nm of the flavin-containing starting DNA (retention time = 13 min) and the "flavin-tagged" product strand (retention time = 11 min). Integration of the peak areas was performed with standard software. t_{ret} = retention time, t_{react} = irradiation time.

duplexes investigated (**1a–e**) gave such clean cycloreversions and no degradation products were observed. Plots of the yield of cleaved DNA versus the irradiation time (Figure 2b) gave straight lines approximately up to 20% conversion. The data was analyzed in this regime by linear approximation. We finally determined the quantum yield of the cleavage reaction in **1a**. A value of $\Phi = 10^{-3}$ was determined, which is among the highest reported for photochemistry in DNA.[33]

Two further experiments were performed to prove the intramolecularity of the repair reaction. 1) Concentration-dependent studies show that the repair rate does not change even after the DNA concentration is decreased by a factor of five or ten. 2) When a noncomplementary dimer-containing single strand was added to a solution of double-stranded

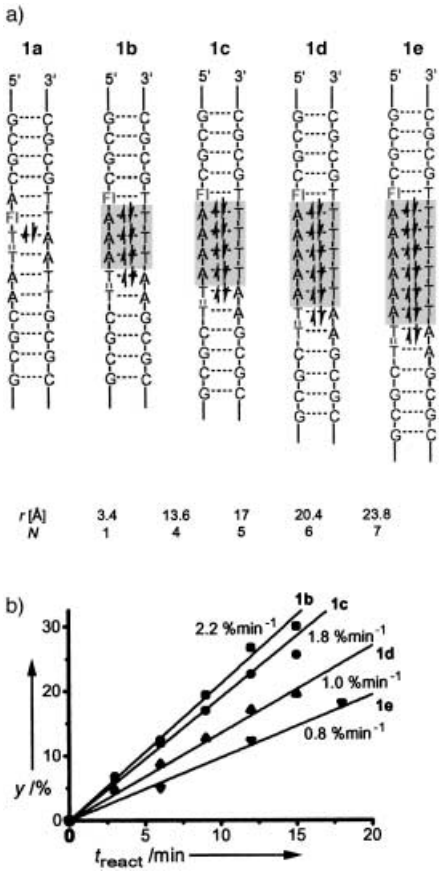

Figure 2. a) Depiction of the oligonucleotides **1a–e** containing a thymine dimer and a flavin at increasing distances. b) A plot of the observed yields versus the irradiation time. The data up to 20% conversion were fitted linearly. ■ = **1b**, ● = **1c**, ▲ = **1d**, ▼ = **1e**. For the determination of the quantum yield, the number of photons emitted onto the cuvette were determined with a two-channel optometer from United Detector Technology. The value $\varepsilon = 4900\ L\,mol^{-1}\,cm^{-1}$ was used as the extinction coefficient of the reduced flavin at 366 nm. y/% = amount of cleaved DNA in percent.

DNA, the double strand was repaired but not the added single strand.

In the double-strand series **1b–e** the flavin donor and the dimer acceptor are separated by a minimum of three A:T base pairs. This ensures that the duplex can adopt a B-conformation between the potentially duplex-disturbing chromophores. Because conformation and stacking might influence the efficiency of excess electron transfer[34] we investigated the structure of the DNA duplexes **1b–e** by circular dichroism (CD) spectroscopy and performed UV-melting studies. All DNA duplexes adopt a B-duplex conformation based on their CD spectra, which show a maximum at 280 nm and a minimum at 250 nm. Thermal denaturing of the duplexes provided in all cases two melting points.[35] One melting point at approximately 25 °C was observed at 260 nm and 460 nm and hence attributed to local melting of the duplex around the flavin. This melting point was confirmed by fluorescence melting studies that showed sigmoidal increase of the flavin fluorescence at the same temperature. This suggests flavin destacking. The second melting point was observed only at 260 nm and hence was attributed to full denaturing of the duplexes. All oligonucleotides **1b–e** show sharp melting

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behaviors and no hysteresis. These initial data suggest that the flavin donor is intercalated into the duplex. For the thymine dimer, stacking inside the duplex caused only small structural perturbations, confirmed by NMR spectroscopy.[36–39] We assume that our DNA duplexes exist in a B-like conformation below the first melting point at about 20 °C. All electron transfer studies were in consequence performed at 10 °C. Analysis of the cleavage efficiency in **1b**–**e** shows that the observed yield decreases from 2.2 % after 1 min of irradiation of **1b** to 0.8 % after 1 min of irradiation of **1e** (Figure 2b). The cleavage yield hence decreases only by about 30 % with every additional A:T base pair between the donor and the acceptor.

If we assume that the negative charge is transferred directly from the donor (D) to the acceptor (A) by coherent superexchange, then the distance dependence of the charge transfer rate k_{CT} would be described by Equation (1).

$$k_{CT} = A\,e^{-\beta' \Delta r} \quad (1)$$

If we assume electron hopping, in which the thymines act as discrete charge carriers, the distance dependence of the charge transfer would be shallow and described in the simplest case by Equation (2), where N is the number of hopping steps and η a proportionality factor, which in the ideal case of a random walk has the value of $\eta = 2$.

$$\ln(k_{CT}) = -\eta \ln(N) \quad (2)$$

As a result of the constant irradiation conditions and under the assumption that the electron injection efficiency is the same in all DNA strands, the observed differences in yield reflect directly differences in the efficiency of the electron transfer from the flavin donor to the dimer acceptor.

A plot of our observed yields as ln(yield per minute) against the distance as Δr (Å) between the flavin donor and the dimer acceptor, assuming the duplex is in the B-conformation, is depicted in Figure 3a. We obtain a straight line and a rather small β' value of just 0.1 Å^{-1}. Such small β' values were determined for long-range hole transfer as well.[40] Our data show that excess electron transfer proceeds with similar efficiency. Plotting our yield data according to the hopping model ln(yield per minute) against ln(N) by assuming that every thymine between the flavin donor and the dimer acceptor can function as a discrete charge carrier, gives a straight line as well (Figure 3b) and a plausible η-value of close to 2.[21–23]

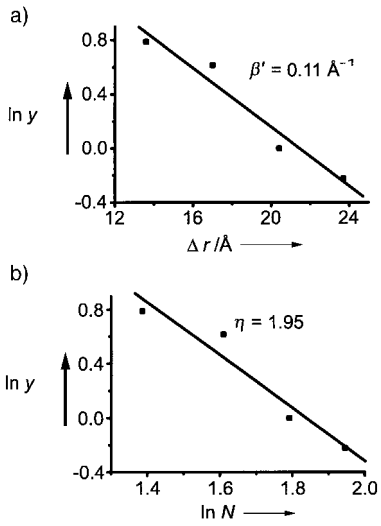

Figure 3. a) A plot of $\ln y = \ln$(yield per minute) against the distance Δr (in Å) between the flavin and the dimer in **1b**–**e**. b) A plot of $\ln y$ against $\ln(N)$, where N is the number of putative electron hopping steps defined by the number of thymines between the flavin and the thymine dimer.

Today, small β' values are generally taken as an indication that the charge transfer proceeds by hopping. In fact, hole-transfer studies with DNA duplexes, in which hopping is not possible, provided β' values between 0.7 Å[41] and 1.4 Å.[42, 43] Our small β' value of 0.1 furnishes therefore evidence that excess electron transfer in DNA might involve thermally activated hopping. This view is supported by thermodynamic considerations. The free energy for the electron transfer steps involved in such an excess electron hopping can be estimated from the singlet energy of the reduced flavin ($E_s = -2.8$ V, vs SCE)[44] and the reduction potentials of thymine ($E_{red} = -2.1$ V, vs SCE in CH_3CN)[44] and of the thymine dimer ($E_{red} = -2.2$ V, vs SCE in CH_3CN).[44] Electron transfer from the reduced flavin to thymine and to the dimer is exergonic. Electron transfer from the thymine radical anion to the thymine dimer should be approximately thermoneutral.

The fact that a single electron can move through a DNA duplex with a rather weak distance dependence—which allowed for the first time remote reductive repair of thymine dimers over a distance of about 24 Å—has important consequences in biology. For example, the harmful effect of UV irradiation responsible for the development of skin cancer in humans[45, 46] could be reduced by compounds that bind to DNA and trigger long-range electron transport.

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A Highly Specific Ca^{2+}-Ion Sensor: Signaling by Exciton Interaction in a Rigid – Flexible – Rigid Bichromophoric "H" Foldamer**

Ayyappanpillai Ajayaghosh,* Easwaran Arunkumar, and Jörg Daub

The design of chemosensors which are specific for the detection of biologically relevant cations, such as Na^+, K^+, Mg^{2+}, and Ca^{2+}, is a topic of considerable interest.[1] Selective detection of one of these cations in the presence of others, particularly the sensing of Ca^{2+} ions in the presence of Na^+, K^+, and Mg^{2+} ions, is a challenging task. Crown-ether based binding sites, which are integrated into the signaling units of organic chromophores, are the usual configuration of a chemosensor.[1b] A recognition event is signaled by perturbation in the absorption or emission properties of the chromophore, which are highly sensitive and easy to detect.[2] Even though a variety of crown-ether and related macrocycle-based chemosensors are known, the corresponding acyclic-polyether- (podand-) based sensors are relatively rare.[3] In most of the latter systems, the binding of a metal ion forces the attached donor and the acceptor moieties to come close enough to interact, thereby triggering either a charge-transfer, electron-transfer, or energy-transfer process, except in a recent report where conformational restrictions and charge transfer are invoked for a dual signaling of cation binding.[3h]

Herein we describe a different approach to the design of a highly specific Ca^{2+} ion sensor, which exploits the principle of a metal-ion induced conformational folding of a rigid – flexible – rigid bichromophore **3** (Scheme 1) to form an "H" foldamer,[4] thereby leading to dramatic perturbations in the optical properties as a result of exciton interactions. To our knowledge, this is the first report of a cation sensor based on the exciton interaction of a Ca^{2+} foldamer, in which the tethered chromophores are positioned akin to the "H" aggregate of an organic dye.

The rationale behind the design of the new sensor is based on the formation of "H" and "J" aggregates of cyanines and squaraines, which show distinctly different optical properties under appropriate conditions.[5, 6] According to the exciton theory of Kasha, the excited-state energy level of a monomeric dye splits into two upon aggregation, one level being lower and the other higher in energy than the monomer excited states.[7] Studies related to the aggregation of squar-

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