Chapter 3

Spectroscopy of 2-Aminopurine/7-Deazaguanine-Modified DNA Duplexes[‡]

[‡]Femtosecond time-resolved laser experiments performed in collaboration with Professor Ahmed H. Zewail, Dr. Chaozhi Wan, and Dr. Torsten Fiebig in the Laboratory for Molecular Sciences at the California Institute of Technology.

3.1 Introduction

Experiments involving reactants appended to DNA have been useful in determining the efficacy of charge transfer through the DNA π stack. Perhaps not surprisingly, charge transfer efficiency through the DNA double helix has been found to depend strongly on how well the reactants are coupled to the DNA base stack.[1] Early investigations aimed at determining the nature of charge migration through the double helix utilized intercalators tethered to the ends of DNA duplexes.[2,3] More recently, as described in Chapter 2, a modified DNA base (which is an integral part of the DNA π stack) was employed to examine charge transfer.[4,5] While these studies have provided much data and valuable insight, the approach of using a pendant chromophore suffers from a few drawbacks. First, it is nearly impossible to fix the tethered intercalator at a specific site within DNA, although it is limited to the end where it is anchored. This gives rise to a distribution of populations when examining charge transfer to a fixed component located at a distance down the helix. Second, limits are placed on the concentrations able to be used in order to maintain the intramolecularity of the charge transfer reactions.

To address these limitations Kelley and Barton examined the photoinduced charge transfer between the base analog 2-aminopurine (Ap, Figure 3.1) and the natural base guanine in DNA.[6] Ap is similar in structure to the natural base adenine. Investigations of Ap-modified duplexes by NMR, fluorescence spectroscopy, and calorimetry have shown that Ap participates in normal Watson-Crick base pairing with thymine and is well stacked within the DNA double helix.[7-11] Importantly, Ap is fluorescent and may be excited selectively over the other DNA bases when

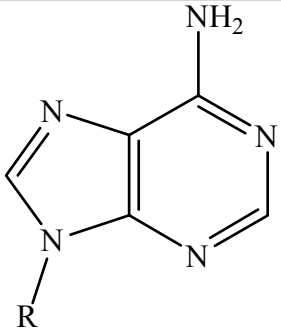

Figure 3.1 Structure and excited-state potential of 2-aminopurine.

incorporated into a duplex. Figure 3.2 displays the absorption and emission spectra of Ap-modified duplexes as well as the absorption of 2-aminopurine in solution. DNA has its characteristic large π-π* absorbance at 260 nm, but in the same duplex the absorbance of Ap (at 325 nm) is separated from that band. This allows for selective excitation of the fluorophore, which emits with λ_{max} = 370 nm.

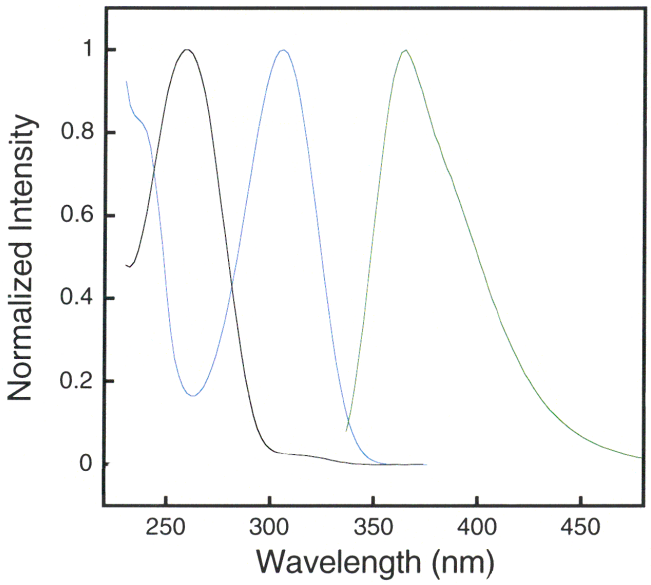

Figure 3.2 Spectral properties of 2-aminopurine (Ap): Ap-modified DNA duplex absorption (left); Ap absorption (middle); Ap-modified DNA duplex emission (right).

Once excited, Ap has enough thermodynamic driving force to oxidize the natural DNA base guanine[12] (G) and the modified base 7-deazaguanine[4] (Z) (Figure 3.3). It does not, however, have enough driving force to oxidize inosine (I).[6] Inosine differs from guanine in having one less amine group. This reduces the number of hydrogen bonds in its base pair with cytosine from 3 to 2. Perturbations to the overall structure and stacking are minimal, though, so Ap/I containing duplexes provide a nice reference to Ap/G containing duplexes in which photoinduced charge transfer is expected to occur.[13–14] This is important if we wish to evaluate solely the contribution of oxidative charge transfer between the 2-aminopurine and guanine or 7-deazaguanine.

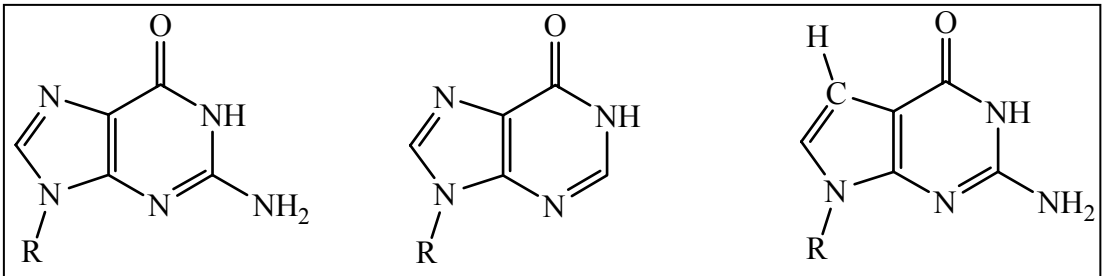

Figure 3.3 Structures and oxidation potentials of guanine (G), inosine (I), and deazaguanine (Z).

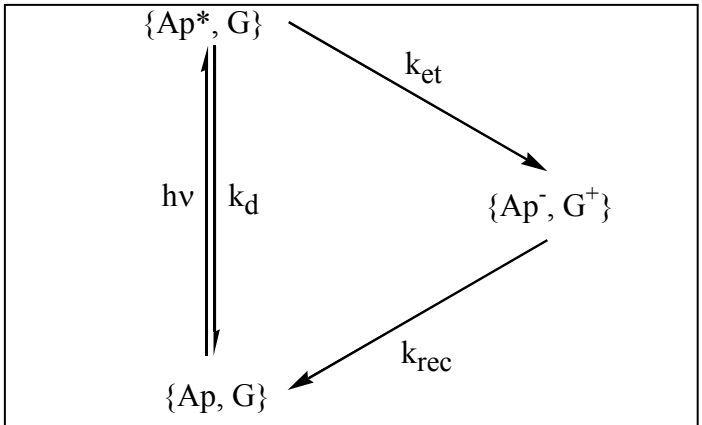

Figure 3.4 Proposed electron transfer cycle between 2-aminopurine (Ap) and guanine (G).

In solution, the fluorescence of excited-state 2-aminopurine is readily quenched by the nucleotide triphosphates of guanine and 7-deazaguanine ($K = 2.2\times10^9$ $M^{-1}s^{-1}$ and 5.2×10^9 $M^{-1}s^{-1}$, respectively).[6] This quenching is attributed to charge transfer, as the lack of spectral overlap between Ap* fluorescence and dGTP and dZTP absorption allow energy transfer to be ruled out as a quenching mechanism. Further, the quenching rate constants parallel the driving force for charge transfer (ΔG = -0.2 V for dGTP and -0.5 V for dZTP). Also consistent with a charge transfer quenching mechanism is the result that quenching is not observed in the presence of dITP, where the driving force for the

reaction is essentially 0 V. The charge transfer cycle for an Ap/G system is outlined in Figure 3.4

Because reactants which are modified DNA bases may be placed at any site in the double helix, intrastrand vs. interstrand charge transfer may be examined. Kelley and Barton looked at this phenomenon for a series of Ap-modified duplexes containing guanine or 7-deazaguanine.[6] In all cases the reaction was found to exhibit a shallow dependence on distance, consistent with the notion that reactants well stacked in the double helix participate in efficient charge transfer. While the dynamics of the interstrand charge transfer reaction were able to be observed via time-correlated single photon counting (TCSPC) spectroscopy, the dynamics of the intrastrand reaction were too fast to be determined.

This chapter describes the use of steady-state and ultrafast time-resolved spectroscopies to study intrastrand DNA-mediated charge transfer between 2-aminopurine and guanine and 7-deazaguanine. Comparisons to previous results from our laboratory are detailed, and conclusions regarding the effect of driving force on the reaction are made. These experiments demonstrate once again the remarkable efficiency of charge migration through the DNA π stack and the effect that occurs when employing 7-deazaguanine instead of guanine as a reactant.

3.2 Experimental Section

Materials. Reagents for DNA oligonucleotide synthesis were obtained from Glen Research. Unless otherwise noted, all other chemicals were purchased from Fluka or Aldrich and used without further purification.

Instrumentation. UV-visible spectra were taken on either an HP 8452A spectrophotometer or a Beckman DU 7400 spectrophotometer. Time-resolved fluorescence and transient absorption measurements on the femtosecond timescale were carried out in the facilities in the Laboratory for Molecular Sciences using a Ti:sapphire

laser (Spectra-Physics), as has been described.[15] Steady-state emission from 340 nm to 500 nm was measured using an ISS K2 spectrofluorometer with λ_{exc} = 325 nm.

Methods. Sample Preparation. Unless otherwise noted, all experiments were performed in a buffer of 100 mM sodium phosphate (NaPi) at pH=7. 2-Aminopurine, inosine, and 7-deazaguanine containing duplexes were synthesized as previously described.[4,6] All oligonucleotides were purified by reverse phase HPLC (0–15% CH_3CN over 35 minutes, C18 Dynamax column). The duplex concentration for all experiments was 100 μM. Duplexes were created by hybridizing the appropriate amounts of complementary single strands based on calculated extinction coefficients for unmodified sequences (ε_{260} ($M^{-1}cm^{-1}$): dC = 7400; dT = 8700; dG = 11,500; dA = 15,400; dI = 11,000; dZ = 10,500; dAp = 2500.[4,6,16,17] Characterization for all oligonucleotides included MALDI-TOF mass spectrometry and UV-visible spectroscopy.

Time-resolved Spectroscopy. Time-resolved data were obtained by Dr. Chaozhi Wan and Dr. Torsten Fiebig in the Laboratory for Molecular Sciences at the California Institute of Technology. A Ti:sapphire laser generated femtosecond pulses (80 fs; ca. 800 nm; 2 mJ at 1 kHz). The 2 mJ pulse was split equally to pump two optical parametric amplifiers (OPAs). The signal output from one OPA was then mixed with the residual 800 nm pulse to generate the pump pulse at 325 nm. The probe pulse at 400–700 nm (typically 600 nm) was generated from another OPA. The probe pulse at ca. 400 nm was generated by doubling the 800 nm pulse. Pump energy pulses at the sample were approximately 0.2 μJ, and the probe energy pulses were attenuated with filters to be approximately 0.1 μJ. Unless otherwise noted, spectra were obtained at ambient temperature (ca. 21 °C).

Steady-state Spectroscopy. Steady-state fluorescence measurements were made at ambient temperature (ca. 21 °C) on an ISS K2 fluorometer. Excitation was at 325 nm, and luminescence intensities were integrated from 340–500 nm and compared to a 100 μM 2-aminopurine standard in a pH=7 buffer of 100 mM NaPi. Measurements were

performed using 5 mm path length cells to minimize inner filter effects that often arise when using high concentrations of chromophore.

Melting Temperature Experiments. Thermal denaturation experiments of DNA duplexes were performed on a Beckman DU 7400 spectrophotometer. Absorbance at 325 nm was monitored every 1 degree from 80 to 10 °C, with a 2 minute equilibration time at each step. The point of inflection in the sigmoidal curve was taken to be the melting temperature of the DNA. Using data at 325 nm, however, provides information about the local melting temperature surrounding the aminopurine nucleotide and should give melting temperatures slightly lower than the DNA duplex as a whole (monitored at 260 nm).

3.3 Results and Discussion

3.3.1 Steady-state spectroscopic investigations of intrastrand charge transfer

Distance Dependence. When incorporated into DNA duplexes, guanine (G) and 7-deazaguanine (Z) are able to be oxidized by excited-state 2-aminopurine (Ap). Since the reaction of the excited state of Ap with the base analog inosine (I) is not thermodynamically favorable, charge transfer between G or Z and photoexcited Ap can be evaluated using inosine containing duplexes as references. Further, because duplexes containing Z differ from those containing G by only one atom, the effect of driving force on DNA-mediated charge transfer behavior may be examined without drastically altering the structure of the assemblies. Controlling the separation between donor and acceptor along the double helix is quite easy, as standard automated oligonucleotide synthesis can be employed to place the reactants at fixed points in the DNA. Fixing the reactants at specific sites eliminates the distribution of distances usually present when charge transfer is examined with intercalators tethered to the end of DNA.

Kelley and Barton found that intrastrand base-base charge transfer between Ap* and G or Z in DNA was quite rapid and efficient.[6] In order to examine this phenomenon in more detail, a series of 14-mer duplexes was created in which inosine, guanine, or 7-deazaguanine was located 3.4–13.6 Å away on the same strand in double helical DNA. Because of the remarkable dependence of DNA-mediated charge transfer on the sequence surrounding the reactants,[4] the flanking sequences for Ap and G or Z were kept constant. Table 3.1 summarizes the chosen sequences and details their melting temperatures (which are a measure of the duplex stability near the Ap). The high inosine content gives melting temperatures 10–20 degrees lower than that for mixed-sequenced oligomers of similar length. This is unfortunate; however, using inosines instead of guanines is necessary to insure Ap* is reacting with only the single G or Z we have placed at chosen sites down the helix. The differences in melting temperatures for the G or Z containing duplexes and the reference I containing duplexes is only a few degrees, consistent with

Duplex	Ap-X	T_m (I)	T_m (G)	T_m (Z)
5'-TAIA$_p$**X**ITITTATIA-3' 3'-ATC T CCACAATACT-5'	3.4 Å	28	32	32
5'-TAIA$_p$A**X**ITATTAIA-3' 3'-ATC T TCCATAATCT-5'	6.8 Å	29	33	32
5'-TAIA$_p$AA**X**ITITAIA-3' 3'-ATC T TTCCACATCT-5'	10.2 Å	30	32	32
5'-TAIA$_p$AAA**X**ITATIA-3' 3'-ATC T TTTCCATACT-5'	13.6 Å	32	33	34

Table 3.1 Melting temperature data (°C, λ_{obs}=325 nm) for a series of Ap$(A)_nX$ duplexes where X = inosine (I), guanine (G), or 7-deazaguanine (Z). Measurements taken with 0.1 mM duplex in 100 mM NaPi buffer at pH=7.

there being one less hydrogen bond in the interior of the helix. It should be noted, however, that the melting temperatures reported here were determined at 325 nm, where Ap is the major contributor to the overall absorbance. Thus, we are actually monitoring the melting temperature of the duplex near the Ap. As we would expect, the melting temperatures indicate stabilization in the duplex when a reference inosine at position X is moved further from the Ap along the DNA double helix. Also, not surprisingly, the melting temperatures of the G and Z containing duplexes are similar and maintain a nearly constant value over the entire range of Ap-X donor-acceptor distances studied in these experiments.

Table 3.2 summarizes the luminescence quenching of Ap* by G and Z in the series of duplexes outline above. The fraction quenched (Fq) was determined in the following manner. Ap containing duplexes were selectively excited with 325 nm light with simultaneous recording of the Ap* fluorescence intensity from 340–500 nm. The

Duplex	Ap-X	Fq (X=G)	Fq (X=Z)
5'-TAIA$_p$**X**ITITTATIA-3' 3'-ATC T CCACAATACT-5'	3.4 Å	0.94 (2)	0.95 (2)
5'-TAIA$_p$A**X**ITATTAIA-3' 3'-ATC T TCCATAATCT-5'	6.8 Å	0.72 (2)	0.86 (1)
5'-TAIA$_p$AA**X**ITITAIA-3' 3'-ATC T TTCCACATCT-5'	10.2 Å	0.47 (2)	0.53 (1)
5'-TAIA$_p$AAA**X**ITATIA-3' 3'-ATC T TTTCCATACT-5'	13.6 Å	0.10 (3)	0.21 (2)

Table 3.2 Steady-state luminescence quenching values (Fq = fraction quenched) for a series of Ap(A)$_n$X duplexes, where X = G or Z. λ_{exc}=325 nm and λ_{obs}=340-500 nm. All measurments performed on 0.1 mM duplexes in a buffer of 100 mM NaPi at pH=7.

fluorescence signal over that range for Ap/I duplexes (X = I), in which there is negligible charge transfer, was used as the reference for Ap/G and Ap/Z duplexes in which charge transfer was expected to occur. The quantum yields for the Ap/G and Ap/Z duplexes were then divided by the quantum yields for the corresponding Ap/I duplexes to determine Fq ($Fq = 1-\phi_G/\phi_I$ or $Fq = 1-\phi_Z/\phi_I$). A higher number for Fq indicates more luminescence quenching and thus more efficient DNA-mediated charge transfer.

The results for both Ap/G and Ap/Z duplexes indicate intrastrand charge transfer through an adenine tract is fairly efficient over 13.6 Å. Figure 3.5 illustrates the distance dependences of the quenching yields for the Ap/G and Ap/Z series. The distance dependences for the two reactions are quite similar. At all donor-acceptor separations the amount of quenching observed in Ap/Z duplexes is greater than that observed in Ap/G duplexes. This is consistent with the notion that, all else being equal, an increase in the

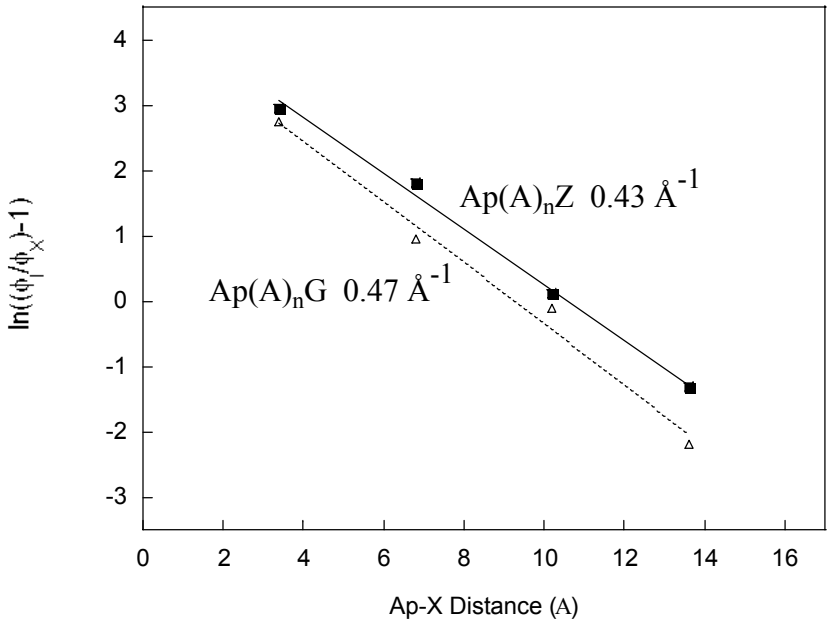

Figure 3.5 Distance dependences of intrastrand charge transfer between Ap and G (triangles, dashed line) and Z (squares, solid line).

thermodynamic driving force for the reaction should produce a larger amount of quenching. Donor-acceptor separations greater than 13.6 Å were not studied. The quenching values in such systems are expected to be small and difficult to observe, especially in the Ap/G assemblies. Nevertheless, intrastrand base-base charge transfer is indeed an efficient process.

Intervening Sequence Effects. Charge transfer in DNA mediated by an adenine tract was expected to be relatively facile due to the enhanced coupling of purines in DNA.[18] Base-base charge transfer allows this to be easily evaluated. The question arises, then, of the ability of pyrimidines to mediate charge transfer. Table 3.3 summarizes the luminescence quenching in a series of Ap/Z duplexes in which the intervening base stack consisted of thymines or adenines. The dropoff with distance is quite steep for the assemblies in which Ap and Z are separated by a thymine bridge. At a 2 base pair separation (10.2 Å donor-acceptor distance) luminescence quenching is nonexistent. This is in stark contrast to Ap/Z charge transfer mediated by an adenine

Duplex	Ap-X	Fq (Y=A)	Fq (Y=T)
5'-TAIA$_p$**Z**ITITTATIA-3' 3'-ATC T CCACAATACT-5'	3.4 Å	0.95 (2)	0.95 (2)
5'-TAIA$_p$Y**Z**ITATTAIA-3' 3'-ATC T NCCATAATCT-5'	6.8 Å	0.86 (1)	0.69 (2)
5'-TAIA$_p$YY**Z**ITITAIA-3' 3'-ATC T NNCCACATCT-5'	10.2 Å	0.53 (1)	-0.02 (3)
5'-TAIA$_p$YYY**Z**ITATIA-3' 3'-ATC T NNNCCATACT-5'	13.6 Å	0.21 (2)	---

Table 3.3 Steady-state luminescence quenching values (Fq = fraction quenched) for a series of Ap(A)$_n$X and Ap(T)$_n$X duplexes, where Y and N = A or T, and n = 0-3.

bridge, in which >50% of the luminescence is quenched at that donor-acceptor distance. The quenching yield as a function of distance for the two sets of Ap/Z assemblies is shown in Figure 3.6 (*vide infra*). An adenine bridge for oxidative charge transfer gives rise to a 0.43 $Å^{-1}$ distance dependence; whereas, a thymine bridge reveals a steeper (0.63 $Å^{-1}$) distance dependence.

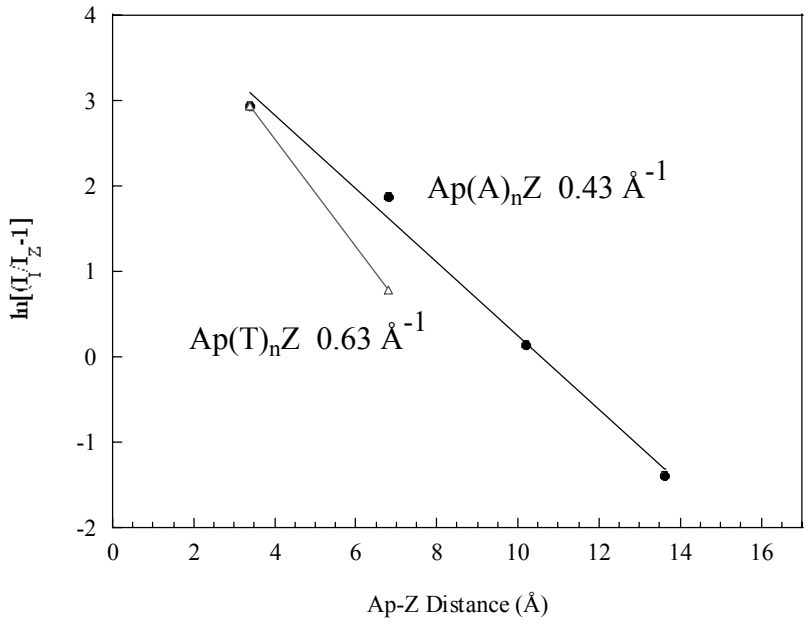

Figure 3.6 Distance dependences of intrastrand charge transfer between Ap and Z mediated by adenine (triangles) and thymine bridges.

These results are consistent with a picture in which purines are more well coupled electronically in the DNA base stack than are pyrimidines. The $Ap(T)_nZ$ series of duplexes, granted, allowed only two points to be used in determine the distance dependence of the luminescence quenching yield. This was necessary, however, because fluorescence quenching yields were negligible at longer donor-acceptor distances. A possible complication in out interpretation of the results involves the *reductive* quenching reaction between thymine and excited-state 2-aminopurine. Previous experiments determined that Ap excited state was quenched by thymine in DNA and in solution.[19] While this quenching of the excited state by thymine in DNA could reduce the population

of 2-aminopurine chromophores available for oxidative reaction with 7-deazaguanine, it is important to note the fraction quenched we measure is solely a function of *oxidative* charge transfer, because any interfering *reductive* charge transfer that may be occurring takes place in both the inosine (reference) and 7-deazaguanine containing duplexes.

To further examine the effect of the intervening bridge on base-base charge transfer in DNA, another series of duplexes was created in which Ap and G or Z (and I) were separated by one base (6.8 Å donor-acceptor distance). The base in between the donor and acceptor was then varied, and the luminescence quenching yields were measured in each case (Table 3.4). The oxidative quenching in Ap/G duplexes correlates with the oxidation potential of the intervening base. In contrast, the oxidative quenching in Ap/Z duplexes can be divided into two groups, (1) charge transfer in which a purine separates the donor and acceptor and (2) charge transfer in which a pyrimidine separates the donor and acceptor. In all duplexes the luminescence quenching yields are greater for ApXZ compared to ApXG. This is especially apparent in the assemblies in which Ap

Duplex	E(+/0) for the intervening base	Fq (X=G)	Fq (X=Z)
5'-TAIA$_p$A**X**ITATTAIA-3' 3'-ATC T TCCATAATCT-5'	1.4 V	0.72 (2)	0.86 (1)
5'-TAIA$_p$I**X**ITATTAIA-3' 3'-ATC T CCCATAATCT-5'	1.5 V	0.48 (3)	0.87 (2)
5'-TAIA$_p$C**X**ITATTAIA-3' 3'-ATC T ICCATAATCT-5'	1.6 V	0.23 (4)	0.72 (3)
5'-TAIA$_p$T**X**ITATTAIA-3' 3'-ATC T ACCATAATCT-5'	1.7 V	0.12 (5)	0.69 (3)

Table 3.4 Effect of the intervening base on the luminescence quenching of the 2-aminopurine excited state by guanine or 7-deazaguanine.

and G or Z are separated by the pyrimidines cytosine (C) and thymine (T). An explanation for this is the increased driving force for oxidative charge transfer in the ApXZ assemblies. In the ApTG and ApCG cases, the driving force for oxidative charge transfer is approximately the same as for reductive charge transfer. In the ApXZ cases, the driving force for oxidative charge transfer is 0.3 V greater, and thus more charge transfer is observed in these assemblies. These results demonstrate quite clearly that the structure and energetics of the intervening bridge greatly affect DNA-mediated charge transfer.

Effect of Driving Force. To explore the subtleties of the driving force dependence of the base-base charge transfer reaction, a series of duplexes was created in which guanines (or a single 7-deazaguanine) with slightly different oxidation potentials were fixed at a given distance from 2-aminopurine on the same strand in DNA (Figure 3.7). The oxidation potentials at these 5'-G sites were tuned by placing additional guanines flanking the site to the 3' side. Theory and experiment predict that the 5'-G in 5'-GG-3' doublets and 5'-GGG-3' triplets should be easier to oxidize than an isolated 5'-G.[20–22] 7-deazaguanine, which is even easier to oxidize, was also placed at the site. An inosine reference system was used as a control.

Figure 3.7 illustrates the results obtained by varying the redox potential at the hole acceptor site. In accordance with theory, the amount of 2-aminopurine luminescence quenching (and hence charge transfer) increases as the driving force for the reaction increases from ΔG = -0.2 V with a single 5'-G to ΔG = -0.5 V with a single 5'-Z. The data also suggest that a 5'-GGG-3' site has a true oxidation potential much closer to 1.3 V than 1.0 V. The fact that it is easy to distinguish the different sites based on such small changes in oxidation potential is quite remarkable and a testament to the sensitive nature of charge transfer through the DNA π stack.

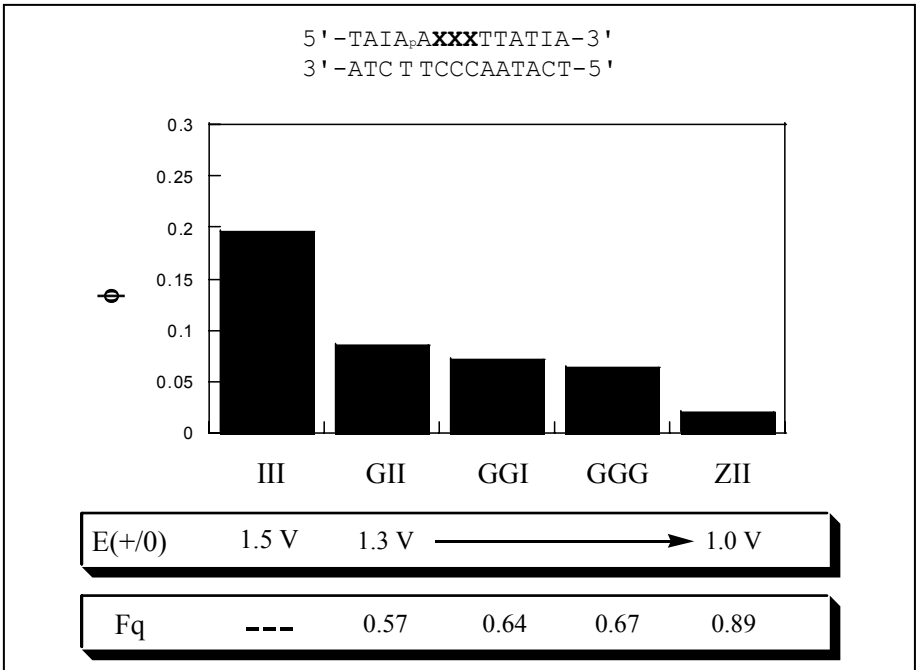

Figure 3.7 Quantum yields and quenching values (Fq) for a series of Ap-modified duplexes in which the oxidation potential of the base donor is varied in small increments.

3.3.2 Time-resolved spectroscopic investigations of intrastrand charge transfer

Previous experiments revealed that intrastrand base-base charge transfer occurred on a fast timescale when the reactants were well stacked within the DNA helix.[6] In order to examine the dynamics of fluorescence quenching in intrastrand base-base (specifically Ap-G and Ap-Z) systems, it is necessary to take measurements using an ultrafast laser setup. Such a setup is available in the Laboratory for Molecular Sciences at the California Institute of Technology, and the time-resolved data presented here is the result of a collaboration with the Zewail group at Caltech.

To examine intrastrand charge transfer dynamics as a function of distance and driving force in base-base assemblies, we utilized the same series of $Ap(A)_nX$ sequences outlined at the beginning of Section 3.3.1. The spectral properties and stabilities of these duplexes have been well characterized, so it would be beneficial to see how the dynamics relate to the steady-state properties in the assemblies. Table 3.5 (*vide infra*) shows the short decay times of the 2-aminopurine excited states in the I, G, and Z containing duplexes at various distances as monitored by transient absorption (λ_{exc} = 310 nm, λ_{obs} = 600 nm). The actual decays are shown in Figures 3.8 and 3.9. Because of the limitations of the apparatus, the longer multiexponential lifetimes typical of 2-aminopurine in DNA were not observed.[23-24]

Duplex	Ap-X	τ (I)	τ (G)	τ (Z)
5'-TAIA$_p$**X**ITITTATIA-3' 3'-ATC T CCACAATACT-5'	3.4 Å	509 ps	10 ps	4 ps
5'-TAIA$_p$A**X**ITATTAIA-3' 3'-ATC T TCCATAATCT-5'	6.8 Å	250 ps	65 ps	29 ps
5'-TAIA$_p$AA**X**ITITAIA-3' 3'-ATC T TTCCACATCT-5'	10.2 Å	209 ps	155 ps	209 ps
5'-TAIA$_p$AAA**X**ITATIA-3' 3'-ATC T TTTCCATACT-5'	13.6 Å	190 ps (209 ps)	179 ps	148 ps

Table 3.5 Transient absorption decay times for a series of $Ap(A)_nX$ duplexes where X = inosine (I), guanine (G), or 7-deazaguanine (Z) and n=0-3. The number in parentheses for ApAAAI is the value reported in the literature.

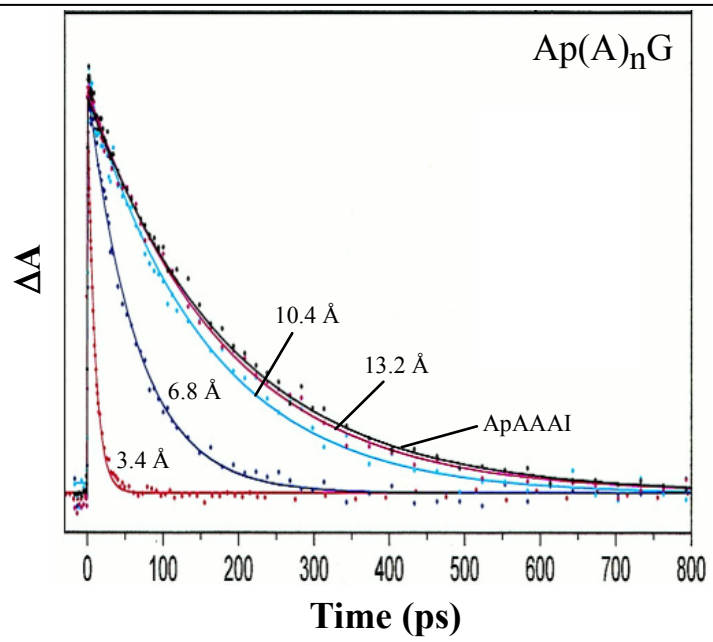

Figure 3.8 Transient absorption decays at 600 nm for a series of $Ap(A)_nG$ containing duplexes. The top trace is of a reference ApAAAI duplex. Ap-G distances are given for the other decays (n=0-3).

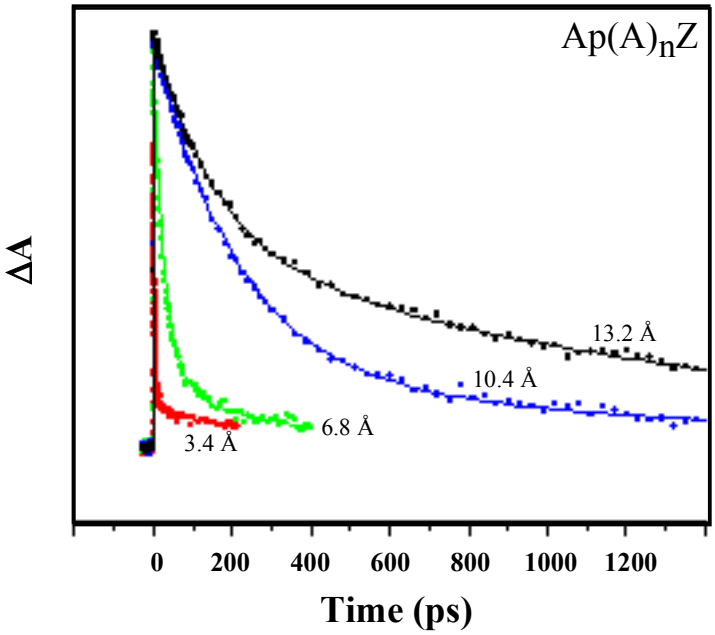

Figure 3.9 Transient absorption decays at 600 nm for a series of $Ap(A)_nZ$ (n=0-3) containing duplexes. Ap-Z distances are given for each trace.

The data clearly demonstrate that the charge transfer rates decrease with increasing distance between donor and acceptor. A few key points stand out when examining the data. First, the decay of Ap* is twice as fast in the "reference" ApAI duplex as in the ApI duplex, and the decay time gradually decreases as more adenines are introduced into the intervening bridge. It is quite likely that some amount of charge transfer occurs between adenine and excited-state aminopurine, given that the driving force for the reaction (ΔG = -0.1 V) is slightly favorable. Therefore, the inosine reference assemblies must always be taken into account when trying to isolate decays based solely on charge transfer quenching of Ap* by G or Z. Second, the observed rates are generally faster for Ap/Z duplexes than Ap/G duplexes at a given distance. This would mean that the observed reactions are taking place in the "normal" region for electron transfer, in which the driving force for the reaction is less than the reorganization energy at a given distance.[25] Since the driving force for charge transfer from Ap* to Z is ca. -0.5 V, this value can be considered as a lower limit for the reorganization energy in base-base DNA-mediated charge transfer.

One peculiarity present in the data is evident at an Ap-X distance of 10.2 Å. In this instance the observed rate is slower for the 7-deazaguanine compared to the guanine containing duplex. Given the increased driving force with the reaction of Ap* for Z over G and the *decreases* in rates observed at the other distances, there must be something structurally unique about that specific sequence that prevents ultrafast DNA-mediated charge transfer. Switching the intervening bridge to AT instead of AA for this distance had virtually no effect on the steady-state yields (Table 3.6) or decay dynamics. This is quite remarkable considering the abundance of data demonstrating the importance of stacking within the helix upon DNA mediated charge transfer. This sequence may unique in enhancing some of the typical nanosecond and picosecond molecular motions that occur in DNA[9,10,26,27] and prevent rapid charge transfer through the π stack.

Duplex	Φ (X=I)	Φ (X=Z)	Fq
5'-TAIA$_p$AA**X**ITITAIA-3' 3'-ATC T TTCCACATCT-5'	0.19	0.09	0.53
5'-TAIA$_p$AT**X**ITITAIA-3' 3'-ATC T TACCACATCT-5'	0.25	0.11	0.56

Table 3.6 Steady-state luminescence quantum yields and quenching values (Fq = fraction quenched) for selected aminopurine-modified duplexes. Quantum yields are relative to an aminopurine standard and X = I or Z.

Plots of the rate of charge transfer for both the Ap/G and Ap/Z duplex series vs. the donor-acceptor distance (minus the anomalous 10.2 A point) are shown in Figure 3.10. The data reveal an exponential distance dependence for the rate for charge transfer between Ap* and G or X that follows the empirical relationship $k(r) \propto \exp(-\beta r)$. Both the Ap/G and Ap/Z series exhibit similar distance dependences (0.46 $Å^{-1}$). Also notable is the fact that these distance dependences are in agreement with those determined from steady-state quenching values.

How does the behavior compare to that observed in the ethidium/7-deazaguanine DNA mediated charge transfer described in Chapter 2? In contrast to the results described for Ap/G and Ap/Z intrastrand DNA-mediated charge transfer, the observed rates in ethidium/7-deazaguanine containing duplexes exhibited little variation with distance. An important difference between the ethidium and aminopurine system is that the ethidium cation transferring a hole to 7-deazaguanine is a true charge transfer system (i.e., the positive charge on the ethidium is transferred to the 7-deazaguanine). In the Ap/G and Ap/Z systems, charge separation is the process that occurs. Thus, the reorganization energies might be different, leading to different behavior in DNA-

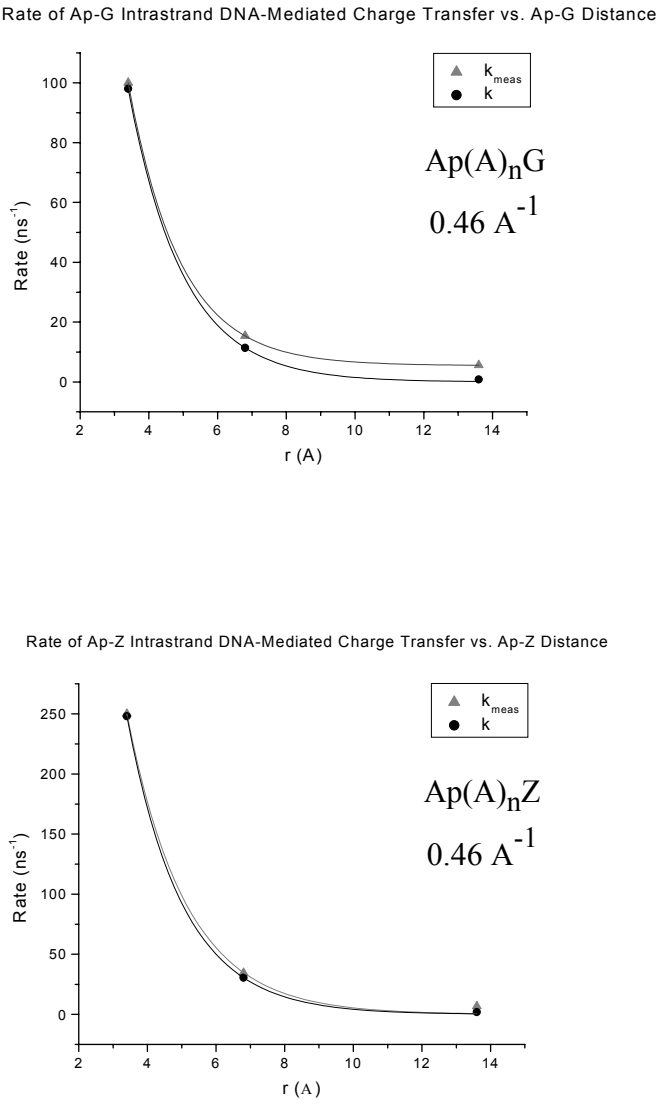

Figure 3.10 Plots of the charge transfer rate vs. distance for a series of Ap/G (top) and Ap/Z (bottom) modified duplexes.

mediated charge transport. Also, the ethidium intercalator presents a larger surface area and has the freedom to conformationally adjust to maximize its electronic interaction with the π stack as a whole. This is not the case for 2-aminopurine, which is restricted to one side of the helix and has little (if any) freedom to reorient within the stack if base-pairing and structural integrity are to be maintained. A combination of these factors, as well as the intervening base stacking and dynamics of the bases and reactants, plays a role in determining the true nature of charge migration through the DNA double helix.

3.4 Conclusions

These spectroscopic investigations of base-base charge transfer through the DNA π stack emphasize the importance of stacking and energetics to DNA-mediated charge transfer. Using guanine and the modified base 7-deazaguanine in a series of 2-aminopurine-modified DNA duplexes has allowed us to determine how driving force affects charge transfer through the double helix. Significantly, this variation in driving force was examined without introducing any large structural variations, a key point if one wishes to isolate the energetic (as opposed to structural) parameter. Even though intrastrand base-base charge transfer was found to exhibit a fairly shallow distance dependence, the kinetics were quite different than those observed for intercalator-base assemblies.

3.5 References

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