Magnetic Field Effect on Photoinduced Electron Transfer between $[Cu(phen)_2]^{2+}$ and DNA

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The magnetic field effect (MFE) on the photoinduced electron transfer (PET) reaction between the $[Cu(phen)_2]^{2+}$ complex and DNA has been studied in homogeneous buffer medium and in reverse micelles. The copper complex on photoexcitation can oxidize DNA in a deoxygenated environment. A prominent MFE is found even in a homogeneous aqueous medium for the triplet born radicals. The process of partial intercalation of $[Cu(phen)_2]^{2+}$ complex within DNA is responsible for such a rare observation. In reverse micelles, the MFE is not very much prominent because of the large separation distance between the component radicals of the geminate radical ion pairs generated through PET.

Introduction

The role of electron transfer in drug−DNA interaction has become a very interesting problems in recent years.[1] It has been found that transition metal complexes are such an important class of compounds that undergo electron transfer with DNA. The 2:1 1,10-phenanthroline (phen)−copper (I) complex is the first synthetic coordination complex, which acts as a "chemical nuclease" with an efficient nucleolytic activity in presence of reducing agents, e.g., thiol or ascorbic acid, and molecular oxygen or hydrogen peroxide.[2] The complex binds noncovalently to double stranded DNA in a sequence specific manner.[3] The mode of binding of the tetrahedral $[Cu(phen)_2]^+$ complex is not very conclusive. Somewhere it has been proposed that the complex binds to DNA by intercalation between base pairs.[4−6] There is also an alternative suggestion regarding the mode of binding; that is, insertion of one phen ligand into the minor groove.[7] Another possibility of an external binding mode of the complex and DNA[8] has been suggested in which one phen lies in the minor groove, whereas the other phen extends outside and remains parallel to the helix axis.[9] Thus there exists a fuzziness regarding the binding mode of the complex with DNA. On the other hand, the mechanism of action of this first chemical nuclease is quite lucid. During nuclease activity, the monovalent complex binds to DNA reversibly and the metal center toggles between +1 and +2 oxidation states.[10] There are comparatively fewer reports on binding of the corresponding cupric complex to double stranded DNA. Competitive studies on emission of ethidium bromide and viscometry reveal that the corresponding cupric complex also binds to calf thymus DNA.[11] Kinetic analysis showed that the dissociation constant of the cupric complex from DNA is greater compared to the cuprous complex,[10] which infers stronger binding of Cu(I) with DNA than Cu(II).

In this paper, we report the mechanism of an electron-transfer phenomenon occurring between DNA and the $[Cu(phen)_2]^{2+}$ complex on photoexcitation. In this photoinduced electron transfer (PET) phenomenon, neither reducing agent nor the presence of oxygen or H_2O_2 is required or necessary for the oxidation of DNA. The PET reactions involve formation of radical ion pairs (RIPs) initially and, in general, can be affected by an internal or external magnetic field (MF) due to the presence of two spin-correlated free electrons in the geminate RIPs.[12−16] Magnetic field effect (MFE) is basically interplay between spin dynamics and diffusion dynamics. By diffusion, the RIPs can separate to an optimum distance where the exchange interaction (J) becomes negligible. In this situation, the electron−nuclear hyperfine coupling induces efficient mixing between the triplet ($T_{\pm}$, T_0) and the singlet (S) states. The application of an external MF of the order of hyperfine interaction (HFI) removes the degeneracy of the triplet states and reduces intersystem crossing (ISC), thus resulting in an increase in the population of the initial spin state. This is reflected from the increase in absorbance and decrease in decay rate constant of the transients produced. Thus MFE importantly serves to identify the initial electronic spin state of the RIPs. Again, the MFE is very much sensitive to the distance between the participating radical ions because the hyperfine induced spin flipping depends on *J*, which in turn has exponential distance dependence. When the RIPs are in contact, the S−T splitting caused by *J* is much stronger than the hyperfine coupling energies so that spin evolution cannot occur by this mechanism. On the other hand, at a distance where *J* is sufficiently small, S−T conversion becomes facile. However, if the separation between the two radicals is too great, the geminate characteristics get lost and, consequently, MFE cannot be observed. Therefore, an optimum separation between the RIPs is required so that both spin flipping and recombination are feasible. Generally MFE experiments on the triplet born transients involve micellar media[10,17,18] or highly viscous solvents[19−21] at low temperature or long chain biradicals[22,23] to reduce fast escape, thus retaining the spin-correlation between the partners of the geminate RIP. However, there are few examples found in the literature, where MFE has been detected in homogeneous medium by transient absorption of the triplets and the radical ions.[24−33] Interestingly, in this case, we have found prominent MFE for the triplet born radicals during the interaction of $[Cu(phen)_2]^{2+}$ with DNA even in a homogeneous aqueous medium, which is a rare phenomenon. Obviously when we used organized assemblies, e.g.,

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reverse micelles instead of water, as the reaction medium, MFE is observed. The process of partial intercalation of the complex within DNA might be responsible for the observation of MFE in homogeneous medium.

Experimental Section

Materials. Tris buffer was obtained from Spectrochem. Heptane (HP) was obtained from Merck (Uvasol). Sodium bis-(2-ethylhexyl)sulfosuccinate (AOT) was purchased from Sigma was used as such. Highly polymerized calf thymus DNA (CT DNA) was purchased from Sisco Research Laboratory, India, and used as received. After the DNA fibers were dissolved in buffer, the purity of the DNA was checked from the absorbance ratio A_{260}/A_{280}. The ratio was greater than 1.9. Water was triply distilled. All the solutions were prepared in 50 mM Tris-HCl and 5 mM NaCl buffer at pH 7.4, which is mentioned as aqueous medium or buffer.

Methods and Instrumentation. The copper complex $[Cu(phen)_2](ClO_4)_2$ **(1)** was prepared in the laboratory by adopting the procedure described for $Cu(phen)_2Cl_2 \cdot H_2O$.[34] It was purified by repeated crystallization. Anal. Calcd for $Cu(phen)_2(ClO_4)_2 \cdot H_2O$: C, 44.96; H, 2.81; N, 8.74. Found: C, 45.05; H, 2.66; N, 7.88.

Preparation of Reverse Micelles. AOT reverse micelles were prepared in HP.[35] The complex and DNA was mixed in buffer and the desired amount of this buffer was added for W_0 variation (W_0 = [H_2O]/molar concentration of the reverse micelle) as described by Imre and Luisi.[36] The final concentration of the complex was 40 μM. The concentration of the surfactant was 0.2 M.

The transient absorption spectra were measured by using a nanosecond flash photolysis setup (Applied Photophysics) having an Nd:YAG laser (DCR-11, Spectra Physics) described elsewhere.[37] The sample was excited by 266 nm laser light with ~8 ns fwhm. Transients were monitored through absorption of light from a pulsed Xe lamp (250 W). The photomultiplier (IP28) output was fed into a Tektronix oscilloscope (TDS 3054B, 500 MHz, 5Gs/s), and the data were transferred to a computer using the TekVISA software. MFE on the transient absorption spectra was studied by passing dc through a pair of electromagnetic coils placed inside the sample chamber. The strength of MF can be varied from 0.0 to 0.08 T. The software Origin 5.0 was used for curve fitting. All the samples were deaerated by passing pure argon gas for 20 min prior to the experiment. No degradation of the samples was observed during the experiment.

In general, cupric complexes do not exhibit any intense charge transfer or a sufficiently intense d−d band, suitable to monitor their interaction with DNA. So the ligand based intense ($\pi \rightarrow \pi^*$) absorption band is used to monitor the interaction of **1** with CT DNA. Both complex **1** and DNA have significant absorbance at 266 nm wavelength. Therefore when we excite a mixture of **1** and DNA with 266 nm laser light, there is the possibility of excitation of both **1** and DNA. However, the extinction coefficient of DNA at 266 nm is ~6600 mol^{-1} cm^2, whereas that of **1** is ~56974 mol^{-1} cm^2. Therefore, when a mixture of **(1)** and DNA is excited by 266 nm laser light the probability of excitation of DNA is very much lower compared to **1**.

Results and Discussion

Figure 1 shows the transient absorption spectra of pure complex **1** (4×10^{-5} M) and **1** in presence of DNA in tris-HCl medium at 0.6 μs after laser flash. The maximum around 420 nm for **1** corresponds to its triplet−triplet absorption. In the presence of DNA, the spectrum shows substantial quenching of 420 nm peak with a rising peak around 550 nm, which might be for radical anion of 1,10-phenanthroline,[38] $phen^{\bullet -}$. Therefore, electron transfer might occur between photoexcited complex and DNA, which is further confirmed from the small hump around 370 nm due to the formation of DNA radical cation,[39] $DNA^{\bullet +}$. As we know that guanine base is the most easily oxidizable component of DNA, the DNA radical cation might be of guanine radical cation[39] $G^{\bullet +}$. This hump becomes much more prominent with increasing DNA concentration. The quenching phenomenon of **1** in the presence of DNA also proves that in the presence of **1**, DNA itself may not absorb the laser light.

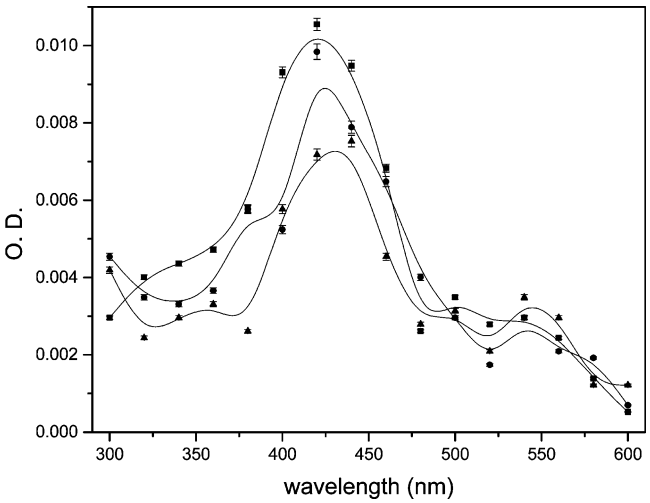


Figure 1. Transient absorption spectra of the complex (40 μM) at different DNA concentrations 0.0 (■), 40 (●), and 120 μM (▲) in buffer at 0.6 μs after the laser flash.

The photoinduced electron transfer (PET) phenomenon between DNA and photoexcited complex and the formation of corresponding radical ion pairs is further confirmed by magnetic field effect (MFE). When an external MF is applied then we have found a prominent MFE in homogeneous aqueous medium. The absorbance values of radical ions are enhanced in the presence of an external MF (0.08 T), which indicate that all these transients have a triplet origin (Figure 2). The decay of the RIP is expected to be biexponential. The change in absorbance A(t) with time follows the expression, $A(t) = I_f \exp(-k_f t) + I_s \exp(-k_s t)$ where k_f and k_s are the rate constants for the fast and slow components of the decay profiles respectively.[40] The fast component corresponds to the decay of geminate RIPs and the slower one corresponds to the reaction of the escaped radicals. The k_f values obtained by biexponential fitting from the decay profiles in absence and in presence of MF are given in Table 1. The relative escape yield after 5 μs are also calculated (Table 1). It is observed that on application of an external magnetic field, the decay rate decreases and correspondingly the escape yield increases. This also implies that the RIPs are generated in the triplet spin state. On application of a magnetic field the conversion of the triplet RIP to the singlet RIP is retarded and consequently the decay rates become slower and escape yield gets enhanced.

On photoexcitation initially the $^1([Cu(phen)_2]^{2+})^*$ is formed, which then undergoes a rapid ISC to produce $^3([Cu(phen)_2]^{2+})$. In presence of DNA, one electron transfer occurs from DNA to complex **1** and RIPs are generated. When by diffusion, the inter-radical distance becomes such that the exchange interactions between the two free electrons of the geminate RIP become negligible and maximum ISC occurs between the triplet and

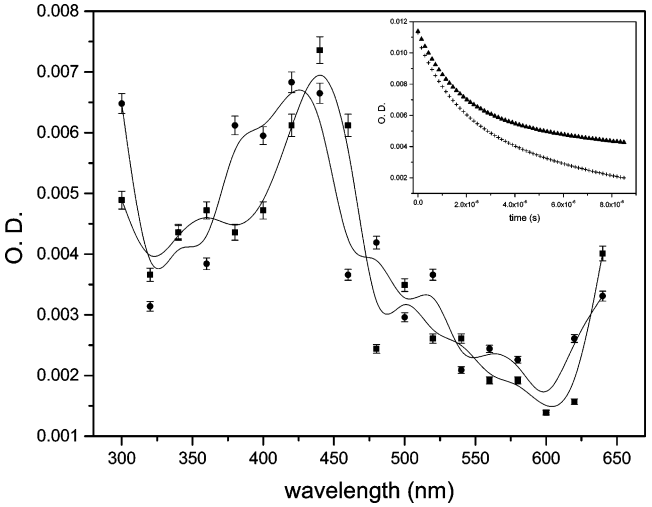


Figure 2. Transient absorption spectra of the complex (40 μM) − DNA (120 μM) in buffer in the absence (■) and presence (●) of 0.08 T MF at a delay of 0.6 μs after the laser flash. Inset shows the decay profile of the transient at 380 nm in the absence (+) and presence (▲) of MF.

TABLE 1. Fast Rate Constants (k_f) and Relative Radical Escape Yield after 5 μs (Y), in the Absence and Presence of an External Magnetic Field in Homogeneous Buffer Medium and AOT Reverse Micelle

medium	magnetic field (T)	$k_f \times 10^{-5}$ (s^{-1})[b]	Y
buffer	0.00	5.04	1.00[a]
	0.08	2.22	1.55
AOT reverse micelle	0.00	17.2	1.00[a]
	0.08	12.8	1.13

[a] Arbitrarily taken. [b] At 380 nm.

the singlet state. Application of an external MF on the order of HFI suppresses the ISC by introducing Zeeman splitting in the triplet sublevels, which in turn increases the yield of the free ions in the initial spin state. The mechanism of the reaction is shown as follows

$$[Cu(phen)_2]^{2+} \xrightarrow{h\nu} {}^1([Cu(phen)_2]^{2+})^* \xrightarrow{ISC} {}^3([Cu(phen)_2]^{2+})$$

$${}^3([Cu(phen)_2]^{2+}) + DNA \xrightarrow{ET} {}^3\{[Cu(phen)(phen^{\bullet-})]^{2+}\cdots DNA^{\bullet+}\} \xrightleftarrows[ISC]{MF} {}^1\{[Cu(phen)(phen^{\bullet-})]^{2+}\cdots DNA^{\bullet+}\}$$

Now, the question is, why MFE is observed in this aqueous homogeneous medium for the triplet born radical ion pairs? It is known that when cationic Cu(I) and Cu(II) complexes bind to double helical DNA, most likely they replace a cation from the compact inner (Stern) layer or the diffused outer layer surrounding DNA.[41] Earlier, it was proved from the crystal structures of $[Cu(phen)_2(H_2O)](NO_3)_2$[42] and $[Cu(phen)_2Cl]ClO_4$[43] that the coordination chemistry around copper(II) is distorted trigonal bipyramidal in which the water molecule or Cl^- ion occupies the trigonal plane. The two phen ligands when bind to the copper(II) ion deviate from coplanarity because of steric repulsion between ortho 2 and 9 hydrogens (Chart 1). Because the phen ligands in complex **1** are coordinated to copper(II) in a nonplanar configuration, the complete intercalation of phen ring between a set of adjacent base pairs is sterically impossible. However, some sort of partial intercalation involving one of the phen ligands can be envisioned.[44]

Because of partial intercalation, the $phen^{\bullet-}$ and $DNA^{\bullet+}$ cannot remain very close to each other, which maintains the optimum

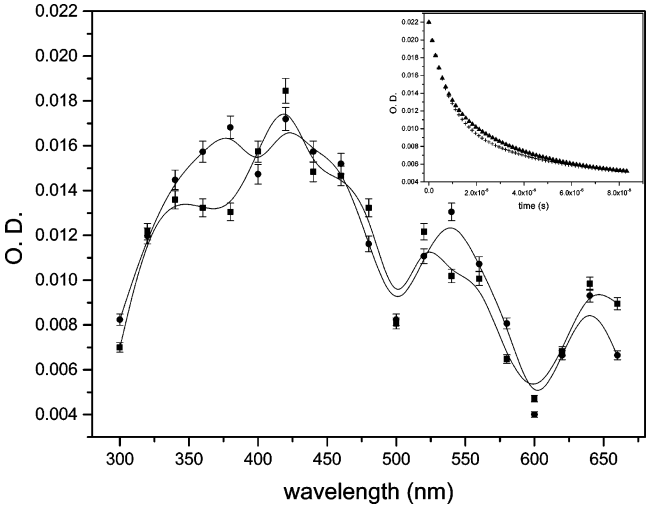
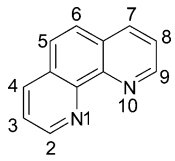


Figure 3. Transient absorption spectra of the complex (40 μM) − DNA (120 μM) in AOT reverse micelle in the absence (■) and presence (●) of 0.08 T MF at a delay of 0.6 μs after the laser flash. Inset shows the decay profile of the transient at 380 nm in the absence (+) and presence (▲) of MF.

CHART 1



distance between donor and acceptor with negligible exchange interaction favoring the occurrence of the MFE even in a homogeneous buffer medium. If the complex is a perfect intercalator, then the DNA base radical cation and ligand radical anion should be very near to each other; because of the presence of sufficient exchange interaction, MFE could not be observed.

We have repeated the experiments in AOT reverse micelles. Reverse micelles consist of a homogeneous thermodynamically stable solution of nanodroplets of water surrounded by a surfactant monolayer and dispersed in an organic solvent. These water nanodroplets are used as confined system. In reverse micelles, we have also observed MFE for the electron-transfer reaction between complex **1** and DNA. For a given concentration of AOT, the size of the entrapped water pool and hence that of the reverse micelle depends on the ratio between water and AOT molecules ($W_0 = [H_2O]/[AOT]$). The water pool size is given by $2W_0$.[35] Figure 3 shows the transient absorption spectra of the complex−DNA system in presence and in absence of MF. It is evident from the Figure 3 that in presence of the MF the yield of the $DNA^{\bullet+}$ radical cation and $phen^{\bullet-}$ radical anion increases. The decay constants of the fast component and the escape yields in the absence and presence of MF are given in Table 1. It is evident from the rate constant (k_f) and the escape yield (Y) that the MFE is not very much strong in AOT reverse micelle as that in buffer. We have found maximum MFE at $W_0 = 10$. Above and below this W_0 value, the MFE dies out rapidly. As mentioned earlier, the observation of MFE involves diffusion, spin flipping and geminate recombination. When the participating radicals are close to each other (small W_0), the exchange interaction, J, will hinder spin conversion and at a large distance of separation (large W_0), spin correlation will be lost. So MFE requires an optimum separation between the participating RIP that is attained at an intermediate W_0. This optimum W_0 may not be the same for all the acceptor−donor systems studied. However, the observed MFE in AOT reverse micelle is not very

strong, as expected in a confined system, compared to a homogeneous medium. The reason behind is that the divalent cation of the complex is very much hydrophobic in nature. It more readily dissolves into a organic medium, e.g., heptane, than in water. Thus when we add the complex, dissolving it in water, into AOT reverse micelles in heptane, the complex becomes partitioned between water and heptane. Thus the effective concentration of the complex in the water pool decreases in one hand and on other hand the distance between the donor and acceptor molecules increases, which leads to the decrease in the extent of MFE in AOT.

Conclusion

From this work, we infer that although the complex **1** is not covalently linked with DNA, they behave as a linked system because of the intercalation. The driving force for intercalation is the electrostatic force of attraction between the positively charged complex and negatively charged DNA, and the hydrophobic interaction between phenanthroline ring and DNA base pairs. But because of the nonplanar structure of complex **1**, perfect intercalation between DNA base pairs is not possible. This in turn helps in maintaining the proper distance between the RIPs, generated through PET, so that spin correlation can occur between them. This results to the observance of MFE in a homogeneous aqueous medium. In a confined system like AOT reverse micelles, the MFE is expected to be larger compared to that in aqueous medium, but in this case, the partitioning of complex **1** in organic medium increases the inter-radical distance that breaks the spin correlation of geminate RIPs, and thus MFE gets reduced.

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