Magnetic Fields Facilitate DNA-Mediated Charge Transport

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Supporting Information

ABSTRACT: Exaggerated radical-induced DNA damage under magnetic fields is of great concern to medical biosafety and biomolecular electronic devices. In this report, the effects of an external magnetic field (MF) on DNA electronic conductivity were investigated by studying the efficiencies of photoinduced DNA-mediated charge transport (CT) via guanine damage. Under a static MF of 300 mT, positive enhancements in the decomposition of 8-cyclopropyldeoxyguanosine (8CPG) were observed at both the proximal and distal guanine doublets, indicating a more efficient propagation of radical cations and higher electronic conductivity of duplex DNA. MF-assisted CT has shown sensitivity to magnetic field strength, duplex structures, and the integrity of base pair stacking. Spin evolution of charge injection and the alignment of base pairs to the CT-active conformation during radical propagation were proposed to be the two major factors that MF contributes to facilitate DNA-mediated CT. Herein, MF-assisted CT may offer a new avenue for designing DNA-based electronic devices and unraveling MF effects on redox and radical relevant biological processes.

Electronic coupling among the highly organized arrays of aromatic base pairs along double-helical DNA makes DNA a promising biomaterial for the conduction of electrical charges, a process termed DNA-mediated charge transport (CT).1−4 Efficient DNA CT in a well-stacked duplex was readily observed over 200 Å.5−7 A variety of approaches, from time-resolved spectroscopic measurements8−10 and biochemical assays11−13 to electrochemical methods,14−16 indicated that the integrity of the base pair stacking is a crucial factor in modulating CT efficiencies. The dynamic conformation of duplex domains over 4−5 bp was proposed to be the gating factor for electron hopping between adjacent domains.17,18 Both biological significance and technological ramifications lie in the high sensitivity to base pair integrity. In terms of cellular responses to oxidative stress, DNA CT not only might be harvested as a redox pathway to reallocate genomic damage but also might regulate and reshuffle redox-active proteins rapidly according to genomic anomalies.19−21 Meanwhile, molecular electronics or biosensing nanoapparatus analyze mutagenesis,15,22 and protein–nucleic acids interactions23 via electrochemical observation of DNA CT.

The potential negative effects of a magnetic field (MF) on the genomic stability have long raised health concerns. With the rapid development and wide application of magnetic-based medical instruments for diagnosis and therapeutics, these concerns were further extended to medical practice and healthcare. Despite the progress made in theoretical and experimental studies, efforts to provide conclusive evidence to link the effects of external MF to DNA damage remained a contentious issue, as there was a lack of a consistent pattern in the MF exposure-induced changes or damage to the cellular DNA.24,25 While the underlying mechanism responsible for the biological reactions exerted by the external MF remains controversial, several in vitro magnetic field effects (MFE) can be noted. Exposure to static MF alone induces no detectable lethal effects on the cell viability and/or DNA damage regardless of the MF strength.26 However, MF along with other stimuli, such as biological oxidants, reactive oxygen species (ROS), and ionizing radiation, could cause enhanced oxidative damage to the cellular DNA.27,28 The change in the responsiveness to MF in the presence of the oxidative stress led to the postulation that MF manifests, rather than induces, oxidative consequences of ROS radicals. This was not surprising as it was well-documented that external MF has a substantial influence on the evolution and kinetics of chemical reactions that include radicals.29−32 In addition, conformations of biomacromolecules with magnetic anisotropy can be manipulated by a high external MF in either a solution or immobilized on a surface and consequently affect the flow of electrons through a monolayer of biomacromolecules as observed via conductive AFM.33−35
Although it could provide essential fundamental knowledge for unraveling the biological roles and expanding the technological ramification of DNA-mediated CT, the effects of external MF on the propagation of charge through the DNA duplex and in turn how MFE affects CT-promoted oxidative DNA damage are still not well understood. Herein, we had designed a series of duplex DNAs with a photooxidant, anthraquinone (AQ), and a kinetic fast trap of radical cation, 8-cyclopropyldeoxyguanosine (8CPG), separated by bridge duplexes with defined lengths, to investigate how external MF will affect the electronic conductivity and in turn alter the yields of radical intermediates formed during DNA-mediated CT. Chemical decomposition of 8CPG induced by photoirradiation of distant attached AQ is used to determine the efficiency of CT through a base pair bridge in the absence and presence of an external MF. Here, we observed that the level of oxidative damage of 8CPG mediated by DNA CT through well-matched and mismatched DNA is significantly elevated by MF.

**EXPERIMENTAL PROCEDURES**

**Oligonucleotide Synthesis.** Cyanoethyl phosphoramidite of 8-cyclopropylguanosine<sup>56</sup> was synthesized as described previously, while anthraquinone-5-ethynyl-dU was purchased from Berry & Associates. DNA oligonucleotides with trityl-on were synthesized using standard phosphoramidite protocols on a BioAutomation Mermade 4 DNA synthesizer with reagents from Glen Research. After incubation in an ammonium hydroxide/methylamine mixture [v/v (1:1)] at 37 °C for 2 h, the cleaved DNA strands were purified by reverse-phase high-performance liquid chromatography (HPLC) (microsorb 100-5 C18 Dynamax column, 250 mm × 10.0 mm), detritylated with 80% glacial acetic acid for 15 min, and repurified by reverse-
phase HPLC. All the DNA oligonucleotides were confirmed by ESI mass spectrometry and quantified by UV−vis spectroscopy.

**Photooxidation Experiment.** DNA duplexes [10 μM, 30 μL in 20 mM sodium phosphate buffer (pH 7.0)] were prepared by annealing the modified DNA strands with its complements in a microtube and gradually cooled to room temperature overnight after being heated for 5 min at 90 °C. The duplexes were then irradiated at 350 nm by a 450 W xenon lamp, equipped with monochromator and a 320 nm long-pass filter for 10 min. Exposure to a stationary magnetic field was applied by placing the sample tube between pair(s) of neodymium magnet plates (0.7 cm in diameter). The flux intensity was varied from 7 to 100 mT by adjusting the distance between a pair magnetic plate, while two pairs of magnetic plates were utilized to obtain an intensity of 300 mT. A gaussmeter, model HGM09 (MAGSYS magnet systeme), equipped with a thin transversal probe, was used to measure the magnetic flux density. As the surface area of the magnetic disc was larger than that of the sample tube, the magnetic field strength between the magnetic disc considered to be was homogeneous. For irradiation under an external MF, 300 mT of MF was applied unless otherwise stated. Following, the samples were digested into free nucleosides by incubation at 37 °C with phosphodiesterase I and alkaline phosphatase for 24 h. The nucleosides were subsequently separated and analyzed by reverse-phase HPLC (Chromocobond 5-ODS-H column, 4.6 mm × 150 mm). The percentage decomposition of 8CPG was determined by subtracting its peak area in an irradiated sample from that in a nonirradiated sample, normalizing to thymidine determined by subtracting its peak area in an irradiated sample, normalizing to thymidine.

![Figure 1a](image1.png)

**RESULTS AND DISCUSSION**

**Experimental Design.** Anthraquinone (AQ) was selected as the photooxidant to trigger efficient injection of charge into DNA. The attachment of AQ to deoxyuridine via an acetylene linker (AQdU) would ensure strong electronic coupling with DNA π-stack and restrict the charge injection at the anchoring site. AQ1 and AQ2 have the same sequence, which contains two guanine doublets, 2 and 10 bp from AQdU, respectively. S- G of the proximal GG doublet in AQ1 and that of the distal GG in AQ2 are replaced with 8CPG to report on the formation of guanine radical cation at the corresponding GG sites via DNA CT (Figure 1a). Upon being annealed to either complementary DNA (DD) or RNA strands (DR), B-form DNA or A-form hybrid duplexes can be obtained. Upon irradiation at 350 nm, the excited AQ would be competent for abstracting an electron from deoxyuracil and injecting an electron hole, also known as a charge, into π-stack of DNA base pairs. The resulting radical cation would propagate along the base pair bridge until it reached guanine doublet sites and was trapped by the 8CPG rapid ring opening reaction of 8-cyclopentyl group to form a permanent oxidative product via oxidation reactions (Figure 1b).

**Figure 1b.** DNA CT under an external magnetic field (MF) was performed by placing the samples between pair(s) of permanent neodymium magnets, which provide a highly homogeneous magnetic flux across the aqueous samples (Figure 1b).

![Figure 2](image2.png)

**Magnetic Field Enhances 8CPG Decomposition via DNA CT.** We first elucidate the influence of an applied external MF on DNA-mediated CT by monitoring 8CPG decomposition in DNA duplex, AQ1-DD and AQ2-DD (Figure 2). In the absence of an applied MF (B = 0 mT), 7% of 8CPG at the proximal (AQ1-DD) and 12% of 8CPG at the distal (AQ2-DD) guanine doublets were decomposed after irradiation for 10 min. Under an external MF (B = 300 mT), both 8CPG decompositions were remarkably elevated to 33% in AQ1-DD and 44% in AQ2-DD. A slightly higher decomposition of 8CPG was observed over a longer propagation distance in AQ2-DD (32%) than in AQ1-DD (26%). Irradiation of either GG2-DD, a duplex without a photooxidant, or a mixture of AQ0-DD and GG2-DD, in which AQ and 8CPG are placed in separate duplexes (Figure S1 of the Supporting Information), was conducted at both 0 and 300 mT. No decomposition of 8CPG was observed above the noise level in either control experiment. The fact that 8CPG remained intact in the absence of AQ, regardless of the external MF, indicated that 8CPG is decomposed only by photoinduced oxidation from distant AQ and MF does not cause nonredox damage to guanines. In the second control, AQ in AQ0-DD does not induce any 8CPG decomposition in the separate duplex, GG2-DD, even in the presence of an external MF. This suggested that no diffusible oxidants, such as ROS, were causing damage to 8CPG under the experimental conditions in the absence or presence of MF. Thus, the effects of external MF on stabilizing diffusible radical oxidants and consequently enhanced guanine damage were invalid here. The elevation of 8CPG decomposition in AQ-DQ duplexes after the application of an external MF was the sole consequence of MF effects on DNA-mediated charge transfer.

**Dependence of 8CPG Decomposition on Magnetic Flux Intensity.** 8CPG decomposition under a varying magnetic flux intensity (B = 0−300 mT) was induced upon irradiation of duplexes, AQ1-DD and AQ2-DD (Figure 3). It appeared that the increment of 8CPG decomposition was dependent on the strength of the magnetic flux density. Obvious MF effects on accelerating DNA CT can be detected at B values as low as 7 mT. CT yields, in form of 8CPG decomposition, increased linearly under low flux (<50 mT) and approached plateau saturation after 100 mT for both AQ1-DD and AQ2-DD. Throughout the entire flux intensity range, the elevation of 8CPG decomposition efficiency at distal G doublets was always more pronounced than at the proximal site. Better appreciation
of the flux intensity increment over a longer DNA bridge in AQ2-DD further confirmed that enhancement of guanine damage as 8CPG decomposition was due to the effects of external MF on oxidative DNA CT.

**Structural Dependence.** It is well-known that CT exhibits different efficiencies in various secondary structures of nucleic acids as measured by guanine oxidation, fluorescence quenching, electrochemistry, and transient absorption spectroscopy. Among the various helical structures that DNA can adopt, A-form DNA/RNA hybrids are of great interest as they are essential to genetic transduction. Hence, we explored whether the magnetic field effect was also applicable to the A-form DNA/RNA hybrid duplex. AQ1-DR and AQ2-DR were formed by annealing the RNA version of complementary strands, DR, to AQ1 and AQ2 (Figure 1a). CD spectra of AQ1-DR and AQ2-DR showed a characteristic A-form structure (Figure S2 of the Supporting Information). Upon irradiation, AQ1-DR and AQ2-DR showed decompositions of 2 and 3%, respectively (Figure 2). A lower 8CPG decomposition observed in the A-form hybrid duplex was not unexpected, because the appreciable interstrand stacking, due to low twist and large positive tilt, in A-form helices may not be optimal for DNA CT, as compared to intrastranded stacking in the B-form duplex. Albeit there was a lower decomposition of 8CPG, under an external MF (B = 300 mT), AQ1-DR and AQ2-DR showed decent increments in the damage of 8CPG to 6 and 7%, respectively. Elevation of CT yields was not as significant as those in the B-form duplex, which is probably due to the hybrid helix, with wider grooves and a more compact structure, being less flexible and dynamic to be tuned toward the CT-optimal conformation by an external MF. Furthermore, the higher melting temperatures for the hybrids (AQ1-DR and AQ2-DR) versus those of the canonical B-form duplexes (AQ1-DD and AQ2-DD) excluded the possibility that the lower CT yield was associated with a weaker structural stability (Table S1 of the Supporting Information). Regardless, MFE can also be observed in A-form hybrid duplexes and was not restricted specifically to the B-form helix.

**Origins of MFE on DNA CT.** The complicated nature of photoinduced DNA CT makes it challenging to identify the reaction steps that might be affected by the external MF. Scheme 1 shows a simplified diagram of an electron pathway through three major stages of DNA CT in the current system. Charge injection (CI, stage 1) was initialized upon photo-irradiation of AQ to the singlet excited state, $^1\text{AQ}$, which would rapidly undergo intersystem crossing (ISC) to yield the excited triplet state, $^3\text{AQ}$. Subsequent charge separation (CS) between $^3\text{AQ}$ and electronically conjugated deoxyuracil would generate the initial triplet radical pair ($[\text{AQ}^-\text{−}d\text{U}^+]$, 3RP). A charge as a radical cation was then injected into the base pair from dU$^+$. In stage 2, the radical cation migrated through the bridging base pairs and would eventually oxidize 8CPG to form $[\text{8CPG}^+]$. In the final stage, $\text{8CPG}^+$, as a radical cation, would undergo a rapid ring opening reaction to trap the charge and complete charge transport (Scheme 1). Because it has been well documented that MF could influence the spin dynamics of radical intermediates in biological reactions, we postulated that an external MF might affect charge injection via 3RP and sequential migration of radical cation, while the final stage was unlikely to be altered significantly by an external MF.

In stage 1, charge injection, $[^1\text{AQ}^-d\text{U}^+]$ as a radical pair (3RP) may alternatively cross over to a singlet radical pair...
(\([1\text{AQ}^+−\text{dU}^*], \text{1RP}\) via triplet–singlet (T–S) radical pair intersystem crossing (RP-ISC). \text{1RP} would then decay to the singlet ground state by spin selective charge recombination (CR). A trinucleotide, S'-Q\text{6CP}GT-3' was therefore used to elucidate if MF can facilitate charge injection from \text{aqdU} by redistributing \text{3RP} and \text{1RP} populations via RP-ISC. The trinucleotide is an ideal assembly for such investigation. The electronic coupling through an acetylene bond between AQ and dU is strong enough to ensure that RP remains as a germinate pair, and dissociation of the radicals is suppressed to allow sufficient time for T–S interconversion to develop under an applied MF. The flexible phosphate chain would keep the photooxidant, AQ, and hole trap, \text{8CPG}, close to ensure rapid charge injection without being limited by diffusion. Further charge migration is eliminated because of the lack of duplex formation in the short trinucleotide. Figure 4 shows the decomposition of \text{8CPG} in the trinucleotide in the absence and presence of a 300 mT magnetic flux following irradiation for 0–60 s. In the absence of a magnetic field (B = 0 mT), up to 17% of \text{8CPG} undergoes irreversible oxidative ring opening with the duration of irradiation increasing to 60 s, whereas when B = 300 mT, the trinucleotide consistently exhibits a higher decomposition (up to 23%) during the entire irradiation time course. Although our experimental setup may not be able to distinguish whether CI originated from \text{3RP} or \text{1RP}, but \text{3RP}, which has a lifetime much longer than that of \text{1RP}, should be the major species for charge injection. Hence, any variation in the oxidative decomposition of \text{8CPG} could represent the quantum yield of \text{3RP}. The appreciable enhancement of \text{8CPG} decomposition under B = 300 mT indicated that the stabilization of spin-correlated RP, specifically the triplet state, is one effect of an MF on DNA CT. Such MFE can be interpreted with reference to a simple but well-known RP model.\footnote{29–32} Assuming that the exchange interaction energy between the \text{3RP} and \text{1RP} was very small, at zero applied field, the singlet (S) and the three triplet sublevels (T₀, T₁, and T₂) are nearly isoen energetic, and there would be unrestricted RP-ISC among the four states, induced by electron–nuclear hyperfine interaction (HFI). As the applied MF strength increased, the electronic Zeeman interaction would slow and uncouple T₀ and T₁ states from the conversion process. Eventually, only the interconversion between T₀ and S states would remain at high flux intensity (hyperfine mechanism). Hence, the conversion of \text{3RP} to \text{1RP} was diminished and radical annihilation via charge recombination of \text{1RP} would be impeded. Consequently, the populations of \text{3RP} would increase and charge injection from \text{3RP} as an "escape" product from CR would be enhanced and be revealed as more charge trapping at the \text{8CPG} site. Hence, in the trinucleotide model, a higher efficiency of \text{8CPG} decomposition under an external MF implied that charge injection is facilitated by MF via enriching the triplet radical pair, \([\text{AQ}^+−\text{dU}^*],\) during photoinitiated charge separation.

Notably, it was reported that MF could induce orientation rearrangements in organic molecules\footnote{54} and biological macro-molecules.\footnote{55–58} The preferential orientation originates from the external magnetic induction and the anisotropy of magnetic susceptibility (\(\chi\)) of nucleobases.\footnote{59} Although the \(\chi\) values for neutral nucleobases are weak, base pair domains, which are the propagating species during DNA CT, carry delocalized paramagnetic radical cations and led to a much stronger response to MF-induced conformational rearrangements. An intervening mismatch is a severe disruption to the integrity of DNA base pair stacking and could significantly diminish the overall CT yields.\footnote{22,60,61} Hence, we challenge the ability of MF to promote a more optimal conformation for DNA CT by introducing different mismatches into the DNA bridge before and after \text{8CPG} trap. Upon being annealed to complementary strands with substitution of G with either A, C, or T, duplexes containing CA, CC, and CT mismatches between proximal and distal GG were formed (Figure 1a). When AQ1-CA, AQ1-CC, and AQ1-CT were excited in the absence of a MF (B = 0 mT), \text{8CPG} at the proximal GG site in all three mismatched duplexes showed a decomposition of \(~7\%\). When a B of 300 mT was applied, the level of \text{8CPG} damage was increased to \(~30\%\) (Figure 5). These results were almost identical to those of \text{AQ1-DD}. None of the mismatches diminished DNA CT between photooxidant and proximal GG, which was not unexpected as the mismatched base pair was positioned after the charge trap and the intervening base pair stack between AQ and \text{8CPG} was not disturbed. With the photoinduced DNA CT remaining intact and a similar degree of enhancement in CT efficiency being observed, it implied that MFE was not restricted by the presence of a mismatched site in the duplex, whereas in the cases of AQ2-CA, AQ2-CC, and AQ2-CT, the damage yield of distal \text{8CPG} in the absence of MF was significantly diminished by every single-base mismatch. Similar to previous studies of CT chemistry,\footnote{52,60,61} base replacements of G with A, C, or T disrupted the integrity of the \(\pi\)-stack and inhibited the propagation of charge to the distal \text{8CPG}. This showed that CT in our DNA assemblies showed sensitivity to the integrity of base pair stacking as reported in the literature.

Figure 4. Time course of the decomposition percentage of \text{8CPG} in S'-Q\text{6CP}GT-3' in the absence (○) and presence (×) of an external MF (300 mT).

Figure 5. Decomposition percentage of \text{8CPG} for AQ1- and AQ2-CA, -CC, -CT in 20 mM sodium phosphate buffer (pH 7.0) in the absence (crossed) and presence (shaded) of an external MF.
Interestingly, under a B of 300 mT, decomposition yields of \(^{8}CG\) were recovered in AQ2-CA (4%), AQ2-CC (11%), and AQ2-CT (11%). In the cases of two pyrimidine/pyrimidine mismatches, the CT yield was restored to an efficiency similar to that of the well-matched DNA under a background MF. The reinstallation of DNA CT by external MF suggested that MF had applied a well-pronounced compensation effect to repair or to shield the distortion of base pair stacking. Characterization studies using X-ray crystallography and NMR methods had shown that a mismatched base pair causes minimal alterations in the global conformation of B-form DNA duplex, but instead, the distortions were localized in the vicinity of the mismatched site.22−66 Hence, it is likely that a MF as weak as 300 mT might be sufficient to partially tune the base orientation of the mismatched and neighboring base pairs so that the optimal π-stacking in the local environment can be recovered temporarily to the CT-active conformations comparable to a well-matched DNA. 

The ability to enhance CT in duplex DNA by the application of a weak MF could be the basis for designing a magnetically triggered DNA electronic switch. Using a mismatched duplex as a molecular wire, the electron current could be turned on and off by applying and removing MF, respectively. By manipulation of a combination of CI sources and MF, an “AND” gate could be readily constructed with DNA wire containing disrupted base pairs. Reciprocally, such a magnetic switch could be useful for sensing of proteins that disrupt base pair stackings and studying reactions and/or processes between DNA and other biomolecules in which radicals are involved.

It had been proposed that DNA-bound proteins with a metal cluster as the redox cofactor might exploit DNA CT to signal and communicate with each other, to detect lesions efficiently across long genomic DNA and/or regulate transcription.19−21 Interestingly, our results suggested that with the assistance of an MF, local alignment near the mismatch site might permit the charge migration to proceed without obstruction. As such, an electron may still be able to funnel through the damaged DNA between the two repair proteins in the presence of a mild MF. Consequently, the proteins may detach and bind to an alternative DNA site without rapidly locating and repairing the damage in time. In essence, the proteins will erroneously pass on false information about DNA structural integrity to one another through the restored DNA CT by an MF. Potentially, this could be an alternative justification for why more DNA damage was detected in the biological systems after application of an external static MF. In fact, the number of lesion sites generated by ROS could remain unchanged except that in the presence of MF, the repair system was compromised because repair enzymes were misled by MF-assisted DNA CT and bypass the lesion sites, leading to an “accumulation” of damage spots.

**CONCLUSION**

Here by using anthraquinone as a photooxidant and \(^{8}CG\) as a hole trap, CT efficiencies through duplex DNA were explored in the presence of an external MF. The application of MF caused an increased level of damage to \(^{8}CG\) via photoinitialized DNA CT over various lengths of duplex bridges. Such effects were also observed in the DNA/RNA hybrid duplex, albeit lower yields. The acceleration of CT is closely related to the strength of the applied MF, where damage to \(^{8}CG\) was detectable as low as 7 mT. Two factors may account for the observed MF-induced acceleration of DNA CT. Application of an external MF interfered with the spin evolution of RP and, consequently, enriched the population of the triplet state and enhanced hole injections into the DNA π-stack. Second, MF can manipulate the alignments of base pair stacking to promote high accessibility of the DNA bridge to the CT-active conformation. Herein, our results suggest that an external MF could be a promising approach to enhancing the electronic conductivity of duplex DNA. This approach would open a new venue for the design of molecular devices and inspire a better understanding of biological and medical processes relevant to DNA CT and reactions in which radicals are involved.

**ASSOCIATED CONTENT**

**Supporting Information**

Light control and cross strands control, CD spectra of DNA assemblies, and thermal analysis of DNA assemblies. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biochem.5b00295.

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**Notes**

The authors declare no competing financial interest.

**ABBREVIATIONS**

CT, charge transport; MF, magnetic field; MFE, magnetic field effects; ROS, reactive oxygen species; \(^{8}CG\), 8-cyclopropyldeoxyguanosine; AQ, anthraquinone; CI, charge injection; ISC, intersystem crossing; CS, charge separation; RP, radical pair; CR, charge recombination.

**REFERENCES**


