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J. Am. Chem. Soc., **2005**, 127 (49), 17130-17131• DOI: 10.1021/ja054955z • Publication Date (Web): 17 November 2005

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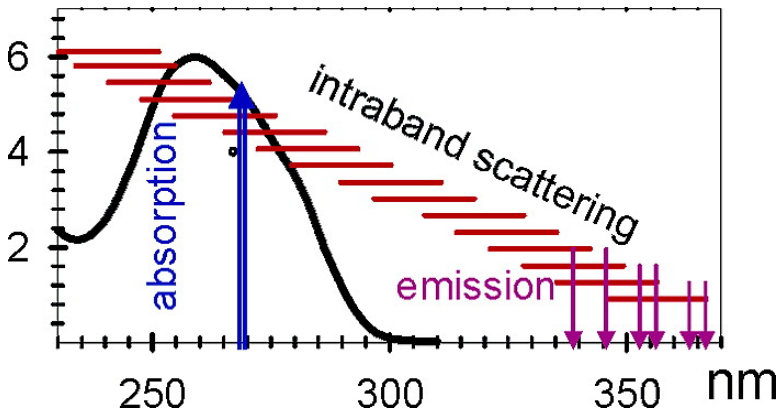

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Published on Web 11/17/2005

Collective Behavior of Franck−Condon Excited States and Energy Transfer in DNA Double Helices

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Absorption of UV radiation by DNA bases is known to induce carcinogenic mutations.[1] The lesion distribution depends on the sequence around the hotspots, suggesting cooperativity between bases.[2] Such cooperativity could intervene at the very first step of a cascade of events by formation of Franck−Condon states delocalized over several bases. Yet, the observation that the DNA UV absorption spectra closely resemble the sum of the spectra of the constituent bases has led to the conclusion that photons are absorbed by single bases.[3] However, recent theoretical calculations have shown that the absorption spectra of model double helices in which the excited states are delocalized over a few bases exhibit only a slight shift with respect to the spectra of noninteracting monomers,[4,5] in agreement with the experimental spectra.[5−7] However, as the observed spectral changes are very subtle, stronger evidence for the formation of delocalized Franck−Condon states is needed. This type of information can be obtained from combined steady-state and time-resolved studies. Here, we present such an investigation of the double helix poly(dA)·poly(dT), whose fluorescence, induced by femtosecond pulses at 267 nm, is probed by two different techniques, fluorescence upconversion and time-correlated single photon counting (TCSPC) over a large time domain (100 fs to 100 ns). To obtain signals devoid of contamination due to thymine dimer formation[8] and biphotonic ionization of nucleic acids,[9] which is the major difficulty of laser experiments with nucleic acids, specific protocols (cf. Supporting Information) had to be developed. We show that the duplex properties are not consistent with photon absorption by a single base, adenine, or thymine. In contrast, they are readily explained by the formation of delocalized excited states directly upon photon absorption and subsequent energy transfer via intraband scattering occurring in less than 100 fs.

The absorption spectrum of poly(dA)·poly(dT) is presented in Figure 1a, together with the spectra of the monomeric chromophores, 2′-adenosine monophosphate (dAMP) and thymidine monophosphate (TMP). The maximum of the duplex spectrum, peaking at 258.8 ± 0.2 nm, is hypsochromically shifted by 3.2 nm with respect to the spectrum obtained with an equimolar mixture of monomers,[7] and the maximum molar coefficient (6000 M^{-1} cm^{-1})[10] is decreased by about 50%. Despite the fact that the dAMP and TMP spectra are very different, the emission spectrum of poly(dA)·poly(dT) does not depend on the excitation wavelength and is very similar to the TMP spectrum (Figure 1b). These observations, reported previously[11] and confirmed by us (cf. Supporting Information) has led to the conclusion that fluorescence of this double helix stems only from thymine.[11] According to this reasoning, the lack of sensitivity of the double helix fluorescence on the excitation wavelength precludes any significant site dependence of the thymine Franck−Condon excitation energy.

The simplified picture of thymine moieties being the only emitting species and all having identical excitation energy is altered

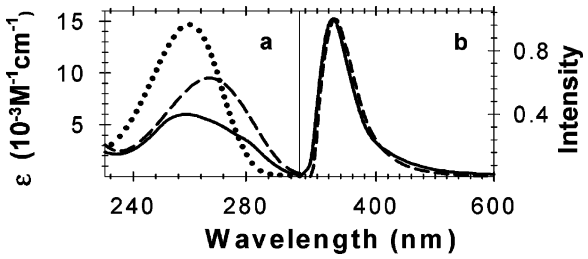

Figure 1. Steady-state absorption (a) and normalized fluorescence (b) spectra. Dotted, dashed, and solid lines correspond to dAMP, TMP, and poly(dA)·poly(dT), respectively. The molar extinction coefficient (ϵ) of the duplex is given per base. The fluorescence spectra of both TMP and poly(dA)·poly(dT) do not depend on excitation wavelengths ranging from 245 to 285 nm.

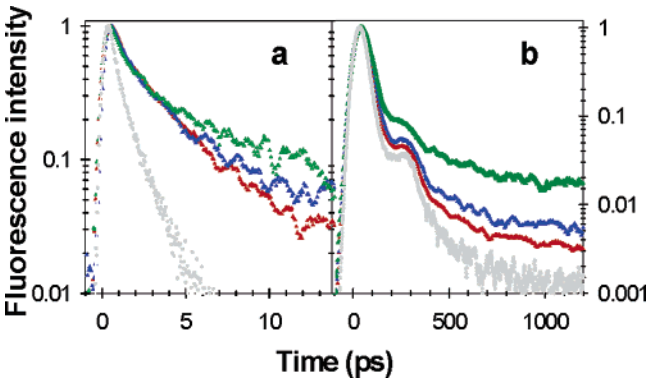

Figure 2. Normalized fluorescence decays of poly(dA)·poly(dT) recorded by the upconversion technique (a) and TCSPC (b) at 330 nm (red), 380 nm (blue), and 420 nm (green). Gray points correspond to the decay of TMP at 330 nm; its lifetime is 0.5 ps,[12] and its decay corresponds to the instrumental response function in (b). The TCSPC decays were obtained without polarizer at the emission side.

by the results of time-resolved measurements. The fluorescence decays of poly(dA)·poly(dT) are much longer than those of TMP (Figure 2a), in agreement with what was found previously for the shorter duplex $(dA)_{20}$·$(dT)_{20}$.[13] In contrast to TMP, for which signals are identical at all wavelengths, the decays of the polymer become longer upon increasing wavelength. Within the time-resolution of our setup, 100 fs after deconvolution, the upconversion decays show no rise-time. The decays obtained by TCSPC (Figure 2b) also exhibit strong wavelength dependence. The long time components have low amplitude, but they represent an important part of the total emitted photons; at 330 nm, about 20% of the photons are emitted at times longer than 100 ps. At least five exponentials are needed to correctly fit the decays from the femtosecond to the nanosecond time scale, and the time constants vary with the observation wavelength.

The fluorescence anisotropy decays of poly(dA)·poly(dT) determined by the two detection techniques at 330 nm are plotted in

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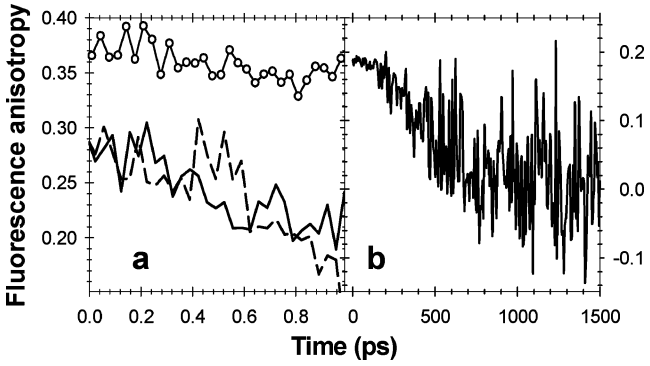

Figure 3. Decays of fluorescence anisotropy obtained from the upconversion (a) and TCSPC (b) measurements for poly(dA)·poly(dT) (solid lines) at 330 nm. For comparison, the anisotropy of TMP (open circles) and that of poly(dAdT)·poly(dAdT) (dashed line) recorded by upconversion are also shown.

Figure 3 together with that of TMP. The anisotropy of the polymer obtained from upconversion measurements at zero-time is 0.28 ± 0.01 (Figure 3a). This value is lower than that observed for TMP at the same time (0.38 ± 0.02). After 1 ps, the TMP anisotropy has only slightly changed (0.36 ± 0.02), whereas that of poly(dA)·poly(dT) has dropped down to 0.20 ± 0.02. The anisotropy of the polymer is completely lost at about 1 ns (Figure 2b), which is still too fast for a physical rotation of such a large system to occur. The rapid decay of the fluorescence anisotropy of poly(dA)·poly(dT), observed at the subpicosecond time scale, can only be understood in terms of energy transfer taking place within the double helix.

Energy transfer may occur via either intraband scattering or Förster-type transfer among thymine moieties. In the case of Förster transfer, the anisotropy decay corresponds to the hopping rate, which is proportional to the square of the electronic coupling and should vary with interchromophore distance. Therefore, we found it instructive to compare the upconversion anisotropy decays of poly(dA)·poly(dT) and poly(dAdT)·poly(dAdT). The dipolar coupling between neighboring thymines, located in the same strand for the homopolymer and on opposite strands for the alternating polymer, is 220 and 80 cm^{-1}, respectively.[5,7] Consequently, the anisotropy decay corresponding to the homopolymer should be about 8 times faster than that of the alternating polymer. Figure 3a shows that the fluorescence anisotropy of both duplexes exhibits exactly the same time dependence, which is incompatible with Förster transfer.

The ensemble of the observations can be explained in the frame of the exciton theory as follows. Laser excitation creates a large number of collective states associated with different conformations or segments of the double helix,[7] as well as with the homogeneous broadening of the monomeric transitions.[4] Intraband scattering, occurring in less than 100 fs, brings each system to the bottom of the exciton band. Consequently, emission stems from a large number of low-lying states, each one associated with the geometry of the system. The polarizations of the electronic transitions associated with different pairs of the excited states composing the exciton band form a variety of angles, resulting in different fluorescence anisotropy values (Figure 10 in ref 7). Conformational changes of the double helix occurring at time scales probed by TCSPC may lead to changes of the electronic coupling and affect the properties of the excited states.[7] Finally, we are tempted to correlate the long time behavior of the poly(dA)·poly(dT) emission (Figure 2b) to states built with participation of charge transfer interactions.[14] Such interactions are known to change the oscillator strength[15] and could be responsible for the hypochromism characterizing the absorption spectra of poly(dA)·poly(dT) (Figure 1a).

After submission of the present work, a study on the excited states dynamics of $(dA)_{18}$·$(dT)_{18}$ was published.[16] On the basis of transient absorption decays, the authors conclude that intrastrand adenine excimers, having a lifetime of 150 ps, are formed in high yield. If adenine excimers were the dominant species created during excited state relaxation in poly(dA)·poly(dT), the steady-state fluorescence spectrum of this double helix should be similar to that of poly(dA) because the fluorescence quantum yield of poly(dA) (7.3×10^{-4})[17] is higher than that of poly(dA)·poly(dT) (3×10^{-4}). This is not the case since the fluorescence spectrum of poly(dA)·poly(dT), peaking at 327 nm, is clearly different than that of poly(dA) peaking at 360 nm (cf. Supporting Information).

Supporting Information Available: Details on materials, experimental setups and procedures, steady-state spectra, fits. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA054955Z