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Excess Electron Trapping in Duplex DNA: Long Range Transfer via Stacked Adenines

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Abstract

An understanding of charge transfer (CT) in DNA lies at the root of assessing the risks and benefits of exposure to ionizing radiation. Energy deposition by high-energy photons and fast charged particles creates holes and excess electrons (EE) in DNA and the subsequent reactions determine the complexity of DNA damage and ultimately the risk of disease. Further interest in CT comes from the possibility that hole transfer and/or excess electron transfer (EET) in DNA might be used to develop nanoscale circuits. In order to study EET in DNA, EPR spectroscopy was used to determine the distribution of EE trapping by oligodeoxynucleotides irradiated and observed at 4K. Our results indicate that stretches of consecutive adenine bases on the same strand serve as an ideal conduit for intrastrand EET in duplex DNA at 4K. Specifically, we show that A is an efficient trap for EE at 4K if, and only if, the A strand of the duplex does not contain one of the other three bases. If there is a T, C, or G on the A strand, trapping occurs at T or C instead of A. This holds true for stretches up to 32 A's. While T competes effectively against A for the EE, it does not compete effectively against C. Long stretches of T pass the majority of EE to C. Our results show that AT stretches channel EE to cytosine, an endpoint with significance to both radiation damage and the photochemical repair of pyrimidine dimers.

Keywords

EPR; Electron Transfer; DNA; Superexchange

Introduction

Charge transfer (CT) in DNA is of long standing interest to radiation science because the holes (h) and excess electrons (EE) generated in DNA by ionizing radiation result in free radical reactions that play a central role in the biological response to ionizing radiation. Considerable progress in characterizing these radicals has stemmed from EPR/ENDOR spectroscopic studies of X- and γ -irradiated DNA and nucleic acid constituents in the solid state.¹ Early progress on hole transfer (hT) and excess electron transfer (EET) in DNA came

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ASSOCIATED CONTENT

Supporting information includes the tensors used to simulate the EPR spectra of each base free radical used in component analysis; one-electron reduced ODN EPR spectra in which contributions from one-electron reduced benzoyl have been accounted for, illustrating the role influence of one-electron reduced adenine on spectral characteristics; and EPR spectra of three samples synthesized without using benzoyl as a protection group. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Author Contributions

The manuscript was written through contributions of all authors./All authors have given approval to the final version of the manuscript.

by observing the distribution of radical trapping sites and the evolution of thermally activated reactions upon annealing.² In such experiments, the radical intermediates are frozen in time, providing a snapshot of processes that are transient at room temperature (RT). The suggestion that DNA might be a molecular wire³ spurred applications of fast-detection optical methods to further advance our understanding of charge separation (CS) in DNA.⁴ But advances in understanding EET have lagged that of hT,^{4a, b, 5} in part because the quantum yield for EE obtained by photochemical injection is very small, $\sim 10^{-3}$ ⁶ due, in part, to charge recombination with the electron donor. This problem does not arise in EPR studies of trapped EE, where a covalently bound electron donor does not supply the attached electron. Using this system, information on tunneling⁷ and the distribution of EE trapping have been measured^{2a, 8}.

DNA is known to be effective at trapping EE and h at temperatures below 100K with measured free radical yields documented at $\sim 600\text{nmol/J}$.⁹ The range of CT under these conditions was found to be < 8 bp.^{2c, 2f} The properties that make DNA an excellent trap also make DNA an insulator as opposed to a molecular wire.¹⁰ EE are trapped primarily at C, secondarily at T, and possibly at A.¹¹ The resulting radical anions and subsequent protonation reactions are shown in Scheme 1. EPR studies have shown that electron attachment to T results in the radical anion,¹² which does not protonate in DNA.^{2a} In contrast, the cytosine radical anion, $\text{C}^{\bullet-}$, is not observed in monomers, DNA, or at 4K. However, $\text{C}^{\bullet-}$ is consistently protonated at N3 giving the neutral $\text{C}(\text{N3+H})^{\bullet}$ radical.^{1d, 13}

These findings agree with Steenken's predicted EE trapping efficiencies, $\text{C} > \text{T} \sim \text{A} > \text{G}$ in duplex DNA, based on reduction potentials and pK_a 's of the nucleobase/nucleobase radical anion couples.¹⁴ A key element in understanding why EE trapping is favored by C over T is that capture by T does not have the driving force of proton transfer across the Watson-Crick (WC) bond.¹⁵ In C:G, the EE is trapped such that the unpaired electron and charge are localized on separate strands, $\text{G}(\text{N1-H})^{\bullet-}:\text{C}(\text{N3+H})^{\bullet}$. In A:T, the charge and unpaired electron are localized on the same strand, $\text{T}^{\bullet-}$. The same conclusion emerged from the work of Wagenknecht, Fiebig, and coworkers in their studies on aqueous solutions of diradicals generated by photolysis of pyrene-modified pyrimidine nucleosides.¹⁶ They found that $\text{dU}^{\bullet-}$ (U being a good analogue for T) was not protonated above pH 5.5; even with ps resolution, the nonprotonated $\text{C}^{\bullet-}$ was not observed. Proton transfer to $\text{C}^{\bullet-}$ was estimated to occur in a few ps or less. Also, they observed proton transfer to $\text{dA}^{\bullet-}$ at pH 7¹⁷ as predicted by Steenken^{14a}. This agreed with the earlier work of Fiebig that showed A appears to be uniquely positioned because, with its oxidation potential greater than G and reduction potential less than T, it is "redox ambivalent".^{16a}

EE move through DNA via a combination of superexchange (SE) and hopping.^{4b, 5} Anderson and Wright studied CT using the reaction of aqueous electrons generated by pulse radiolysis at RT. They determined transfer distances of $\sim 3\text{--}7$ bp which are governed by a free energy of activation of $\sim 26\text{ kJ mol}^{-1}$.¹⁸ Using debromination of 5BrdU to monitor CS and EE arrival, Ito and Rokita concluded that T was the primary carrier and observed a sharp decrease in transfer efficiency when T was replaced by C and, based on a deuterium isotope effect, ascribed this effect to proton transfer to $\text{C}^{\bullet-}$.¹⁹ In a similar system, Daublain *et al* observed a shallow distance dependence over 3 to 8 AT bp and concluded that CS was governed by hopping.^{6a} This hopping rate from T to T, has been measured directly by Majima and coworkers and found to be surprisingly fast at $k_{\text{intra}}(\text{TT}) = (4.4 \pm 0.3) \times 10^{10}\text{ s}^{-1}$.²⁰

Below 80K, EE trapped by DNA are stable with long lifetimes. Detrapping from T occurs upon warming to 100K–140K and from C above 170K.^{2a, 2c} Migration of EE terminates by irreversible protonation at the C5=C6 bond of $\text{T}^{\bullet-}$, $\text{C}(\text{N3+H})^{\bullet-}$, and $\text{C}^{\bullet-}$ (Scheme 1).^{2a, 21} On

product analysis of DNA irradiated in the solid state it was found that the ratio of product formation due to reductive damage at cytosine (DHU in Scheme 1) compared to thymine (DHT) depends strongly on sequence.⁹ Irreversible trapping by T is significantly enhanced if it is flanked on either side by purines in the same strand. If T is adjacent to C, the electrons are preferentially trapped at C.

SE has been observed in DNA containing intercalated mitoxantrone (MX). From EPR measurements, EET was shown to be temperature independent between 4K and 77K, proving that transfer was by SE, a single step event also referred to as tunneling.^{7b} Because the time span was over hours it means SE was either over long distances and/or through high barriers. SE to MX was observed from T^{•−} but not from C(N3+H)[•]. Using $k_{SE} = k^0 e^{-\beta \Delta x}$, where Δx is the SE distance, and k^0 the pre-exponential factor, a distance dependence constant β of *ca.* 9 nm^{−1} was determined.^{7, 22}

The results summarized above can provide insight into the framework put forward in Harriman's paper on "Electron tunneling in DNA."²³ Using fluorescence quenching on a ns time scale, he found that intrastrand EET was short range with $\beta = 10 \pm 0.6$ nm^{−1} for both poly(dAdT)•poly(dAdT) and poly(dGdC)•poly(dGdC) but the rates of tunneling for poly(dAdT) were faster than for poly(dGdC). The calculated rates at orbital contact, k^0 , were $2.5 \times 10^{14} \text{ s}^{-1}$ for poly(dAdT) vs. $5.8 \times 10^{13} \text{ s}^{-1}$ for poly(dGdC). He argued that the value of β reflects poor coupling between stacked base pairs and that different rates with the same β arise from a better blending of energy levels or coupling coefficients in poly(dAdT).

Here we provide evidence that stacked adenines at 4K, a system with strong coupling and low reorganization energy, give rise to long distance transfer with a possibility that transfer is by SE. In contrast, interstrand EET, which involves weakly coupled bases and high reorganization energy, was found to depend strongly on whether the transfer is from A to T or from A to C.

Methods

Synthesized oligodeoxynucleotides (ODN) were purchased from either Integrated DNA Technologies or SynGen, at desalted or HPLC purity, and used without further modification. LiCl was purchased from Sigma Aldrich and dissolved to a concentration of 8M in nano-pure water distilled using a Thermo Scientific Barnstead Easy Pure II water purification system. Samples were dissolved in LiCl solutions at a monomer concentration of 27.7mM.

Solution volumes of 3 μ l were pulled down into a 0.7mm outer diameter (OD) Charles Supper quartz tube using a microcentrifuge. Tube wall thickness was 0.1mm. Samples were cooled from room temperature to 4K using a Janis liquid helium cryostat. Upon cooling, the solutions form a glass.

Irradiation and EPR Spectroscopy

Electron paramagnetic resonance (EPR) was performed on X-irradiated glasses containing ODN, at 4K without warming, using a Varian E12 Q-band spectrometer and Janis Dewar Accessory.²⁴ The X-ray source, a Varian OEG-76 with tungsten target, was operated at 70kV and 20mA. Dose rate at the sample was 0.75kGy/min. When irradiated at 4K in a LiCl glass, only one-electron reduced precursor radicals are formed in ODNs present. This is due to the hole scavenging ability of Cl[−], which is present in the system in high abundance relative to DNA. The resultant Cl₂^{•−} radical gives a broad EPR signal that is easily discernable from the spectral characteristics due to one-electron reduced ODN.

Simulations

EPR spectral simulations were performed using PowderSim software developed in house. PowderSim uses the elements of hyperfine, g , and line width tensors as variable parameters, constrained by boundaries based on the literature, to simulate the experimental spectra.²⁵ Benchmark spectra for one-electron reduced thymine, adenine, and cytosine are shown in Figure 1. These were calculated by simulating the spectra generated in LiCl glasses containing T₁₆, A₁₆, and C₁₆, respectively. In each case, published hyperfine tensors²⁶ were used and not allowed to vary. Elements of the g -tensor were permitted to vary but bounded by information from the literature.²⁷ Elements of the line width tensor adequately incorporate weak couplings from hydrogen and nitrogen. The tensor parameters are given in Table 1S in Supplementary Information.

Benchmark spectra were used to deconvolute spectra of irradiated oligomers of varying sequence using methods described previously.¹² During analysis the EPR spectral characteristics of one-electron reduced benzoyl were incorporated to account for a 2–9% benzoyl contamination in synthesized oligomers containing adenine.²⁸

Results

EPR of AT Series

We prepared 31 distinct double stranded ODNs containing only A & T bases in a LiCl glass and irradiated each in order to test the effect sequence on the distribution of trapped EE at 4K. Early studies on duplex DNA in high concentrations of LiCl concluded equilibrium of conformations between B- and Z-DNA, with B-DNA favored at lower temperatures.²⁹ We expect that during the cooling process our sample would favor B-DNA conformation. All samples were irradiated to a dose of 3kGy, which produces a favorable signal-to-noise ratio and is a factor of 2 lower than the onset of dose saturation. Component analysis of these sequences revealed a consistent difference in the EPR spectra between samples that contained only adenine on one strand and only thymine on the complimentary strand (T:A sequences) as opposed to samples that contained thymine and adenine on the same strand (T-A sequences). Figure 1 illustrates the differences in the T^{•−} and A(N3+H)[•] benchmarks, as well as the fit for A₁₄:T₁₄ using 26% A and 74% T. Directly above this the fit for A₃₂C₄:G₄T₃₂ is provided using 0% A, 38% T, and 62% C.

T:A sequences exhibit a larger EPR spectral extent than the T-A sequences. As shown in Figure 2, this larger spectral extent is due to the contribution of one-electron reduced adenine. Because the spectrum of Thy^{•−} is significantly narrower, the covariance between the A(N3+H)[•] spectrum and that of the reduced pyrimidines is very small. The percentages of one-electron reduced adenine and thymine, determined by component analysis of the EPR spectra of irradiated T:A samples are given in Figure 3 and indicate that interstrand transfer from A to T is slow relative to the trapping rate of the EE at A. For the A₁₆:T₁₆ sample, monitoring the spectrum for 18 h at 4K provided evidence that no transfer from A to T occurred over this time span. The analysis of T-A sequences is provided in Figure 4. We found that whenever T is added to either end of the Ade-strand, EE trapping by A is quenched. Importantly, this behavior persists in samples that contain as many as 32 sequential adenines at the 5' or 3' end of the ODN. In sum, these results indicate that EET through the A-stack is extremely efficient while transfer across the WC bond from A to T is very inefficient.

EPR of AT-mers with CG Ends

We tested 13 samples containing CG at either the 5' or 3' end using an identical protocol, in order to test whether the EET behavior observed in AT-mers prevailed in ODNs containing

cytosine. In addition to the $T^{\bullet-}$ and $A(N3+H)^{\bullet}$ benchmarks in Figure 1, the $Cyt(N3+H)^{\bullet}$ benchmark is provided as well as the fit for an irradiated ODN of sequence $A_{32}C_4:G_4T_{32}$. This fit used 0% A, 38% T and 62% C. Figure 5 provides the component analysis results for all irradiated ODNs containing C. Due to similarities between the T and C benchmarks, the relative contribution of each of these components was difficult to determine; however, by focusing on the spectral extent of adenine, it was discovered that one-electron reduced adenine was not detected in any samples containing cytosine. This remained true whether C was on the same strand as the A-stretch or on the opposite strand. The rate of interstrand electron transfer across CG, therefore, must be greater than the rate of EE trapping by A at 4K.

In addition, we find that trapping by C predominates in ODNs with CG ends. In $A_{32}C_4:G_4T_{32}$, the molar ratio of C to T is 1:8 yet trapping predominates on C residues compared to Ts by a factor of $\sim 2:1$. The probability of trapping by C is, therefore, $\sim 16\times$ that of Thy. Given the probability of EE formation on both strands of this ODN, C can be viewed as efficiently scavenging all of the EE from the A strand and most of the EE from the T strand. A similar analysis applied to $A_{32}G_4:C_4T_{32}$, indicates that C is preferred over T by >100 -fold. Similarly, for $A_{12}G_4:C_4T_{12}$, $A_4G_2:C_2T_4$, $A_4C_2:G_2T_4$, $C_4A_{32}:T_{32}G_4$, and $G_4A_{32}:T_{32}C_4$, trapping by C is more probable than T by $60\times$, $6\times$, $4\times$, $50\times$, and $10\times$, respectively. Two major conclusions can be drawn from this information. First, shorter ODNs exhibit weaker EE trapping preference at C compared to T. This fact supports the conclusion that interstrand transfer from A to T is forbidden while transfer from A to C through the same mechanism is efficient. Sequences with longer stretches of A in effect provide additional sites of electron attachment, leading to favorable EET to C. A second potential conclusion can be drawn from the factor of 5 increase in C trapping efficiency in $C_4A_{32}:T_{32}G_4$ vs. $G_4A_{32}:T_{32}C_4$. This argues that while both interstrand and intrastrand EET from A to C are efficient, intrastrand transfer from A to C is preferred over interstrand transfer. This observation fits well with the results concerning interstrand and intrastrand transfer from A to T, leading to a hypothesis that intrastrand transfer from purine to pyrimidine bases is favored over interstrand transfer in duplex DNA.

To rule out the possibility that site selectivity for EE trapping is due to a direct attachment of EE to specific bases, we tested samples in which the intrastrand pathway for EET from A to C was denied. For example, $A_{32}G_4:C_4T_{32}$ was compared with equal molar amounts of A_4T_4 and $A_{28}G_5:C_5T_{28}$. Whenever the A stretch was isolated from the CG bridge, trapping of EE by A was observed (Figure 5). Thus, the selectivity for EE trapping is a consequence of EE transfer through the duplex to the preferred trapping site and not direct attachment of thermal electrons to the trapping site.

Discussion

Our results identify consecutive adenine bases as an ideal conduit for intrastrand EET in duplex DNA. Specifically, we have found that A is an efficient trap for EE at 4K if the A-strand of the duplex does not contain one of the other three bases, indicating that EET from A to T across the Watson-Crick bond is effectively forbidden. If there is a T, C, or G on the A-strand, trapping occurs at T or C instead of A. This holds true for stretches of up to 32 A's, indicating that EE traverse long distances in a time span much shorter than the EE trapping time. Although T competes effectively against A for the EE, it does not against C. Long stretches of T pass the majority of EE's to C. Additional EPR spectra of irradiated benzoyl-free ODNs were qualitatively consistent with our conclusions. (See Supplemental Figure 2). Our results show that for any biologically relevant sequence, C will be within range of any site of initial EE attachment to efficiently trap reductive damage. This finding strongly argues short range EE mobility in cellular DNA.

Relative electron transfer rates derived from the trapping distribution at 4K

As summarized in the Introduction, the expected order of trapping rates at 4K is

$$k_{\text{trp}}(\text{C}) > k_{\text{trp}}(\text{T}) \sim k_{\text{trp}}(\text{A}) \gg k_{\text{trp}}(\text{G}) \quad (1)$$

where $k_{\text{trp}}(\text{X})$ indicates the rate constant of EE trapping at base X. As illustrated in Scheme 2, the trapping rate at a given base, X, competes with EE transfer to another base, Y, either on the same strand (intrastrand rate $k_{\text{intra}}(\text{XY})$) or opposite strand (interstrand between bases that are paired $k_{\text{inter}}(\text{X:Y})$ or unpaired $k_{\text{inter}}(\text{X/Y})$). Using our findings on the sequence dependent distribution for EE trapping, the following relative rates at 4K can be deduced.

ATmers

$$k_{\text{intra}}(\text{AT}) \gg k_{\text{intra}}(\text{TA}) \quad (2)$$

$$k_{\text{trp}}(\text{T}) \gg k_{\text{intra}}(\text{TA}) \quad (3)$$

$$k_{\text{intra}}(\text{AT}) > k_{\text{intra}}(\text{AA}) \gg k_{\text{trp}}(\text{A}) \gg k_{\text{inter}}(\text{A:T}) \quad (4)$$

Long stretches of A with C on the A strand

$$k_{\text{intra}}(\text{AC}) \gg k_{\text{intra}}(\text{CA}) \quad (5)$$

$$k_{\text{trp}}(\text{C}) \gg k_{\text{intra}}(\text{CA}) \quad (6)$$

Long stretches of A with G on the A strand

$$k_{\text{intra}}(\text{AA}) \geq k_{\text{inter}}(\text{A/C}) \gg k_{\text{trp}}(\text{A}) \quad (7)$$

$$k_{\text{inter}}(\text{C/A}) \ll k_{\text{inter}}(\text{A/C}) \quad (8)$$

$$k_{\text{trp}}(\text{C}) \gg k_{\text{inter}}(\text{C/A}) \quad (9)$$

Using Eqs. (4) and (8)

$$k_{\text{inter}}(\text{A/C}) \gg k_{\text{inter}}(\text{A:T}) \quad (10)$$

Long stretches of T with C on the T strand

$$k_{\text{intra}}(\text{TT}) \sim k_{\text{intra}}(\text{TC}) > k_{\text{trp}}(\text{T}) \quad (11)$$

$$k_{\text{trp}}(\text{C}) > k_{\text{intra}}(\text{CT}) \quad (12)$$

The observation that EE traps exclusively at T in the AT ODN if a T is on the A-strand is described by Equations 2–4. Under the assumption that trapping rates by T and A are comparable (Eq. 1), selective trapping at T must arise from a strong preference for EET from A to T relative to the reverse direction (Eq. 2). This is in accord with the high electron affinity of T compared to A.^{8b} Dielectric relaxation at $T^{\bullet-}$ traps EE faster than back transfer to A (Eq. 3). The observation that the EE traverses up to 32 adenines to be trapped by T and not by A, indicates that $k_{\text{intra}}(\text{AA})$ is very fast. In contrast, observation of EE trapping by A in all of the A:T ODNs shows that interstrand EE from A to its conjugate base T ($k_{\text{inter}}(\text{A:T})$) is much slower than trapping by A (Eq. 4). Interstrand transfer from A to T was not observed over an 18 h period, indicating that $k_{\text{inter}}(\text{A:T})$ is effectively zero.

When C is on either end of an A strand, the EE is captured selectively by C, as expected from Equation 1 and expressed in Equations 5 and 6. When G is on either end of an A strand, all EE are still funneled from A to C, which is on the opposite strand. This requires EET between strands from A to C at a rate faster than trapping by A (Eq. 7). Interstrand transfer from A to C may occur by intrastrand transfer from A to G followed by interstrand transfer from G to C, but we do not rule out the possibility of other mechanisms (e.g. a direct “diagonal” or “wobble pair” interstrand transfer). Eqs. 4 and 8 describe the finding that EET across an AT base pair is far slower than transfer mediated by a GC base pair (Eq. 10).

The finding that the probability of trapping by C (*i.e.*, trapping yield normalized by the molar ratio of C to T) was $4\times$ to $100\times$ higher than trapping by T proves that at 4K intrastrand EET from T to T and from T to C is fast relative to the rate of trapping by T (Eq. 11), and that EET from C to T is slow compared to trapping by C (Eq. 12). The factor of $4\times$ obtained for the short ODN agrees well with our earlier work that found 80% of EE are trapped by C and 20% are trapped by T in a palindromic tetramer and hexamer.^{2c} Taken together, the extremely efficient EET via A's and the less efficient EET via T's, means that **long stretches of AT efficiently funnel EE to C.**

Possible Insights into EET at RT

The RT and low temperature studies discussed in the Introduction report a short range for EET, limited to $\sim 7\text{--}8$ bp.^{2c, 2f, 18} Transport efficiency is higher through T compared to C in RT CS studies. A factor that can be considered here is reversible proton transfer from G to $C^{\bullet-}$. This could contribute to slower EET compared to the anion base pair $\text{A:T}^{\bullet-}$, where a comparable proton transfer is absent. Our results at 4K can provide insight into the tunneling behavior of this system, which is temperature independent. Speaking exclusively in terms of the tunneling component of EET, C will efficiently compete for the EE regardless of whether or not it is on the same or opposite strand of the initial site of electron attachment. Importantly, hopping at RT from C to any other base is slowed by the activation energy needed for reverse proton transfer ($\text{C}(\text{N3+H})^{\bullet-} \rightarrow C^{\bullet-}$) giving time for the competing reaction of irreversible proton transfer to the $\text{C5}=\text{C6}$ bond. While the rate of protonation at C6 of $\text{C}(\text{N3+H})^{\bullet-}$ is not known, it does occur at very low temperatures²¹ and, therefore, occurs at higher temperatures. This means that in systems that utilize CG pairs to flank stretches of AT,^{19a, b} scavenging of EEs by CG should be taken into consideration.

In a study of one-electron reduced AT ODNs using pulse radiolysis, Yamagami et al. observed $\text{dA}^{\bullet-}$, as evidenced by a 480 nm absorption, in $\text{A}_{12}:\text{T}_{12}$ but not in $(\text{AT})_6:(\text{AT})_6$.³⁰ This is consistent with our finding that the probability of trapping EE by A increases when other bases are not present on a strand, which allows time for trapping at A by proton transfer. Evidence that $\text{dA}^{\bullet-}$ is likely to be protonated at pH7 comes from studies of protonation of radical pairs generated by photo-excitation of pyrene (Py) nucleosides³¹ and arguments put forward by Steenken^{15a}. The reduction potential of the $\text{dA}/\text{dA}^{\bullet-}$ couple is 0.3–0.4 V higher than $\text{dU}/\text{dU}^{\bullet-}$ couple.¹⁷ Fiebig and co-workers concluded that while $\text{dT}^{\bullet-}$

could play a major role as a “stepping stone” for EE in DNA, $C^{\bullet-}$ would not and that while ET favors C and T, that A has ambivalent reduction/oxidation properties.^{16a, 32}

Comparison of EET and hT

The primary carriers of excess electrons and holes, C and G respectively, are thermodynamically favored for their respective roles, and a significant part of what makes them primary carriers is related to reversible proton transfer. However, the driving force due to proton transfer from $G^{\bullet+}$ is much smaller than that due to proton transfer to $C^{\bullet-}$.³³ As a result, the hopping rate is faster for hT from G to G compared to EET from C to C. In addition, the rate of $6 \times 10^4 \text{ s}^{-1}$ for irreversible hydroxide addition to $G^{\bullet+}$ ³⁴ is certainly slower than irreversible proton addition to $C^{\bullet-}$.³⁵ Thus G is a better charge carrier than C because reversible proton transfer offers less “resistance” to CT and because competition due to terminating reactions is much smaller.

Although T and A are thermodynamically disadvantaged as charge carriers, once the charge is injected they are as good, or better, carriers than C and G. EET via T is not impeded by reversible proton transfer while C is. But both T and C are favorable sites for irreversible proton transfer and, as such, are unlikely to serve as long range conduits for EE in protic media.

Adenine is unique, and as predicted by Fiebig^{16a}, it is redox ambivalent. In contrast to data taken on adenine in solution³⁶, reversible proton transfer from $A^{\bullet+}$ is unfavorable in adenine stacked systems, making hT by A efficient in biologically relevant environments.³⁴ A is also an extraordinary conduit for EE and offers the only path by which the interior of DNA might conceivably act as a molecular wire. The attributes that make it so must include strong π - π coupling and an EET rate that is much faster than the rate of $A^{\bullet-}$ protonation.

One-electron Reduction vs. Photo-excitation Studies

Our results provide crucial insight into the tunneling behavior of excess electrons in DNA at low temperature. Using trapping behavior we have extrapolated an important hierarchy of EET at low temperature, dependent on base context, sequence, and the physical nature of base-base association. These results can provide possible insight into the tunneling behavior at biologically relevant temperatures, but should be used for prognostic conclusions involving room temperature photo-excitation studies. We do not discount the possibility of a correlation between 4K and room temperature EET, but the systems used to study each of these behaviors possess distinct differences from one another. Most importantly, at room temperature, EET is dependent on both tunneling and charge hopping, while 4K studies are dependent only on the former. In addition, experiments utilizing photo-excitation and subsequent charge injection measure **charge separation not charge transport**. This is an important distinction, since the long range electrostatic properties of the modified DNA hairpin system differs greatly from a duplex ODN that has been one-electron reduced. It is possible that charge transport properties will differ greatly under these distinct conditions. What is needed is a thorough study of CS at low temperature. Direct measurement of rate constants at low temperature and subsequent comparison of these rates to those measured at room temperature would help to answer the hotly debated issue of whether CT behavior of DNA at low temperature can be used to provide insight into similar behavior at RT.

In addition, these results offer a potential avenue of important research involving the conductivity of synthesized DNA at low temperature. Determination of the distance dependence of CT through stretches of consecutive adenine bases could provide important advances to nanowire research currently ongoing and using DNA. It is possible that stacked adenine bases in the duplex have the ability to shuttle charge efficiently to a preferred

trapping site at temperatures higher than 4K. It is important to investigate the temperature dependence of this behavior in conjunction to the distance dependence previously mentioned.

Conclusions

We observed that EET through stacked A's, in DNA at 4K, is fast compared to EE trapping by A. Whether the mechanism is via SE or hopping, EET occurs over distances of at least 32 bp.

While EET from A to T on the same strand is fast relative to EE trapping by A, the transfer rate is significantly smaller between A and T on opposite strands. In contrast, transfer from A to C on the opposite strand is very fast, even faster than EE trapping by A. The GC base pair provides a bridge for interstrand EET whereas the AT base pair does not.

In DNA consisting of a mixture of all 4 bases, EET from A, T, and G favors selective capture of EE by C at 4K.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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ABBREVIATIONS

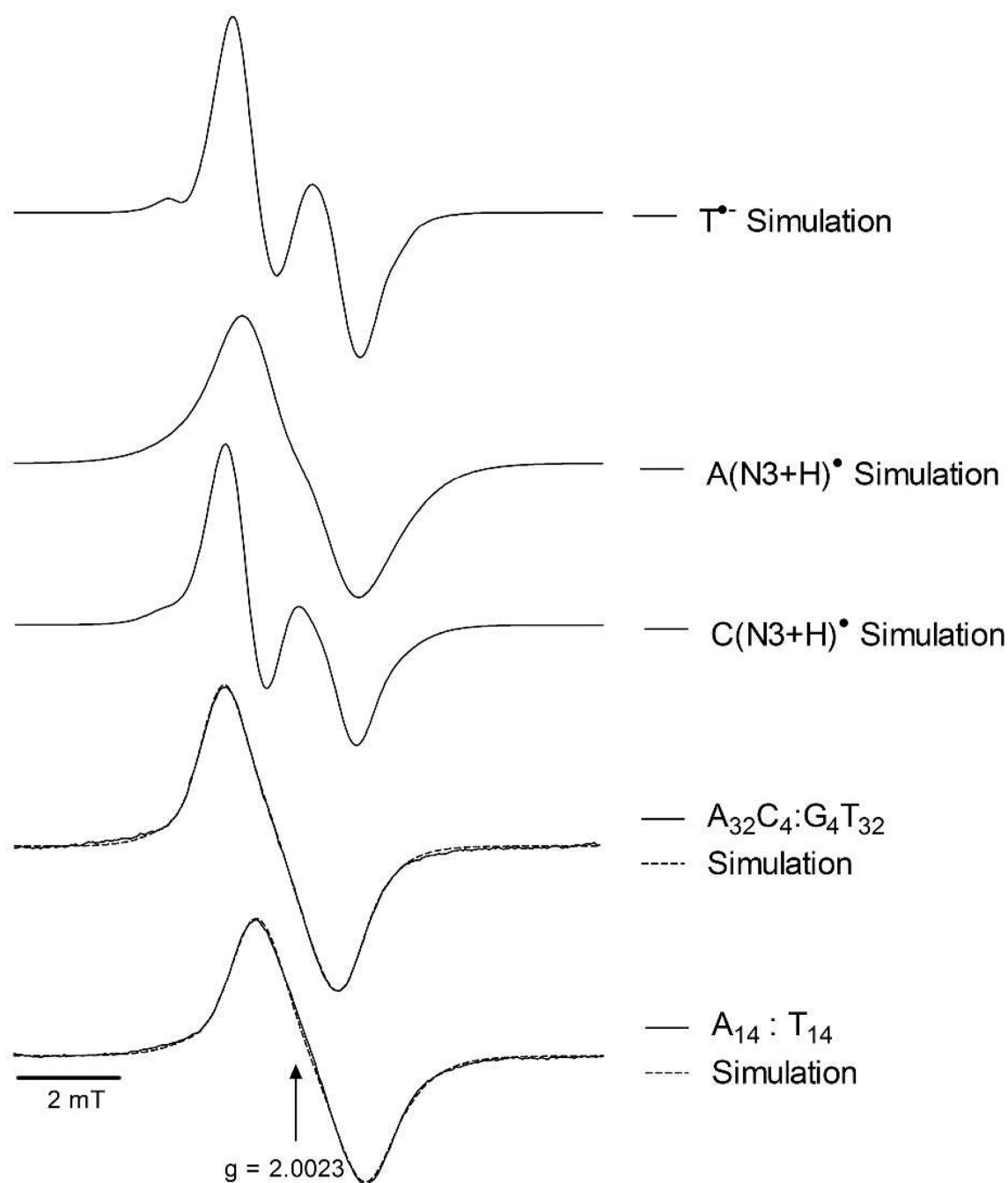
CT	charge transfer
EE	excess electron
h	hole
hT	hole transfer
EET	excess electron transfer
RT	room temperature
CS	charge separation
DS	double stranded
SE	superexchange
EPR	electron paramagnetic resonance
ENDOR	electron nuclear double resonance
A	Adenine
T	Thymine
G	Guanine
C	Cytosine
ODN	oligodeoxynucleotides

WC	Watson-Crick
ps	picosecond
bp	base-pair

REFERENCES

- (a) Sevilla, MD.; Becker, D. ESR studies of radiation damage to DNA and related biomolecules. Vol. Vol. 19. Cambridge, UK: The Royal Society of Chemistry; 2004. p. 243-278.(b) Becker, D.; Adhikary, A.; Sevilla, M. Recent Trends in Radiation Chemistry. Wishart, JF.; Rao, BSM., editors. Singapore: World Scientific Publishing Co. Pte. Ltd.; 2010. p. 509-542.(c) Bernhard, WA.; Close, DM. Charged particle and photon interactions with matter. Mozumder, A.; Hatano, Y., editors. New York: Marcel Dekker; 2003. p. 471-489.(d) Bernhard, WA. Radical and radical ion reactivity in nucleic acid chemistry. Greenberg, MM., editor. Hoboken, New Jersey: John Wiley & Sons; 2009.
- (a) Wang W, Sevilla MD. Radiat. Res. 1994; 138(1):9–17. [PubMed: 8146305] (b) Wang W, Yan M, Becker D, Sevilla MD. Radiat. Res. 1994; 137(1):2–10. [PubMed: 8265784] (c) Sevilla MD, Becker D, Razskazovskii Y. Nukleonika. 1997; 42(2):283–291.(d) Bernhard, WA. Advances in Radiation Biology. Lett, JT.; Adler, H., editors. Vol. Vol. 9. New York: Academic Press; 1981. p. 199-280.(e) Bernhard WA. J. Phys. Chem. 1989; 93(6):2187–2189.(f) Spalletta RA, Bernhard WA. Radiat. Res. 1992; 130(1):7–14. [PubMed: 1313984] (g) Spalletta RA, Bernhard WA. Radiat. Res. 1993; 133(2):143–150. [PubMed: 8382367] (h) Milano MT, Hu GG, Williams LD, Bernhard WA. Radiat. Res. 1998; 150(1):101–114. [PubMed: 9650607] (i) Debije MG, Bernhard WA. J. Phys. Chem. B. 2000; 104(32):7845–7851.(j) Roginskaya M, Bernhard WA, Razskazovskiy Y. J. Phys. Chem. B. 2004; 108(7):2432–2437. [PubMed: 17375182] (k) Sharma KKK, Tyagi R, Purkayastha S, Bernhard WA. J. Phys. Chem. B. 2010; 114:7672–7680. [PubMed: 20469885]
- Arkin M, Stemp E, Jenkins Y, Barbara P, Turro NJ, Barton JK. Polym. Mater. Sci. Eng. 1994; 71:598–599.
- (a) Kawai, K.; Majima, T. Radical and radical ion reactivity in nucleic acid chemistry. Greenberg, MM., editor. Hoboken, New Jersey: John Wiley & Sons; 2009. (b) Varghese, R.; Wagenknecht, H-A. Radical and radical ion reactivity in nucleic acid chemistry. Greenberg, MM., editor. Hoboken, New Jersey: John Wiley & Sons; 2009. (c) Vura-Weis J, Wasielewski MR, Thazhathveetil AK, Lewis FD. J. Am. Chem. Soc. 2009; 131:9722–9727. [PubMed: 19558185]
- Wagenknecht HA. Angew. Chem. Int. Ed. Engl. 2003; 42:2454–2460. [PubMed: 12800162]
- (a) Daublain P, Thazhathveetil AK, Wang Q, Trifonov A, Fiebig T, Lewis FD. J. Am. Chem. Soc. 2009; 131:16790–16797.(b) Tainaka K, Fujitsuka M, Takada T, Kawai K, Majima T. J. Phys. Chem. B. 2010; 114:14657–14663. [PubMed: 20509700]
- (a) Cai Z, Li X, Sevilla MD. J. Phys. Chem. B. 2002; 106(10):2755–2762.(b) Cai, Z.; Sevilla, MD. Long Range Transfer in DNA Vol II. Schuster, G., editor. Vol. Vol. 237. New York: Springer-Verlag; 2004. p. 103-128.
- (a) Bernhard WA. Free Radical Res. Commun. 1989; 6(2–3):93–94. [PubMed: 2545567] (b) Barnes JP, Bernhard WA. Radiat. Res. 1995; 143(1):85–92. [PubMed: 7597149]
- Swarts SG, Gilbert DC, Sharma KK, Razskazovskiy Y, Purkayastha S, Naumenko KA, Bernhard WA. Radiat. Res. 2007; 168:367–381. [PubMed: 17705640]
- Debije MG, Milano MT, Bernhard WA. Angew. Chem. Int. Ed. 1999; 38(18):2752–2756.
- Barnes JP, Bernhard WA. J. Phys. Chem. 1995; 99(28):11248–11254.
- Bernhard WA, Patrzalek AZ. Radiat. Res. 1989; 117(3):379–394. [PubMed: 2538858]
- Debije MG, Bernhard WA. J. Phys. Chem. A. 2002; 106(18):4608–4615.
- (a) Steenken S. Free Radical Res. Commun. 1992; 16(6):349–379. [PubMed: 1325399] (b) Steenken S. Biol. Chem. 1997; 378(11):1293–1297. [PubMed: 9426189]
- (a) Steenken S. Free Radical Communications. 1989; 6(2–3):117–120.(b) Bernhard WA. J. Phys. Chem. 1989; 93:2187–2189.(c) Sevilla MD, Becker D, Yan M, Summerfield S. J. Phys. Chem. 1991; 95(8):3409–3415.

16. (a) Fiebig T, Wan C, Zewail AH. *Chem. Phys. Chem.* 2002; 3:781–788. [PubMed: 12436905] (b) Raytchev M, Mayer E, Amann N, Wagenknecht H-A, Fiebig T. *Chem. Phys. Chem.* 2004; 5:706–712. [PubMed: 15179723] (c) Wagner C, Wagenknecht H-A. *Chem. Eur. J.* 2005; 11:1871–1876. [PubMed: 15685707]
17. Huber R, Fiebig T, Wagenknecht H-A. *Chem. Commun.* 2003:1878–1879.
18. Anderson RF, Wright GA. *Phys. Chem. Chem. Phys.* 1999; 1(20):4827–4831.
19. (a) Ito T, Rokita SE. *J. Am. Chem. Soc.* 2004; 125:11480–11481. [PubMed: 13129334] (b) Ito T, Rokita SE. *J. Am. Chem. Soc.* 2003; 125:11480–11481. [PubMed: 13129334] (c) Ito T, Rokita SE. *Angew. Chem. Int. Ed.* 2004; 43:1839–1842.
20. Park MJ, Fujitsuka M, Kawai K, Majima T. *J. Am. Chem. Soc.* 2011; 133:15320–15323. [PubMed: 21888400]
21. Debije MG, Close DM, Bernhard WA. *Radiat. Res.* 2002; 157(3):235–242. [PubMed: 11839084]
22. (a) Cai Z, Gu Z, Sevilla MD. *J. Phys. Chem. B.* 2000; 104(44):10406–10411. (b) Cai Z, Sevilla MD. *J. Phys. Chem. B.* 2000; 104(29):6942–6949. (c) Messer A, Carpenter K, Frozley K, Buchanan J, Yang S, Razskazovskii Y, Cai Z, Sevilla M. *J. Phys. Chem. B.* 2000; 104(5):1128–1136.
23. Harriman A. *Angew. Chem. Int. Ed.* 1999; 38:945–948.
24. Mercer KR, Bernhard WA. *J. Magn. Reson.* 1987; 74(1):66–71.
25. Fouse GW, Bernhard WA. *J. Chem. Phys.* 1979; 70(4):1667–1670.
26. (a) Sagstuen E, Hole EO, Nelson WH, Close DM. *J. Phys. Chem.* 1992; 96(3):1121–1126. (b) Close DM, Hole EO, Sagstuen E, Nelson WH. *J. Phys. Chem. A.* 1998; 102(34):6737–6744. (c) Close DM, Nelson WH, Sagstuen E, Hole EO. *Radiat. Res.* 1994; 137(3):300–309. [PubMed: 8146272]
27. Sagstuen E, Hole EO, Nelson WH, Close DM. *J. Phys. Chem.* 1992; 96(21):8269–8276.
28. Black PJ, Bernhard WA. *J. Phys. Chem. B.* 2011; 115:8009–8013. [PubMed: 21627082]
29. Patel DJ, Kozlowski SA, Nordheim A, Rich A. *Proc. Natl. Acad. Sci. USA.* 1982; 79:1413–1417. [PubMed: 6951185]
30. Yamagami R, Kobayashi K, Tagawa S. *Chem. Eur. J.* 2009; 15:12201–12203. [PubMed: 19834944]
31. Amann N, Pandurski E, Fiebig T, Wagenknecht H-A. *Angew. Chem. Int. Ed.* 2002; 41(16):2978–2980.
32. Fiebig T, Wan C, Kelley SO, Barton J. *Proc. Natl. Acad. Sci. U. S. A.* 1999; 96(4):1187–1192. [PubMed: 9989999]
33. Steenken S. *Top. Curr. Chem.* 1996; 177(Electron Transfer II):125–145.
34. Giese B, Spichy M. *Chem. Phys. Chem.* 2000; 1:195–198.
35. Deeble DJ, Das S, von Sonntag C. *J. Phys. Chem.* 1985; 89(26):5784–5788.
36. Adhikary A, Kumar A, Khanduri D, Sevilla MD. *J. Am. Chem. Soc.* 2008; 130(31):10282–10292. [PubMed: 18611019]

**Figure 1.**

Simulated benchmark spectra of one-electron reduced thymine, adenine, and cytosine. The electron adducts of both adenine and cytosine are stabilized by proton transfer while thymine is stable in the anionic form. These 3 spectra were used to simulate the spectra of one-electron reduced ODN, two examples of which are shown here.

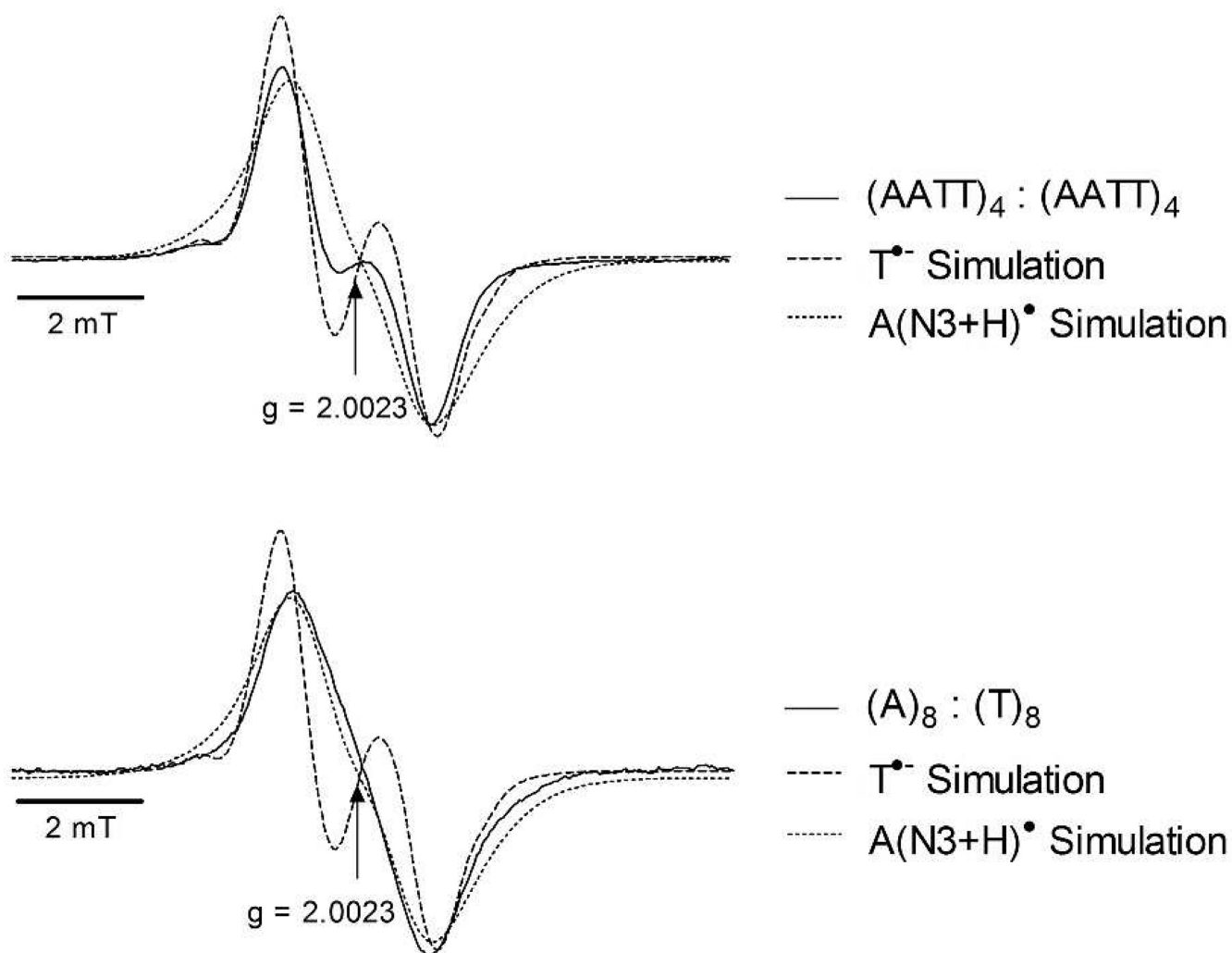


Figure 2. Spectral overlays illustrate the larger spectral extent of the Ade(N3+H)[•] spectrum relative to the T^{•-} spectrum. This difference serves to hold the covariance between these two spectra low. Particularly discriminating is the difference on the high field (right) side as can be seen in the comparison of the one-electron reduced (AATT)₄:(AATT)₄ spectrum with that of (A)₈:(T)₈.

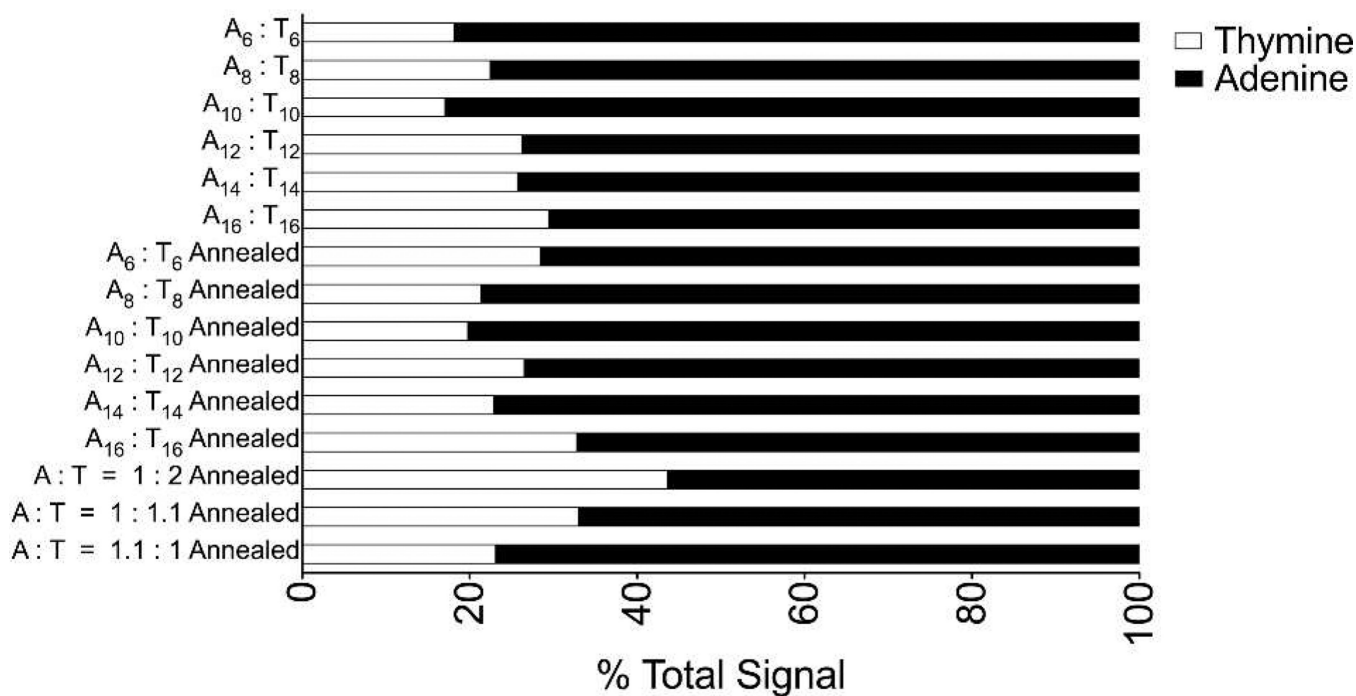


Figure 3.

The result of component analysis of 15 different ODNs, all of the A:T type, for which there was no evidence of EET from the A strand to the T strand. EE were efficiently trapped on the A strand. Included is a component analysis of A_6 and T_6 annealed at different molar ratios in order to rule out the possible presence of single stranded ODNs

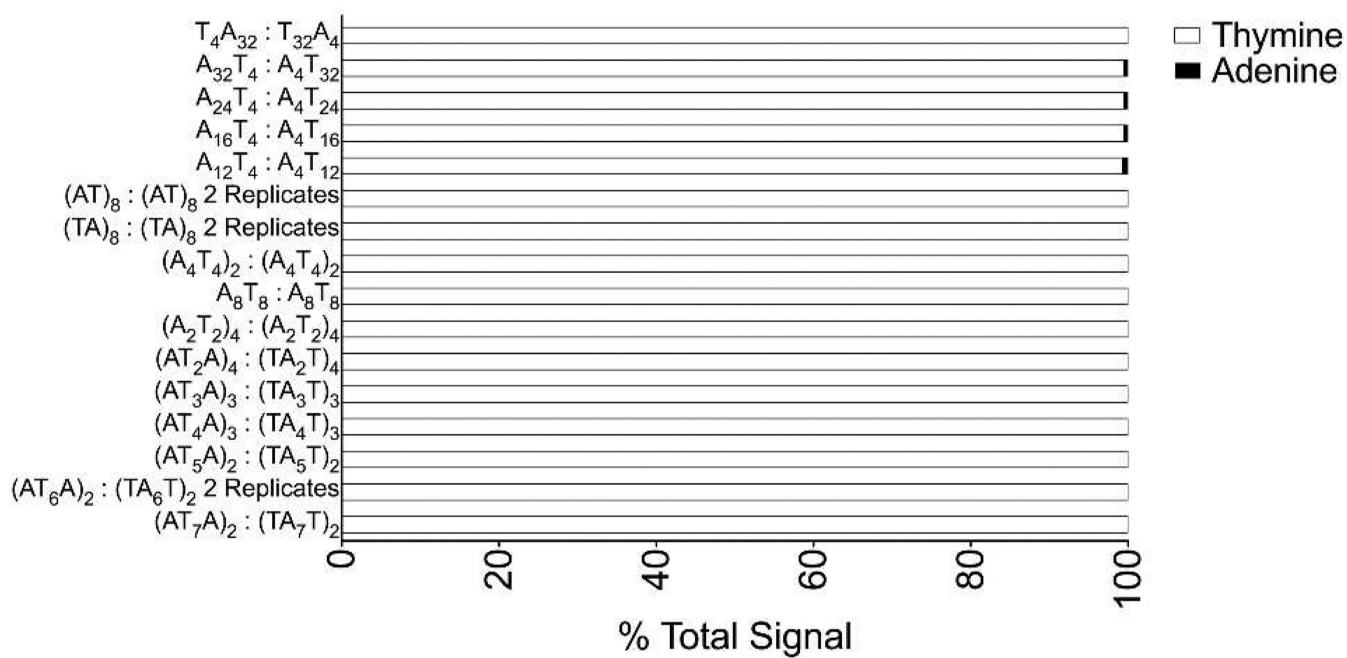
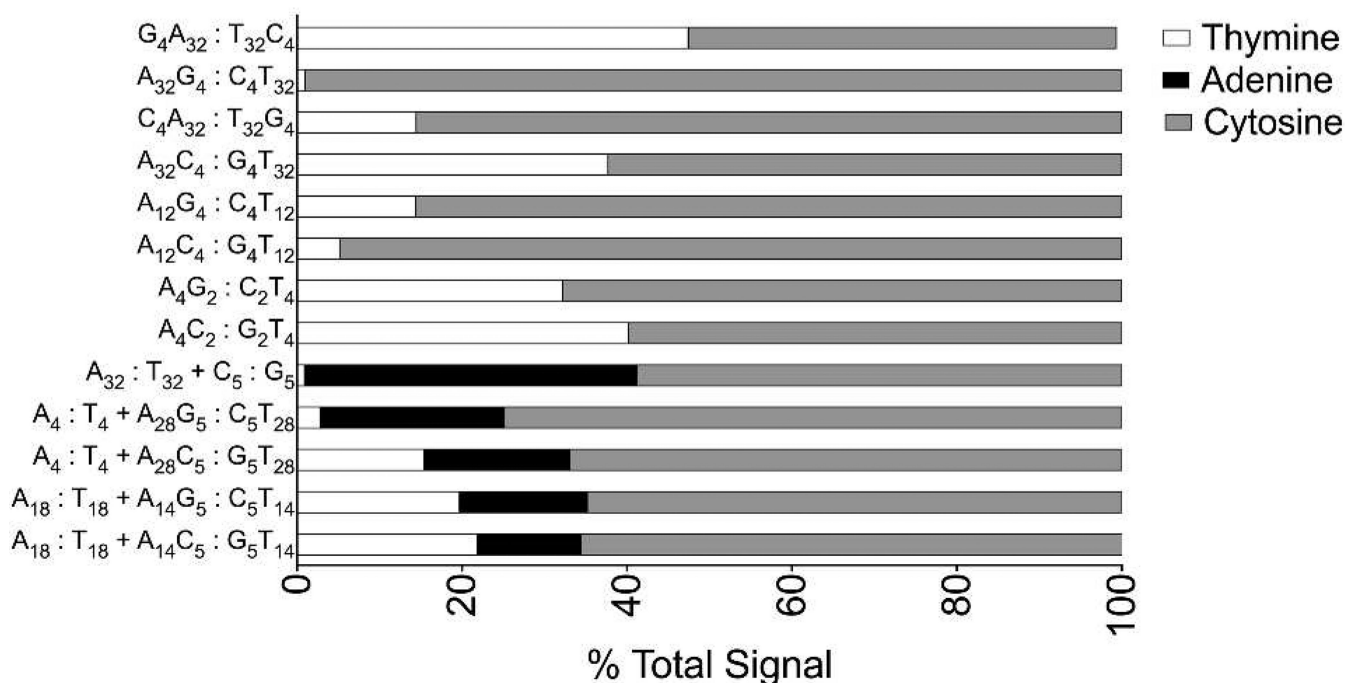


Figure 4.

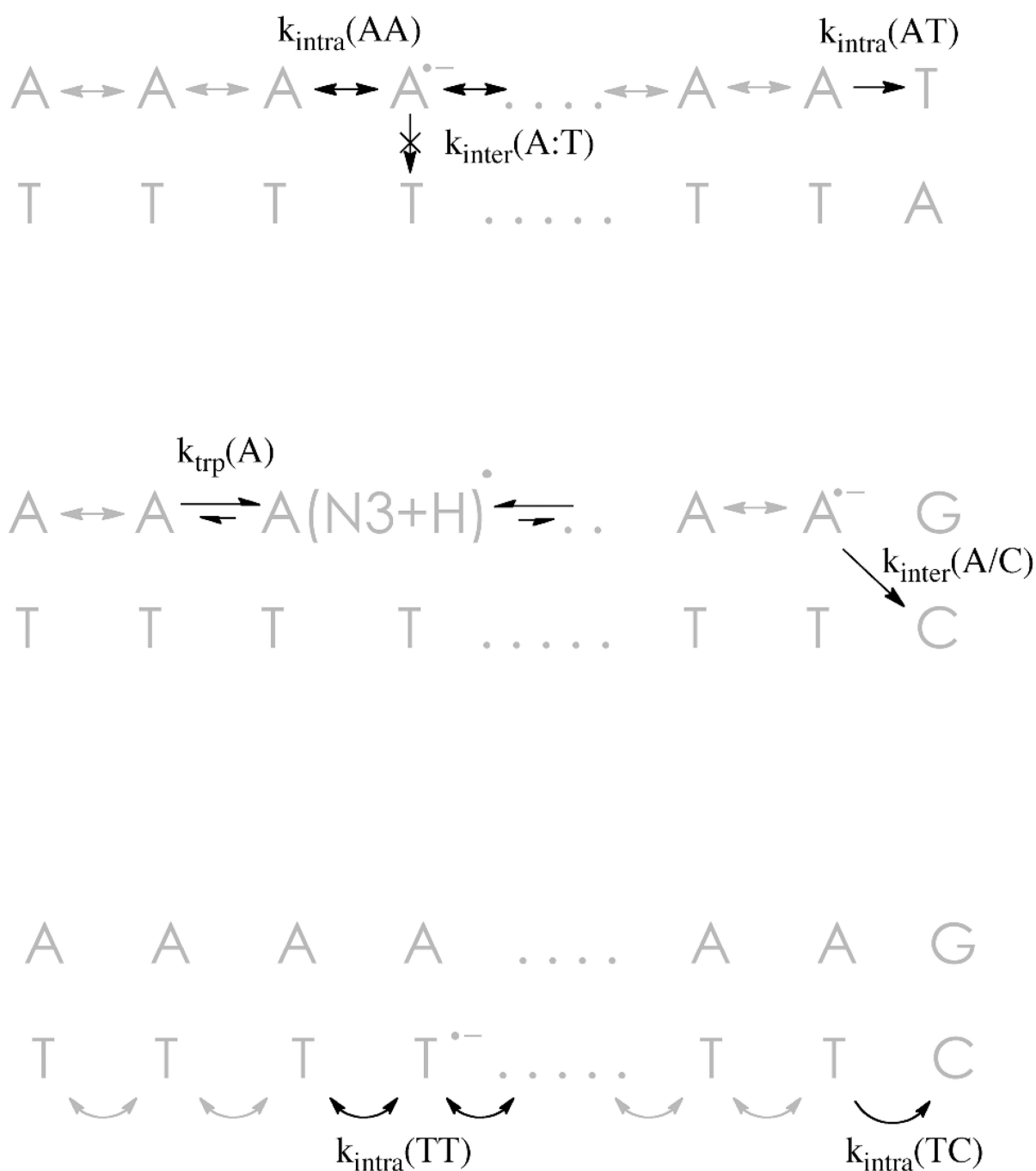
Component analysis results for 16 separate A-T type ODNs. The presence of a very small adenine component in four of the samples is within the error of the fit. These results indicate the dominance of intrastrand transfer from adenine to thymine at distances up to 32 sequential adenines.

**Figure 5.**

The top 8 bars shown here are for ODNs containing CG ends. In all cases, no EE trapping by A is observed and the majority of trapping is by C. Beyond these two main observations, it is difficult to attach significance to the variation from sequence to sequence because of the relatively high covariance between the T^{*-} and $C(N3+H)^*$ spectra. The 5 bottom bars are for ODNs constructed to address the question of whether or not initial attachment of EE is site selectivity. Cleavage of the duplex, so as to isolate A's from the other three nucleobases, resulted in EE trapping by A, showing that attachment initially occurs at all nucleobases and that site selective trapping is a consequence of EET.



Protonation reactions following electron addition to adenine, cytosine and thymine. The radical anion of thymine, $T^{\bullet-}$, is stable at 4K. This is not the case for A and C for which the radicals are trapped at 4K by reversible proton transfer, structures $A(N3+H)^{\bullet}$ and $C(N3+H)^{\bullet}$. The stable end products are 5,6-dihydrothymine (DHT) and 5,6-dihydrocytosine (DHC). In mixed sequences of DNA a stable end product for reduced adenine is not observed because it competes poorly for the EE.



Scheme 2. Charge Transfer Model in DNA

The notation used for rates of EET between the nucleobases is defined here. Transfer is divided into three types: intrastrand transfer (k_{intra}), interstrand transfer (k_{inter}), and EE trapping (k_{trp}). Two cases for k_{inter} are distinguished by using a colon for a conjugate base pair, $k_{\text{inter}}(\text{A:T})$, and a slash for a non-conjugate base pair, $k_{\text{inter}}(\text{A/C})$.