Chapter 5

Ultrafast Charge Transfer Dynamics in 2-Aminopurine Modified Double Helical DNA

Abstract: The ultrafast dynamics of charge transfer in double helical DNA is studied by using femtosecond fluorescence upconversion and time correlated single photon counting techniques. 2-Aminopurine (2AP), an isomer of adenine, was used to modify the double stranded DNA. In the steady state fluorescence spectra we observed a charge transfer complex in one of the duplexes (--2APG--) where the 2AP and guanine are directly linked. In both upconversion and time correlated single photon counting methods we observed an ultrafast decay and the timescale of this component varies with the nature and position of the bridge between 2AP and guanine. The fluorescence time profile of free 2AP was found to obey the typical one-exponential behavior with a nanosecond lifetime. However, a three-exponential decay model was needed to fit the decay kinetics of 2AP in DNA duplexes and in single strands. A 420 fs ultrafast decay component was observed in the duplex where there is no bridge base between 2AP and guanine (2APG). This initial ultrafast component is proposed as the charge transfer time scale in the double helical DNA. In all the selected duplexes and single strands, a second picosecond and a third nanosecond components were identified. In single strands, the initial component decayed with a longer time scale compared to the corresponding double stranded DNA.
Publications from this chapter


5.1 Charge Transport in DNA

Ultrafast dynamics of charge transport in double helical DNA has been an area of active research during the past few decades. An in-depth study of charge transport in DNA is important in understanding the cellular mechanism of oxidative damage which may lead to mutagenesis, apoptosis or cancer. It is also significant in the design and fabrication of DNA chips or micro arrays for the detection of single base mismatches or various DNA lesions by electrochemical methods. The knowledge about the charge transport process in DNA could be employed in the development of nanochemical technologies such as DNA based devices. However, the ultra short excited state life times of the natural DNA bases, which are only a few hundreds of femtoseconds, makes the study of electron transfer (ET) in DNA a difficult task. Fluorophores like 2-aminopurine (2AP), an isomer of adenine, have been widely used to explore the ET dynamics in DNA. 2-AP is a highly fluorescent molecule having a long excited state life time (ns time scale) and forms base pairs with thymine making it a nearly non-perturbative replacement of adenine in DNA. The absorption band of 2AP extents up to around 320 nm and hence it can be selectively excited when incorporated in DNA and therefore it is extensively used as a site-specific probe for the local structure of oligonucleotides. It has been observed that the 2AP fluorescence is strongly quenched in both single
and double stranded DNA and this property has been exploited in probing the electron transfer dynamics of DNA. This strong quenching of fluorescence has been attributed to the ET processes between the normal bases in DNA and excited 2-aminopurine. Among the normal DNA bases, guanine (G) is the easily oxidizable one and hence the most promising site for transferring an electron to an excited 2AP residue. Few reports are available on the electron transfer reaction between 2AP* and the natural nucleobases in DNA. But still a clear picture of the ultrafast dynamics of ET in DNA is lacking. Recently O’Neill et al. demonstrated that the ultrafast dynamics play a vital role in DNA mediated ET. By using femtosecond transient absorption techniques it was observed that the ET occurs in 10 ps when 2AP and G are in direct contact. However, in the presence of an adenine bridge, the ET was found to occur at 100 ps timescale. But in transient absorption and transient grating techniques, the signal has contributions from both the ground and excited states and hence for a precise understanding of the timescale, they need to be unambiguously resolved. Fluorescence upconversion is a technique in which one can observe the dynamic information precisely from the excited state. Therefore, we investigated the ultrafast dynamics of charge transfer in 2-aminopurine modified double helical DNA by using femtosecond fluorescence upconversion technique. In order to study the long decay component in the
nanosecond time scale, time correlated single photon counting (TCSPC) technique has been used. 15-mer DNA duplexes and single strands of the following forms were selected for the study.

<table>
<thead>
<tr>
<th>5'-GATTT[2AP]GTTTACCTC-3'</th>
<th>3'-CTAAATCAAATGGAG-5'</th>
<th>Duplex 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>5'-GATTT[2AP]AGTTTATCC-3'</td>
<td>3'-CTAAATTCAAATAGG-5'</td>
<td>Duplex 2</td>
</tr>
<tr>
<td>5'-GATTT[2AP]TGTTTATCC-3'</td>
<td>3'-CTAAATACAAATAGG-5'</td>
<td>Duplex 3</td>
</tr>
<tr>
<td>5'-GATTT[2AP]GTTTACCTC-3'</td>
<td>3'-CTAAATCAAATGGAG-5'</td>
<td>Single strand 1</td>
</tr>
<tr>
<td>5'-GATTT[2AP]AGTTTATCC-3'</td>
<td>3'-CTAAATCAAATGGAG-5'</td>
<td>Single strand 2</td>
</tr>
</tbody>
</table>

Duplex 1: 2AP is directly linked to G; Duplex 2: 2AP is connected to G by an adenine bridge; Duplex 3: 2AP and G are connected by a thymine bridge. Single strand 1, 2 and 3 are the corresponding single strands of duplex 1, 2 and 3.

5.2 Steady State Spectroscopy

The UV-VIS absorption and the fluorescence spectra of the three selected duplexes and their single strands were recorded in phosphate buffer at pH 7. Figure 5.1 shows the ground state absorption spectrum of the duplexes and the single strands at 25°C.
Figure 5.1 UV-Vis spectra: The steady state absorption spectrum of (a) 2-aminopurine free base, duplexes and single strands obtained at 25°C (in phosphate buffer, pH 7). (b) 300-340 nm region is magnified.

It is clear from the figure that the DNA is characterized with its large π-π* absorption at 260 nm and the absorbance of 2AP around 320 nm (see figure 5.1 b) is well separated from the 260 nm band. Therefore, selective excitation of the fluorophore, 2AP is feasible in these oligonucleotides. The fluorescence spectra of both the double stranded and single stranded DNA are recorded at 25°C and 60°C. The emission spectrum of free 2AP and that incorporated in DNA obtained at 25°C and 60°C are shown in figure 5.2. Since the melting temperature of all the selected duplexes are around 30-35°C it is expected that at a temperature of around 60°C the duplexes will exist in the single stranded form. The fluorescence spectra of the single strands were also recorded at both the temperature and these are shown in figure 5.3. It is observed that the emission spectrum of duplex 2 and duplex 3 at 25°C is almost similar to that
of the free base 2AP (which peaks around 370 nm) though there is a slight shift in the emission maximum of the spectrum in the longer wavelength region (430-500 nm). However, the spectrum of duplex 1 shows a red shift compared to the free 2AP and it showed an emission maximum around 420 nm (see figure 5.2). But at 60°C the spectra of the duplex 1 is similar to that of free 2AP. Also the emission spectra of the three single strands were similar to that of free base.

Figure 5.2 Fluorescence spectra of duplexes: Fluorescence spectrum of free 2-aminopurine and the duplexes at (a) 25°C (b) 60°C.

Figure 5.3 Fluorescence spectra of single strands: Fluorescence spectrum of free 2-aminopurine and the single strands at (a) 25°C (b) 60°C.
Among the four natural nucleobases, guanine has the lowest oxidation potential ($E^0 = 1.29$ versus NHE) and hence it is expected as the most probable site of charge transfer from 2AP when incorporated in DNA.\textsuperscript{54} It is also reported that the charge transfer process occurs not only locally but also with the mediation of intervening purine bases and hardly via the pyrimidines bases.\textsuperscript{55} Therefore, such a charge transfer (CT) has the highest probability when G is attached next to 2AP. The result with duplex 1 (2APG) may represent such a charge transfer process. On the other hand there is no shift in the wavelength in the case of the corresponding single strand where G is attached next to 2AP. This further highlights the importance of stacking interaction in the charge transfer process.\textsuperscript{12} To our knowledge this is the first observation on the shift in the emission maximum of 2AP when incorporated in DNA. Therefore, we propose that the shift in the spectrum of duplex 1 at 25°C is due to the charge transfer process in this double stranded DNA where the 2AP and G are in direct contact. For the same reason, the probability of CT will be very high. But in duplex 2 and duplex 3 the 2AP and G are separated by an adenine and a thymine bridge respectively. So in these sequences the probabilities of the charge transfer process between 2AP and G may be less, hence the emission spectrum is similar to that of the free base. From figure 5.3 it is clear that the emission spectra of the single strands are also similar to that of free 2AP. Also the emission spectrum recorded at higher temperature (60°C) has similar characteristics as that of the single strands and
peaks around 370 nm (figures 5.2 and 5.3). From these observations it can be concluded that in the steady state fluorescence spectra the new band formed in duplex 1 (centered at 420 nm) may be due to the formation of a charge transfer complex. However, in other two sequences (duplex 2 and duplex 3) because of the adenine or thymine bridge, the CT will be less efficient, therefore the probability for the formation of charge transfer complex will be less compared to that of duplex 1. It is also noteworthy that the spectrum obtained at 60°C (above the melting temperature of the duplexes) of duplex 1 is similar to that of free 2AP hence, the charge transfer complex is likely to be formed only in the duplex form.

5.3 Decay Kinetics: Femtosecond Fluorescence Upconversion Technique

In order to investigate the ultrafast charge transfer (CT) dynamics, fluorescence upconversion technique was employed. The ultrafast components were obtained by using the upconversion technique. However, the long nanosecond decay component is precisely measured by using the time correlated single photon counting technique (TCSPC) because the nanosecond decay component is more reliable in TCSPC measurement than in the upconversion experiment (see section 5.4). We have upconverted the fluorescence time profile of the selected duplexes and single strands at 374 nm at 25°C. The fluorescence decay profiles (at 25°C) obtained from the upconversion experiment of all the duplexes and single strands at 374 nm are shown in figure 5.4.
Figure 5.4 Decay time profile of duplexes and single strands at 374 nm and at 25°C (upconversion): Fluorescence decay temporal profile of duplexes and single strands at 374 nm at 25°C. (a) duplex 1 (2APG), (b) duplex 2 (2APAG), (c) duplex 3 (2APTG), (d) single strand 1 (2APG), (e) single strand 2 (2APAG) and (f) single strand 3 (2APTG).
In upconversion experiments the fluorescence time profile of free 2AP was found to obey the typical one-exponential behavior with a nanosecond time scale (life time of 2AP is also measured by TCSPC technique, where it decays single exponentially with a nanosecond lifetime (see section 5.4)) similar to the previous reports. However, a three-exponential decay model was needed to fit the decay kinetics of 2AP in DNA duplexes and in single strands (in upconversion, the single strands was fitted with a bi exponential function and the third component was obtained from the time correlated single photon counting technique). Hence, in all the three duplexes and single strands, three decay components were identified, one ultrafast (ranging from femtosecond-picosecond), a second picosecond and a long nanosecond components. In duplex 1, where 2AP is directly connected to G (2APG) an ultrafast component is observed with a time constant of 420 fs while in 2APAG duplex (duplex 2) this component decays with a time constant of 2 ps and in 2APTG duplex (duplex 3), it decays with a 3.4 ps time constant. However, in the case of single strand 1 (2APG), this ultrafast decay was found to occur in 1 ps time scale. But in single strand 2 (2APAG), this initial decay component was observed at 8 ps and in single strand 3, where 2AP and G were bridged by a thymine base, the initial decay occurs with a time constant of 10.5 ps. In duplexes, the second decay component also shows a similar trend as that of the ultrafast component.
The second decay time constant obtained in duplex 1 (2APG) was about 9 ps, in duplex 2 (2APAG) it was about 35 ps and in duplex 3 (2APTG) it was observed at 112 ps. Also in duplexes, a third decay component was identified in nanosecond time scale by using the upconversion technique. In duplex 1 the third component decays with a time constant of 3.9 ns. But in duplex 2 and 3 this decay was found to occur at 2 and 1.7 ns respectively. However, a more reliable time constant for this long decay component can be obtained from the TCSPC measurements (see section 5.4). In the case of single strands, the second decay component obtained in upconversion technique was 17 ns in single strand 1 (2APG). In single strand 2 (2APAG), this decay is observed at 304 ps and in single strand 3 (2APTG) it was found to be around 245 ps. In the case of single strands, the decay time profile was fitted with a bi exponential function so the third nanosecond decay component was identified by using TCSPC technique (see section 5.4). The fluorescence decay time constants and the amplitudes of all the duplexes and the single strands obtained from the upconversion experiment at 374 nm are depicted in table 5.1.
Table 5.1  Ultrafast fluorescence decay time constant ($\tau$) and amplitudes ($a$) of duplexes at 374 nm, at 25°C obtained by fluorescence upconversion technique.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$a(1)$</th>
<th>$\tau(1)$</th>
<th>$a(2)$</th>
<th>$\tau(2)$</th>
<th>$a(3)$</th>
<th>$\tau(3)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duplex 1</td>
<td>0.61</td>
<td>0.42 ps</td>
<td>0.33</td>
<td>9.3 ps</td>
<td>0.06</td>
<td>3.9 ns</td>
</tr>
<tr>
<td>Duplex 2</td>
<td>0.26</td>
<td>2.1 ps</td>
<td>0.59</td>
<td>35.2 ps</td>
<td>0.15</td>
<td>2.0 ns</td>
</tr>
<tr>
<td>Duplex 3</td>
<td>0.19</td>
<td>3.4 ps</td>
<td>0.54</td>
<td>112.4 ps</td>
<td>0.27</td>
<td>1.7 ns</td>
</tr>
<tr>
<td>Single strand 1</td>
<td>0.32</td>
<td>1.03 ps</td>
<td>0.47</td>
<td>16.6 ps</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Single strand 2</td>
<td>0.46</td>
<td>8.4 ps</td>
<td>0.54</td>
<td>304.2 ps</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Single strand 3</td>
<td>0.61</td>
<td>10.5 ps</td>
<td>0.39</td>
<td>245.5 ps</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The long decay component ($\tau(3)$) may be more accurate in TCSPC measurement (see table 5.2).

We have also upconverted the fluorescence at 460 nm with the expectation that we could resolve the formation of the charge transfer complex. Figure 5.5 presents the upconverted fluorescence time profile at 460 nm of duplex 1. But in the case of duplex 2, duplex 3 and all single strands it was difficult to upconvert the fluorescence at this wavelength (460 nm) because of the very small signal level. In the case of duplex 1 where the 2AP and G are in direct contact (the probability of being in a charge transfer active conformation is high) a rise time component is expected at this wavelength, but interestingly no rise time component is observed within our experimental resolution. At this wavelength, the ultrafast component in duplex 1 decays with a time constant of 1.6 ps.
The reason for this behavior can be understood as the charge transfer process may be very fast and hence it is difficult to resolve the rise time component within this experimental time resolution. Experimental evidences are available for a sub 3 fs charge transfer process from an aromatic adsorbate to a semiconductor. Therefore, it is probable that in DNA, the charge transfer process is very fast (see section 5.5) and because of this reason it is difficult to resolve the rise time component within the experimental time resolution of our upconversion setup (200 fs).

The fluorescence of duplexes is also upconverted at 60°C. We expected that at higher temperature (around 60°C) the duplexes may
have the single stranded conformation because the melting temperature of all the selected duplexes was in the range 30-35°C. Figure 5.6 presents the upconverted fluorescence decay time profile of the selected duplexes at 374 nm at 60°C.

Figure 5.6  Decay time profile of duplexes at 60°C (upconversion): Fluorescence decay temporal profile of duplexes at 374 nm at 60°C. (a) duplex 1 (2APG) and (b) duplex 3 (2APTG)
The decay was also found to follow a three exponential model, hence we identified three decay components similar to that observed at normal temperature. However, the time constant of these three decay components are longer than that observed at 25°C. In the case of duplex 1 where the 2AP and G are directly connected, the ultrafast decay occurs in 480 fs. The second component was found to decay with a time constant of 12.6 ps at 60°C. In duplex 3 (2APTG), the first component decays with a time constant of 5.4 ps and the second decay was found to occur 48 ps at 60°C. The decay time constants are longer compared to that observed at 25°C. But this decay time constants are not exactly matching with the corresponding single strands. One would expect the time constants very close to the single strands. However, the observation, particularly in the case of the first component contradicts this fact.

5.4 Decay Kinetics: Time correlated Single Photon Counting (TCSPC)

In order to obtain a more reliable time constant for the long component (nanosecond) we measured the decay kinetics of free base, duplexes and single strands by the time correlated single photon counting technique. The life time of the free 2AP is measured by using time correlated single photon counting technique and it decays single exponentially with a nanosecond lifetime. The typical single exponential decay of free 2AP obtained from TCSPC measurement is shown in figure 5.7.
The fluorescence decay kinetics of the three selected duplexes measured by using time correlated single photon counting at 374 nm at 25°C is shown in figure 5.8. In this case also, all the duplexes undergo multi-exponential decay with a similar pattern as that observed in the upconversion experiment. Hence the time profiles of all the three duplexes were fitted with a three exponential function. Thus in TCSPC also we have identified three components- an ultrafast component, second picosecond and a third nanosecond component.

Figure 5.7 Decay time profile of 2AP (TCSPC): Fluorescence decay time profile of the free base, 2-aminopurine obtained by time correlated single photon counting technique at 374 nm at 25°C
In duplex 1, where 2AP is directly connected to G (2APG), the first component decays very rapidly with a time constant of 12 ps. But in the case of duplex 2 (with an adenine bridge, 2APAG) this decay occurs with 44 ps and in duplex 3 (with a thymine between 2AP and G, 2AP TG) this was found to occur at 42 ps. In the first and second duplexes (duplex 1 and duplex 2) the second picosecond component decays with a similar time constant (518 ps in duplex 1 and 511 ps in duplex 2 respectively), and in duplex 3 (2AP TG) it was found to be around 405 ps. The long
nanosecond decay was observed at 4.3, 2.8 and 3.5 ns respectively for duplex 1, duplex 2 and duplex 3. We have also measured the decay kinetics of the corresponding single strands. Figure 5.9 presents the fluorescence decay kinetics of the single strands obtained by using TCSPC at 374 nm at 25°C.

![Fluorescence decay time profile of single strands](image-url)

**Figure 5.9 Decay time profile of single strands at 374 nm and at 25°C (TCSPC):** Fluorescence decay time profile of single strand 1 (S1, open triangle), single strand 2 (S2, filled circle) and single strand 3 (S3, open circle) obtained by time correlated single photon counting technique at 374 nm and at 25°C.
In this case also, we observed a multi-exponential decay. Therefore the fluorescence decay was fitted with a three exponential function and subsequently we identified three decay components. But in all the three single strands, the decay time constants are longer than their corresponding duplexes. In the case of single strand 1, where the 2AP and G are directly connected (2APG) the initial fast decay occurs with a time constant 48 ps. But in single strand 2 (2APAG) this decay occurs at 56 ps and in single strand 3 (2APTG), it decays with a time constant of 37 ps. The second component decays with a time constant of 1 ns in the case of single strand 1, but in single strand 2 and 3 this was found to occur at 636 and 668 ps respectively. In the case of single strands also we observed a third nanosecond component. In single strand 1 (2APG), this decays at 3.6 ns. However, in the case of single strand 2 (2APAG), it decays with a time constant of 2.9 ns. When there is a thymine bridge between 2AP and G (single strand 3) it occurs at 3.3 ns. The fluorescence decay time constants and the amplitudes of all the duplexes and single strands obtained by TCSPC experiments at 374 nm at 25°C are summarized in table 5.2. The general nature of the decay pattern in the TCSPC experiments is similar to that from upconversion experiments, though the magnitudes are different. As the instrument response function of the TCSPC setup is about 50 ps, only the third components (ns components) were considered. As can be seen from table 5.1, that
the second components in the upconversion experiments are mostly in tens of ps, so these life times were selected as the decay time constant of the second components. But the long nanosecond decay component may be more reliable in TCSPC setup. So the aim of our TCSPC experiment was to identify the nanosecond decay component (third component) precisely. This means that the ultrafast component will be more accurate in upconversion experiment, while the long decay component (nanosecond) will be more reliable in TCSPC measurement.

Table 5.2 Fluorescence decay time constants ($\tau$) and the amplitudes ($a$) of duplexes and single strands at 374 nm and at 25°C obtained by the time correlated single photon counting technique

<table>
<thead>
<tr>
<th>Sample</th>
<th>$a(1)$</th>
<th>$\tau(1)$</th>
<th>$a(2)$</th>
<th>$\tau(2)$</th>
<th>$a(3)$</th>
<th>$\tau(3)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duplex 1</td>
<td>0.95</td>
<td>12.3 ps</td>
<td>0.02</td>
<td>517.7 ps</td>
<td>0.02</td>
<td>4.35 ns</td>
</tr>
<tr>
<td>Duplex 2</td>
<td>0.79</td>
<td>44.2 ps</td>
<td>0.14</td>
<td>511.3 ps</td>
<td>0.06</td>
<td>2.76 ns</td>
</tr>
<tr>
<td>Duplex 3</td>
<td>0.49</td>
<td>42.4 ps</td>
<td>0.49</td>
<td>405.2 ps</td>
<td>0.007</td>
<td>3.49 ns</td>
</tr>
<tr>
<td>Single strand 1</td>
<td>0.67</td>
<td>48.5 ps</td>
<td>0.18</td>
<td>1.04 ns</td>
<td>0.14</td>
<td>3.64 ns</td>
</tr>
<tr>
<td>Single strand 2</td>
<td>0.56</td>
<td>55.9 ps</td>
<td>0.31</td>
<td>636.7 ps</td>
<td>0.12</td>
<td>2.86 ns</td>
</tr>
<tr>
<td>Single strand 3</td>
<td>0.75</td>
<td>37.5 ps</td>
<td>0.15</td>
<td>668.2 ps</td>
<td>0.09</td>
<td>3.25 ns</td>
</tr>
</tbody>
</table>

The ultrafast component $\tau(1)$ and $\tau(2)$ are more accurate in upconversion measurement than in TCSPC (see table 5.1).
The decay time profile of all the duplexes and single strands were also measured at 60°C by using the time correlated single photon counting technique. In figure 5.10, the fluorescence decay time profile of the three duplexes obtained at 374 nm at 60°C is presented. It is observed that the decay time constant at 60°C is not exactly matching with that of the corresponding single strands. Therefore we have also monitored the decay kinetics of single strands at 60°C, where the decay time constants are more or less similar to that of the corresponding duplexes at this temperature. The decay kinetics of the single strands at 60°C and 374 nm is shown in figure 5.11.

![Decay time profile of duplexes at 374 nm at 60°C (TCSPC)](image)

**Figure 5.10** *Decay time profile of duplexes at 374 nm at 60°C (TCSPC):* Fluorescence decay time profile of duplex 1 (D1, solid circle), duplex 2 (D2, open triangle) and duplex 3 (D3, open circle) obtained by time correlated single photon counting technique at 374 nm and at 60°C.
Figure 5.11 Decay time profile of single strands at 374 nm at 60°C (TCSPC): Fluorescence decay time profile of single strand 1 (S1, solid circle), single strand 2 (S2, open triangle) and single strand 3 (S3, open circle) obtained by time correlated single photon counting technique at 374 nm and at 60°C.

At this temperature (60°C), the duplexes and single strands undergo multi exponential decay, hence the time profile is fitted with a three exponential function similar to that at 25°C. The fluorescence decay time constants and the amplitudes of all the duplexes and single strands obtained by TCSPC experiments at 374 nm at 60°C are summarized in table 5.3.
Table 5.3 Fluorescence decay time constants ($\tau$) and amplitudes (a) of duplexes and single strands at 374 nm (60°C) obtained by the time correlated single photon counting technique

<table>
<thead>
<tr>
<th>Sample</th>
<th>a(1)</th>
<th>$\tau$(1)</th>
<th>a(2)</th>
<th>$\tau$(2)</th>
<th>a(3)</th>
<th>$\tau$(3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duplex 1</td>
<td>0.68</td>
<td>34.8 ps</td>
<td>0.22</td>
<td>452.9 ps</td>
<td>0.10</td>
<td>1.57 ns</td>
</tr>
<tr>
<td>Duplex 2</td>
<td>0.50</td>
<td>43.4 ps</td>
<td>0.37</td>
<td>487.8 ps</td>
<td>0.12</td>
<td>1.63 ns</td>
</tr>
<tr>
<td>Duplex 3</td>
<td>0.65</td>
<td>37.7 ps</td>
<td>0.24</td>
<td>452.9 ps</td>
<td>0.1</td>
<td>1.57 ns</td>
</tr>
<tr>
<td>Single strand 1</td>
<td>0.61</td>
<td>46.8 ps</td>
<td>0.27</td>
<td>611.8 ps</td>
<td>0.1</td>
<td>2.01 ns</td>
</tr>
<tr>
<td>Single strand 2</td>
<td>0.46</td>
<td>50.2 ps</td>
<td>0.40</td>
<td>498.3 ps</td>
<td>0.13</td>
<td>1.61 ns</td>
</tr>
<tr>
<td>Single strand 3</td>
<td>0.65</td>
<td>34.7 ps</td>
<td>0.24</td>
<td>510.7 ps</td>
<td>0.1</td>
<td>1.61 ns</td>
</tr>
</tbody>
</table>

As can be seen from table 5.3 that the decay time constant of duplexes at 60°C has close similarity with that of the corresponding single strands at 60°C. However, the decay time constant of the duplexes at 60°C does not match with the corresponding single stands at 25°C. This discrepancy may be due to the change in the conformation of the bases in duplexes and single strands at high temperature.

5.5 Dynamics of Charge Transfer: Evaluation from Upconversion and TCSPC Data

The structural heterogeneity can be clearly seen from the triexponential decay of the 2AP incorporated DNA duplexes and single strands. All these decay components are distinctly different and hence these represent well separated states. This means that the excited DNA
duplex has several energy levels, each with a different fluorescence decay time. Since each states are well separated the overlap between these different sates are highly improbable. It is noteworthy that the three component decay pattern is in contrast to the earlier reports in 2AP incorporated DNA where the decay pattern was bi exponential.\textsuperscript{12} We presume that the ultrafast component (0.42 ps) having a population of 61\% obtained in the case of duplex 1 (2APG) is the charge transfer between 2AP and G. This charge transfer, however, occurs in a comparatively longer time scale of 2.1 ps when the 2AP and G are separated by an adenine bridge (duplex 2). This time scale still gets longer (3.4 ps) when the bridge is a thymine (duplex 3). The time scale of ultrafast charge transfer process in the case of single strand 1 (2APG) is comparatively smaller (1.03 ps) to duplex 2 and duplex 3, supporting the efficient charge transfer process when 2AP is directly linked to G. However, in single strand 2 and single strand 3, a correspondingly longer time scale of the charge transfer process is observed (see table 5.4). Many of the earlier studies reported that the electron transfer between the excited 2AP and G in DNA takes place at a higher time scale.\textsuperscript{12} The study by O'Neill \textit{et. al.} reported a time scale of 10 ps for the electron transfer process in a 35-mer 2APG duplexes (similar to the duplex 1 in our study) using the femtosecond transient absorption technique.\textsuperscript{12} However, in 2APAG duplexes (similar to duplex 2 in our study) they
reported that the time scale has gone up to 100 ps.\textsuperscript{12} It appears that the ultrafast component (0.42 ps) observed in the present study could be deduced only from the fluorescence upconversion technique. It should be noted that remarkably different decay dynamics in transient absorption (3.6 ps) and in upconversion technique (0.7 ps) were reported in the case of covalently linked 7-deaza-guanine and 2AP (Z-AP-Z-A).\textsuperscript{57} This clearly supports our observation of 0.42 ps component against the 10 ps component observed in the case of 35-mer 2APG duplexes.\textsuperscript{12} The fluorescence decay time constants and amplitudes of all the duplexes and single strands obtained from the upconversion and TCSPC experiment (374 nm and 25\textdegree C) are summarized in table 5.4.

At this point it should be noted that the decay time profile of these duplexes correlates with our steady state fluorescence data, where we observed a shift in the emission maximum of duplex 1 (2APG). The fluorescence spectrum of free 2AP shows a peak around 370 nm, but in the case of duplex 1 (2APG) it shows a red shift and a new band is formed around 420 nm (figure 5.2). We propose that the shift in the spectrum is due to the charge transfer process between 2AP and G because in this duplex (duplex 1, 2APG) the 2AP and G are in direct contact, so the probability of charge transfer will be very high. Hence the new band centered at 420 nm may be due to the formation of a charge transfer complex. However, in other two sequences (duplex 2 and duplex 3) in
which adenine or thymine form the bridge, the charge transfer may be less efficient, therefore the possibility for the formation of charge transfer complex will be less compared to that of duplex 1. It is also noteworthy that the charge transfer complex is formed only in the duplex form because the emission spectra of the single strands are similar to that of the free base 2AP. This leads to the conclusion that \( \pi \)-stacking will facilitate the charge transfer process in DNA. It is reported that in DNA the \( \pi \)-stacked bases in the duplex may mediate the charge transport.\(^{13}\) In a time resolved experiment it is reported that in the duplex form the rate of electron transfer increases with temperature but at the duplex melting region the rate constant and amplitudes of fast electron transfer decreases considerably.\(^{12}\) This leads to the conclusion that \( \pi \)-stacking is a prerequisite for the efficient charge transfer process in DNA. From our time resolved data also it is very clear that the charge transfer process in duplex 1 is comparatively faster than the corresponding single strand. Hence, it can be concluded that \( \pi \)-stacking plays an important role in the charge transfer process in DNA. The decay time profile at higher temperature (60\(^{0}\)C) also supports the effect of \( \pi \)-stacking in the charge transfer process. The decay time constant of the ultrafast component in DNA duplexes are longer (both in upconversion and in TCSPC measurements) at 60\(^{0}\)C compared to that at normal temperature (25\(^{0}\)C).
Table 5.4 Fluorescence decay time constants (τ) and amplitudes (a) of duplexes and single strands at 374 nm and at 25°C obtained by the fluorescence upconversion technique and time correlated single photon counting technique.

<table>
<thead>
<tr>
<th>Sample</th>
<th>a(1)</th>
<th>τ(1)</th>
<th>a(2)</th>
<th>τ(2)</th>
<th>a(3)*</th>
<th>τ(3)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duplex 1</td>
<td>0.61</td>
<td>0.42 ps</td>
<td>0.33</td>
<td>9.3  ps</td>
<td>0.02</td>
<td>4.3 ns</td>
</tr>
<tr>
<td>Duplex 2</td>
<td>0.26</td>
<td>2.1 ps</td>
<td>0.59</td>
<td>35.2 ps</td>
<td>0.06</td>
<td>2.8 ns</td>
</tr>
<tr>
<td>Duplex 3</td>
<td>0.19</td>
<td>3.4 ps</td>
<td>0.54</td>
<td>112.4 ps</td>
<td>0.007</td>
<td>3.5 ns</td>
</tr>
<tr>
<td>Single strand 1</td>
<td>0.32</td>
<td>1.03 ps</td>
<td>0.47</td>
<td>16.6 ps</td>
<td>0.14</td>
<td>3.6 ns</td>
</tr>
<tr>
<td>Single strand 2</td>
<td>0.46</td>
<td>8.4 ps</td>
<td>0.54</td>
<td>304.2 ps</td>
<td>0.12</td>
<td>2.9 ns</td>
</tr>
<tr>
<td>Single strand 3</td>
<td>0.61</td>
<td>10.5 ps</td>
<td>0.39</td>
<td>245.5 ps</td>
<td>0.09</td>
<td>3.3 ns</td>
</tr>
</tbody>
</table>

* The long decay components are measured by TCSPC technique (see text). Single strands 1, 2 and 3 are the corresponding single strands of duplex 1, 2 and 3.

It is reported that no DNA-mediated charge transfer occurs at 77 K, where all the conformational motion of the DNA bases are arrested.16 But in 2APG duplex where the 2AP and G are in direct contact, the charge transfer process is still observed at 77 K even though the efficiency is lower than at normal temperature.16 The present study also points the efficient charge transfer process in duplex 1 where the 2AP and G are in direct contact. It is also interesting that in the single strand 1 (2APG) this charge transfer process is still faster than in duplex 2 (2APAG) and in duplex 3 (2APTG). This indicates that our observation
also supports the efficient charge transfer process in 2APG duplexes (duplex 1). Both the time resolved fluorescence data and steady state fluorescence are in line with this observation.

The second decay component in duplex 1 (9.3 ps) with a population of 33% is much faster than the possible conformational reorganization of the bases. The clearly distinguishable decay component in the present case is presumably due to a relaxation of the charge transfer states. On the other hand, when the 2AP and G are separated by adenine and thymine, a substantially higher decay time constant are obtained (35.2 ps and 112.4 ps respectively for duplex 2 and 3) which could well be the corresponding conformational reorganization of the bases.

All the selected duplexes and single strands decays are characterized by the ultrafast decay due to charge transfer process, picosecond decay due to the conformational reorganization followed by nanosecond type decays. The conformational motion due to the base moieties is considered for the nanosecond decay components. It should be noted that the nanosecond components are slow with respect to the time scale of conformational motions. It is reported that in DNA fluctuations in local helical conformation occur in picosecond to nanosecond time scale.\(^{56}\) Hence the last nanosecond decay component is assigned to the
conformational motion due to the base moieties in DNA. This conformational motion play a vital role in specific protein-DNA binding by enabling proteins to indirectly probe the base sequence via local changes in mechanical and dynamical behavior.

On the other hand still there exists some ambiguity in the results obtained with the duplexes at 60°C. The expected decay time constant at 60°C must match with the corresponding single strands at 25°C. However, as presented in the upconversion data, in the case of duplex 1, the first decay component has a life time of 480 fs at 60°C which is close to the life time of the decay component at 25°C. A similar result is obtained in TCSPC method where the results from duplexes at 60°C do not match with the results from single strands at 25°C (compare table 5.3 with table 5.2). However, the results obtained from duplexes and from single strands at 60°C (table 5.3) gave much closer values compared to that at 25°C in the case of single strands (table 5.2). Therefore, the only probable reason for this discrepancy is the difference in the conformation of the bases at 25°C and at 60°C. Another reason (though with a low probability) is that at 60°C, the conversion of all the duplexes to single strands may not be complete and hence the duplexes may also contribute to the decay kinetics.
5.6 Conclusion

The spectroscopic investigations of the charge transfer through the DNA π stack highlights the importance of stacking interaction between the bases and the ultrafast dynamics involved in the charge transport process. A study by O'Neill et al. using femtosecond transient absorption technique reported 10 ps for the electron transfer process in a 2AP modified 35 mer 2APG DNA duplex. But with an adenine bridge between 2AP and G the time scale has gone up to 100 ps. However a totally different decay dynamics is observed in transient absorption and in upconversion technique because in transient absorption, the signal has ground state contributions. Fluorescence upconversion is a technique in which one can observe the dynamic information precisely from the excited state. Therefore the ultrafast dynamics involved in the charge transfer can be more specifically observed in upconversion technique. To the best of our knowledge, this is the first observation of the charge transfer dynamics in DNA using upconversion technique. It is observed in the present study that in all the duplexes and single strands, the fluorescence decay is multi exponential. This indicates the structural heterogeneity in DNA duplexes and single strands. In the steady state fluorescence spectrum of duplex 1 (where 2AP and G are directly connected) the emission maximum of 2AP (peaks around 370 nm) is red shifted and a new band is formed around 420 nm. This is due to the formation of charge transfer complex. To our knowledge this is the first
information on the shift in the emission maximum of 2AP when incorporated in DNA. In other two duplexes (duplex 2 and duplex 3) the emission spectrum is similar to that of free 2AP. In these duplexes because of the presence of adenine or thymine bridge, the charge transfer process will be less efficient. It is also observed that the charge transfer complex is formed only in the duplex form because the emission spectra of the single strands are similar to that of the free base 2AP. This leads to the conclusion that \(\pi\)-stacking has a profound effect in facilitating the charge transfer process in DNA. The ultrafast component (0.42 ps) in duplex 1 (2AP is directly connected to G) is the shortest ever reported charge transfer time scale in DNA duplex. However, in duplexes with an adenine and/or thymine bridge between 2AP and guanine (duplex 2 and duplex 3) this time scale gets extended. Also the charge transfer process in the case of single strand 1 (2APG) is faster compared to duplex 2 and duplex 3 which supports the efficient charge transfer process when 2AP is directly linked to guanine. It is noteworthy that our time resolved fluorescence data correlates with our steady state observation. These data provides compelling experimental evidence for the ultrafast charge transfer process in DNA.
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