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Direct observation of radical intermediates during electron transfer between DNA and a ternary copper complex

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ABSTRACT

The photoinduced electron transfer (PET) reaction within a ternary copper complex $[Cu(phen)(Htrp)]^+$ (Htrp: L-tryptophanato; phen: 1,10-phenanthroline) **(1)** and in presence of DNA has been studied in homogeneous buffer medium and in reverse micelles. An intramolecular electron transfer occurs within the photoexcited complex **(1)** from tryptophan to phen. The copper complex can displace ethidium bromide from DNA backbone and on photoexcitation can oxidize DNA in a deoxygenated environment due to intermolecular electron transfer, although the intramolecular electron transfer is thermodynamically favorable. A prominent magnetic field effect (MFE) has been found even in homogeneous aqueous medium for the triplet born radicals both in case of intra and intermolecular electron transfer reactions. In case of intramolecular electron transfer the observation of MFE is similar to that of linked donor-acceptor system. However the observation of MFE for the intermolecular electron transfer between non-covalently bound complex-DNA systems is rather rare. Some non-covalent weak interaction, e.g. hydrophobic interaction between the phen ligand and DNA base pairs and electrostatic force of attraction between $[Cu(phen)(Htrp)]^+$ complex and DNA may lead to partial intercalation of the copper complex within DNA that is responsible for such a rare observation.

1. Introduction

Non-covalent weak interactions like hydrogen bonding, electrostatic force of attraction, aromatic ring stacking, etc. are vital in the processes of biological recognition of molecules and subsequent specific reactions [1]. The nucleic base–base, hormone–receptor, enzyme–substrate and antigen–antibody interactions are among the most important biological processes that involve such weak interactions [2]. However interactions between aromatic rings are very important in proteins and protein–DNA systems for protein stabilization and various regulatory processes [3–5]. Among the four amino acids with an aromatic side chain, phenyl alanine (phe), tyrosine (tyr), tryptophan (trp) and histidine (his), his and tyr have effective metal binding sites, whereas phe contributes mainly to the stabilization of proteins through hydrophobic interactions [6]. Tryptophan has an electron rich indole ring, which has an excellent π electron donating property [7,8]. Tryptophyl residue is present in the active center of enzymes [9] and has often been involved in charge transfer interactions with a variety of substrates such as nucleosides and coenzymes like NAD (or NADP), FAD [10], etc., each bearing one or more heterocyclic aromatic ring. Many of these enzymes additionally contain tightly bound metal atoms or require metal ions as cofactors for their functions. Hence the solution studies of ternary metal complex comprising aromatic amino acid, especially tryptophan, and a second ligand containing an aromatic ring such as 2,2′-bipyridine or 1, 10-phenanthroline (phen), is important to understand the electron transfer reactions between indole and heterocyclic molecules. In our earlier works we have studied interactions between $[Cu(phen)_2]^{2+}$ and calf thymus DNA and it has been found to be a photoinduced electron transfer (PET) reaction [11]. Knowing the biological relevance of tryptophan we have substituted one of the phen ring of $[Cu(phen)_2]^{2+}$ with the tryptophan molecule.

In this paper we report that in presence of tryptophan molecule not only the intermolecular electron transfer reactions may occur between the photoexcited complex and DNA, like the parent $[Cu(phen)_2]^{2+}$ complex, but also there is a possibility of intramolecular electron transfer reaction within the complex itself. Although the intra-molecular electron transfer from tryptophan to phen is thermodynamically favorable, in presence of DNA an intermolecular electron transfer occurs from DNA base to the phen ligand, which is kinetically more favored. The intermediate DNA base radical has been identified by applying an external small magnetic field (MF). The PET reactions produce initially geminate radical ion pairs (RIPs) that contain two spin correlated free electrons [12–15]. By diffusion the RIPs can separate to an optimum distance where the exchange interaction (J) between them 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

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of an internal or external MF of the order of hyperfine interaction removes the degeneracy of the triplet states ($T_{\pm}$, T_0) and reduces intersystem crossing (ISC) between $T_{\pm}$ and S, thus resulting in an increase in the population of the initial spin state of the transient. This phenomenon is manifested from the enhancement of the absorbance and decrease in decay rate constant of the transient species produced. Thus magnetic field effect (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 far the geminate characteristics get lost and consequently MFE cannot be observed. Therefore, an optimum distance of 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 [16,17] or highly viscous solvents [18–20] at low temperature or long chain biradicals [21,22] 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 [23–31]. Interestingly we have found prominent MFE for the triplet born radicals not only in case of intra-molecular electron transfer but also for the intermolecular electron transfer between DNA and $[Cu(phen)(Htrp)]^+$ even in homogeneous aqueous medium. The observation of MFE for the intra-ligand charge transfer within **(1)** is very much familiar in literature just like a linked system. However, the occurrence of MFE for non-covalently linked triplet born radicals in homogeneous medium is rather rare due to fast escape of the RIPs produced. The process of partial intercalation of the complex **(1)** within DNA might be responsible for the rare observation of MFE in homogeneous medium. Thus in this study MFE has been used as a tool to probe the distance between the ternary copper complex and CT DNA during PET reactions.

2. Experimental Section

2.1. 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 dissolving the DNA fibers in buffer the purity of it was checked from the absorbance ratio A_{260}/A_{280}. The ratio was between 1.8 and 1.9. Therefore, further deproteinization of DNA solution has not been needed. Water was triply distilled. All the solutions were prepared in 50 mM Tris–HCl buffer at pH 7.4 that is mentioned as aqueous medium or buffer. The copper complex $[Cu(phen)(Htrp)](ClO_4)$ **(1)** (Chart 1) was prepared in the laboratory by adopting the procedure described in Ref. [32]. It was purified by repeated crystallization. Anal. Calcd for $Cu(phen)(Htrp)(ClO_4)$ 2.5 H_2O: C, 46.69; H, 4.06; N, 9.47. Found: C, 46.67; H, 4.06; N, 8.57.

2.2. Methods and Instrumentation

2.2.1. Studies using absorption spectroscopy

UV–vis absorption spectra have been recorded on a Shimadzu UNICAM-UV-500 absorption spectrophotometer. A pair of 1 cm path length quartz cuvette was used for absorption experiments.

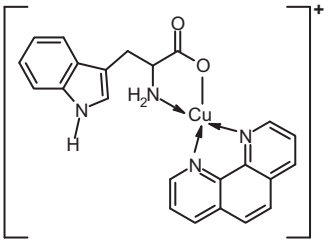

Chart 1. Structure of $[Htrp–Cu–phen]^+$.

All the experiments involving the interaction of the copper complex with DNA have been carried out in buffer. The CT DNA concentration per nucleotide was determined from absorption intensity at 260 nm using the molar extinction coefficient of 6600 M^{-1} cm^{-1} at 260 nm [33].

Absorption experiments have been performed by maintaining the metal complex concentration as constant while varying the CT DNA concentration. As both **(1)** and DNA have absorption in the same region, during measurement of the absorption spectra equal quantity of CT DNA has been added to both the complex solution and in the reference solution to eliminate the absorbance of DNA itself and to monitor the changes of the complex **(1)** in presence of DNA.

2.2.2. Competitive binding study

Competitive binding of complex in presence of EtBr with CT DNA has been studied using fluorescence spectroscopy. EtBr does not show any emission in buffer medium due to fluorescence quenching of the free EtBr by solvent molecules. In presence of CT DNA it shows enhanced emission intensity due to insertion of the molecule within hydrophobic region of the DNA base pairs. A competitive binding of the complex **(1)** to CT DNA in presence of EtBr possibly causes displacement of the intercalated EtBr molecule from DNA backbone. As a result the emission intensity of EtBr reduces on addition of the complex. During this study EtBr bound CT DNA solution in buffer was treated with an increasing concentration of complex. The fluorescence intensity at 600 nm (λ_{ex}=512 nm) of DNA bound EtBr has been plotted against the complex concentration. The apparent binding constant (K_{app}) was calculated from the equation: $K_{EtBr} \times [EtBr] = K_{app} \times [complex]$, where [complex] is the concentration of the ternary copper complex at 50% reduction of EtBr fluorescence intensity [$K_{EtBr} = 1 \times 10^7$ M^{-1} and [EtBr]=4.5 μM] [34].

2.2.3. 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.

2.2.4. Transient absorption measurement

The transient absorption spectra were measured by using a nano-second flash photolysis setup (Applied Photophysics) having an Nd:YAG laser (Lab series, Model Lab 150, 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, 5 Gs/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