Photoinduced Electron Transfer in DNA Matrix from Intercalated Ethidium to Condensed Methylviologen

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We report on an enhancement of the yield of intermolecular electron transfer by half a million as achieved by assembling donor and acceptor in the double helix of DNA. The donor is photoexcited ethidium intercalated in the stack of bases, the acceptor is methylviologen condensed as a mobile coat (Figure 1).

In recent years micelles, vesicles, and polyelectrolytes have been applied to attain efficient electronic charge separation.[1] The spatial organization of pigments by those colloids is rather poor. We take advantage of the double helix of nucleic acid with its well-defined coat/core structure of hydrophilic backbone of ribose phosphate around the hydrophobic pile of stacked bases. This matrix exhibits the combined features of a polymerized micelle and of a liquid-crystalline polymer. As a first step of the synthesis of a molecular machine—designed in analogy to assemblies in a surfactant matrix[2]—we study in the present paper electron transfer from core to coat of DNA, from photoexcited ethidium (ET^+) to methylviologen (MV^{2+}).[3]

Materials and Methods. Calf thymus DNA (Sigma, type III) is purified by phenol extraction.[4] $MVCl_2$ (Serva), EtBr (Sigma), and cacodylate (Sigma) are used as supplied. Binding of MV^{2+} is determined form its optical absorption at 258 nm[5] in the ultrafiltrate of 10^{-4} M DNA (Amicon XM50)[6] that of ET^+ from its change of absorption at 480 nm and its enhanced fluorescence at 600 nm.[7] Fluorescence decay is measured by the sampling technique (DFD-laser,[8] photodiode Oriel 1850, oscilloscope Tektronix 77904/S4). Transient MV^+ is detected by its absorption at 605 nm[9] (signal averaging with Biomation 805/Nicolet 1070) after dye-laser (Lambda-Physik) excitation.

Structure. MV^{2+} binds efficiencly to DNA. With 1.5 mM cacodylate at pH 6 the ratio ν_{MV} of bound MV^{2+} to base pairs is described by a Langmuir isotherm with a binding constant $K_{MV} = 1.8 \times 10^5\ M^{-1}$ and a saturation $\bar{\nu}_{MV} = 1/bp$. We assign this binding to a condensation around the double helix[10] with possible accumulation in the grooves.[11]

Intercalation of ET^+ in DNA[12] is described formally by a Langmuir isotherm with a saturation $\bar{\nu}_{ET} = 0.4/bp$.[7,13] With 1.5 mM cacodylate, the binding constant is $K_{ET} = 4.1 \times 10^6\ M^{-1}$. With $c_{DNA} = 10^{-4}$ M and a total concentration $c_{ET} = 10^{-6}$ M the fraction of bound ET^+ is 98.7%.

The binding constant of ET^+ is lowered in its electrostatic part[14] by condensation of MV^{2+}. It drops to $K_{ET} = 2 \times 10^6\ M^{-1}$ at a bulk concentration $c_{MV}^w = 10^{-4}$ M ($\nu_{MV} = 0.95/bp$). With $c_{DNA} = 10^{-4}$ M and $c_{ET} = 10^{-6}$ M, 97.4% of added ET^+ is still bound. It is the distinctly different binding to core and coat which permits

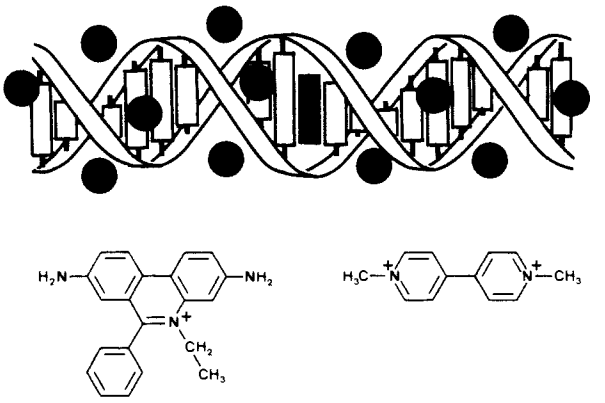

Figure 1. Schematic representation of the assembly of the double helix of DNA with intercalated ethidium (black brick, formula left) and with condensed methylviologen (black dots, formula right). Note the distinctly different nature of binding: Ethidium immobilized in the core, methylviologen as a mobile coat. Electrons are transferred from photoexcited ethidium to methylviologen.

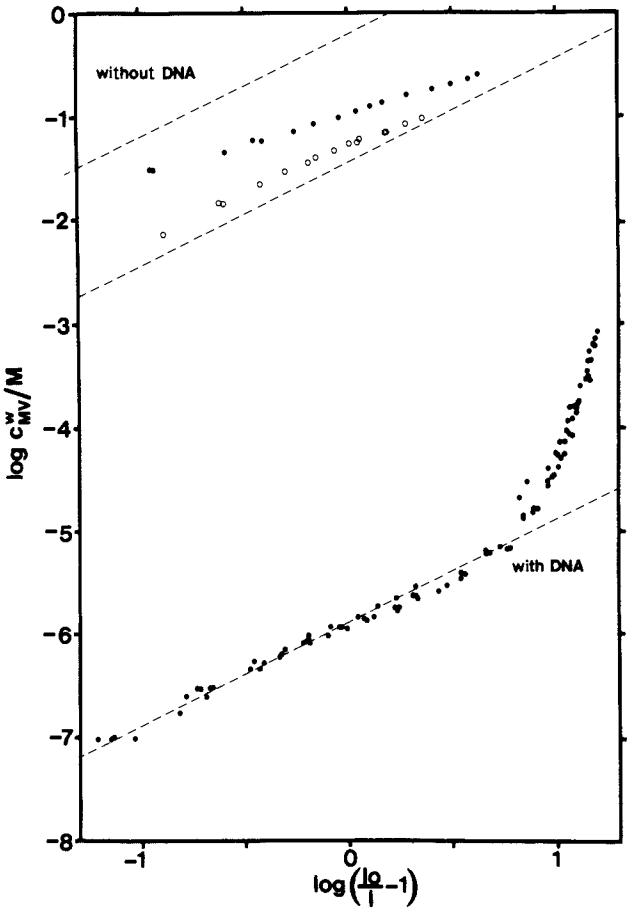

Figure 2. Free concentration of methylviologen c_{MV}^w in water (temperature 298 K, 1.5 mM cacodylate pH 6) required to attain quenching $I_0/I - 1$ of fluorescence intensity I of ethidium (excitation at 510 nm, emission at 600 nm, I_0 intensity without methylviologen). Double-logarithmic Stern-Volmer plot. (Bottom) Data with 10^{-4} M DNA and 10^{-6} M ethidium at a ratio ethidium:base pairs of $\nu_{ET} = 0.02/bp$. Stern-Volmer line fitted to data at low concentration. (Top) Data without DNA with 10^{-6} M ethidium. Dots without salt, circles with 1 M NaCl. Upper Stern-Volmer line refers to vanishing ionic strength, lower line refers to infinite ionic strength.

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a double doping of DNA by ET^+ and MV^{2+} (Figure 1).

Electron Transfer. The interaction of ET^+ and MV^{2+} in water is indicated by the quenching of fluorescence of ET^+ by an increasing concentration c_{MV}^w (Figure 2). After correcting the data for ion-ion interaction (Debye-Hückel), we obtain for the relative intensity I/I_0 a Stern-Volmer relation $I_0/I - 1 = K_Q^w c_{MV}^w$ with $K_Q^w = 1.5\ M^{-1}$ (Figure 2). Quenching is enhanced by NaCl (Figure 2). For infinite concentration of salt we extrapolate $K_Q^s = 27.5\ M^{-1}$. We assign the quenching to electron transfer considering the redox potentials[15] $E'_0(ET^{+*}/ET^{2+}) = -0.52\ V_{NHE}$ (from $E'_0(ET^+/ET^{2+}) = 1.68\ V_{NHE}$) and $E'_0(MV^+/MV^{2+}) = -0.44\ V_{NHE}$. In fact, after flash excitation of ET^+ we observe reduced MV^+. The maximal rate constant with salt is $k_{el}^s = 1.6 \times 10^{10}\ M^{-1}\ s^{-1}$, considering $K_Q^s = k_{el}^s \tau_{ET}^s$ with a lifetime $\tau_{ET}^s = 1.7$ ns.

With ET^+ bound to DNA ($\nu_{ET} = 0.02$/bp), its fluorescence is quenched by MV^{2+} most efficiently (Figure 2). At low concentration we obtain a Stern-Volmer relation with $K_Q^{DNA} = 7.6 \times 10^5\ M^{-1}$. The DNA matrix enhances the yield of electron transfer by a factor $K_Q^{DNA}/K_Q^w = 507000$. Saturation is observed around $I_0/I = 10$ above $c_{MV}^w = 10^{-5}$ M.

Five contributions may be responsible for the dramatic effect: (i) enhanced lifetime of ET^{+*}, (ii) reduced repulsion of approaching reactands, (iii) enhanced interfacial concentration c_{MV}^i, (iv) restriction of diffusion, and (v) modulation of electron transfer. With $\tau_{ET}^{DNA} = 20$ ns the first effect contributes a factor of $\tau_{ET}^{DNA}/\tau_{ET}^s = 12$. The factor of vanishing repulsion is indicated by the salt effect $K_Q^s/K_Q^w = 18$. Accumulation is estimated as $c_{MV}^i/c_{MV}^w = 2.2 \times 10^5$. (From $c_{MV}^i = \nu_{MV}/V_i$ with an interfacial volume $V_i = \pi(2r_H w_i + w_i^2)h_{bp} = 1.34\ nm^3$/bp, with helix radius $r_H = 1$ nm, width of interfacial shell $w_i = 0.5$ nm, and height of base pair $h_{bp} = 0.34$ nm/bp, considering $\nu_{MV} = \bar{\nu}_{MV} K_{MV} c_{MV}^w$ at low concentration). Thus the cumulative effect of the contributions i-iii could be 100 times larger than the enhancement actually observed. The discrepancy may indicate a slowing down of electron transfer itself (diffusion or reaction).

We restrict a mechanistic interpretation to a sketch of two limiting aspects, diffusion control at low occupation and reaction control near saturation. **Diffusion control**: In the limit of low occupation we describe fluorescence quenching by diffusion control in 1D as $I_0/I - 1 = 1.8(4D_{MV}\tau_{ET}^{DNA}/\pi h_{bp}^2)^{1/2}\nu_{MV}$. (The nonstationary term of diffusion[16] dominates in 1D.) Comparison with the data by using $\nu_{MV} = \bar{\nu}_{MV} K_{MV} c_{MV}^w$ yields a diffusion coefficient $D_{MV} = 2.5 \times 10^{-7}\ cm^2/s$. The mobility of MV^{2+} along the double helix appears to be restricted. **Reaction control**: In the limit of saturation we consider electron transfer between localized reactands in the nonadiabatic limit with $k_{el} = k_{el}^0 \exp(-E^*/kT)$ with $k_{el}^0 = V_{el}^2(h^2\lambda kT/4\pi^3)^{-1/2}$ and $E^* = (1 + \Delta/\lambda)^2(\lambda/4)$ (interaction energy V_{el}, Planck's constant h, drop of redox potential Δ, reorganization energy λ).[17] The rate constant is obtained from $I_0/I - 1 = k_{el}\tau_{ET}^{DNA}$. From an Arrhenius plot between 5 and 40 °C we determine $k_{el}^0 = 1.1 \times 10^{12}\ s^{-1}$ with $E^* = 0.180$ eV. With $\Delta = 0.1$ eV, the reorganization energy is $\lambda = 0.95$ eV and the interaction energy $V_{el} = 8$ meV. This large value indicates an intimate contact of donor and acceptor. Without further knowledge of the precise location of MV^{2+} and of the redox potentials in DNA, we avoid a more detailed analysis.[18]

Summary. We use the double helix as a medium for an organized reaction. The well-defined matrix allows a detailed evaluation of photoinduced electron transfer between intercalated ethidium and condensed methylviologen. In preliminary time-resolved studies we have observed that the nonexponential decay of ET^{+*} occurs within 1 ns on one hand and that the lifetime of hole and electron as ET^{2+} and MV^+ is beyond 1 ms on the other hand. We proceed in the assembly of a photosynthetic reaction chain in defined sequences of DNA.

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Photolysis of *N*-(2-Methyl-2-propenyl)phthalimide in Methanol. Evidence Supporting Radical-Radical Coupling of a Photochemically Generated Radical Ion Pair

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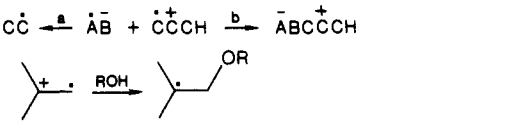
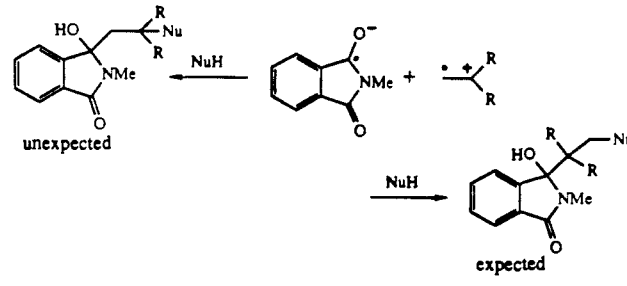
The nature of radical cation-radical anion interactions is a topic of current interest. For photochemically generated radical ion pairs in the absence of nucleophiles the major process is usually back electron transfer or proton transfer to give a radical pair[1] (path a) although radical-radical coupling of the radical ion pair



(path b) to a zwitterion has been suggested in several cases.[2] In nucleophilic solvents, such as alcohols, it is well established that the alcohol adds to the radical cation in an anti-Markovnikov manner[3] to give the most stable radical which can then undergo subsequent reactions. To date there is no convincing data available to support radical-radical coupling of the radical ion pair (path b) and the conclusive demonstration that this process occurs would establish a new reaction of photochemically generated radical ion pairs.

It appeared to us that the most convincing evidence for radical-radical coupling of a photochemically generated radical ion pair would be to trap the resultant zwitterionic intermediate, most conveniently by addition to a nucleophile. To accomplish this, conditions would have to be set up to favor radical coupling over nucleophilic addition to the radical cation and an unexpected product, that which would have been formed from "Markovnikov" addition of the nucleophile to the radical cation, would have to result.



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