

Influence of Secondary Structure on Electronic Energy Relaxation in Adenine Homopolymers

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Electronic energy relaxation in the adenine homopolymers poly(A) and poly(dA), as well as in the adenine mononucleotide, was studied by the femtosecond transient absorption technique and by steady-state absorption and emission spectroscopies in aqueous solution. The excited-state lifetime of the adenine mononucleotide was determined to be 370 ± 40 fs at room temperature. Strikingly, the singlet excited states formed in adenine homopolymers decay on time scales ranging from femtoseconds to nanoseconds, and three decay components are required to adequately describe their transient signals. Increasing the temperature decreases the amplitude of the two slowest decay components, indicating that the long-lived excited states are formed in base-stacked regions of the polymers. At room temperature and neutral pH, the two slowest decay components have significantly larger amplitudes in poly(dA) than in poly(A). No evidence is obtained for excited-state quenching of the adenine chromophore by the nonstandard base pairing that occurs in the double-stranded form of poly(A) at acidic pH. Instead, double helix formation slightly increases the yield of long-lived excitations. This work demonstrates that electronic energy relaxation depends sensitively on the secondary structure of the adenine homopolymers.

1. Introduction

Femtosecond laser experiments have recently shown that the DNA and RNA nucleobases have ultrashort fluorescence lifetimes on the order of hundreds of femtoseconds.^{1–7} Ultrafast internal conversion reduces the probability of photochemical reactions, making the monomeric nucleobases highly photostable. Photostability is a desirable characteristic not only of the nucleic acid building blocks, but also of the polymers themselves. However, progress in understanding the photophysics of these complex, multichromophoric macromolecules has been slow.⁸ In particular, it is still unclear how the noncovalent interactions, which are responsible for nucleic acid secondary structure, affect the singlet excited states created in DNA and RNA model systems by UV radiation.

Here, we present a study of the photophysics of the single-stranded homopolymers poly(riboadenylic) and poly(2'-deoxyriboadenylic) acid, poly(A) and poly(dA), respectively. These two polymers have identical adenine chromophores, and differ only by the presence (poly(A)) or absence (poly(dA)) of a hydroxyl group at the 2'-position of the furanose ring. Both form stacked single-helical structures at neutral pH,⁹ whereas in acidified conditions poly(A) is present in a double-stranded conformation.^{10,11} Adjacent bases in poly(dA) are believed to stack differently than in poly(A). Single-stranded poly(A) adopts an A-type or RNA-like conformation, while single-stranded poly(dA) assumes a B-type conformation similar to that of duplex DNA in solution.⁹ Polynucleotides of the purine base adenine were selected for this initial study because purine monomers yield larger transient absorption signals than pyrimidine ones.² The use of single-stranded homopolymers allows the effects of base stacking on the photodynamics to be studied independently of base pairing. On the other hand, the double-

stranded form of poly(A) at low pH can provide insight into the effect of base pairing on singlet-state dynamics. A preliminary account of some of our results was given elsewhere.¹²

In this report, we show that, although ultrafast internal conversion is the dominant relaxation pathway for monomeric adenines, electronic energy relaxation in single- and double-stranded adenine homopolymers occurs over a wide range of time scales extending from femtoseconds to nanoseconds. Temperature-dependent measurements indicate further that the long-lived excitations are formed in base-stacked regions in yields that appear to reflect differences in the secondary structure of these homopolymers. No evidence is found for quenching of the singlet excited states of double-stranded poly(A) by base pairing at low pH. Instead, our results show that double-stranded poly(A) has a somewhat higher yield of long-lived excited states.

2. Experimental Section

Steady-state absorption and emission spectra were recorded with a Perkin-Elmer Lambda 25 UV/VIS and SPEX Fluorolog-3 spectrometers, respectively, using a 1 cm path length cell. For fluorescence measurements, solutions with an absorbance of 0.30 ± 0.02 at the excitation wavelength of 260 nm were used for both adenine polymers and the mononucleotide adenine 5'-monophosphate (AMP). Emission spectra were recorded in back-to-back experiments on freshly prepared solutions. The band-pass of emission and excitation monochromators was fixed at 2 nm, and emission spectra were recorded in 1 nm steps. The fluorescence spectra were corrected for the instrument response function.

Femtosecond pump-probe experiments were performed using ~ 200 fs UV pump pulses with a center wavelength of 263 nm, as described previously.² Probe pulses at 570 nm were obtained from a white light continuum generated by focusing a weak 800 nm pulse in a 1 cm path length cuvette filled with water.

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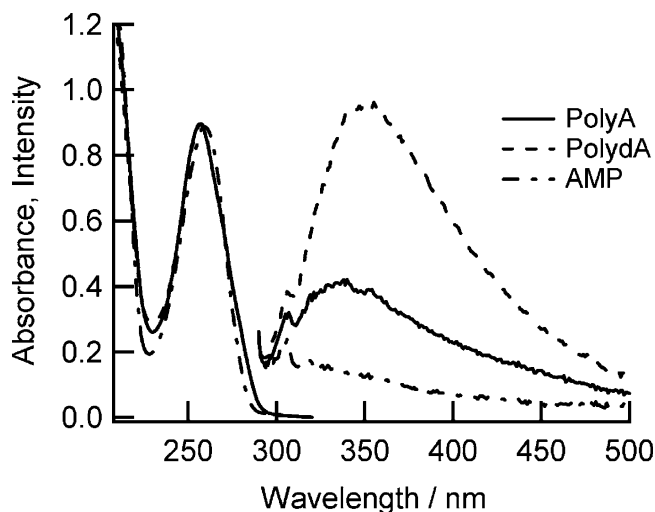


Figure 1. Normalized absorption and corrected emission spectra for poly(A) and poly(dA) in buffered aqueous solution at neutral pH ($\lambda_{\text{exc}} = 260$ nm). The normalized absorption and corrected emission spectra of AMP are presented for comparison.

The polarizations of pump and probe pulses were set to the magic angle, and both pulses were crossed in a 1 mm path length flow cell. The potassium salt of poly(A) and the sodium salt of poly(dA) were purchased from Sigma and used as received. Buffered aqueous solutions at pH 6.8 were prepared by dissolving the polymers in a 25 mM phosphate buffer. Concentrations were adjusted to give absorbance values of 0.75 for both polymers in a 1 mm path length. For some pump–probe experiments on single-stranded poly(A), the ionic strength was increased by addition of NaCl. However, no appreciable changes in the excited-state dynamics were observed even for total salt concentrations of 1 M (data not shown). For the pH-dependent experiments, HCl was added dropwise to a neutral buffered solution of poly(A) until the desired pH was obtained. The pH values have an uncertainty of ± 0.5 pH unit. Below pH 3, precipitation of poly(A) was observed, precluding measurements at lower pH values. Unless otherwise indicated, all experiments were performed at a temperature of 25 ± 2 °C.

3. Results

3.1. Steady-State Photophysical Properties. The steady-state absorption and emission spectra of poly(A) and poly(dA) are shown in Figure 1 together with spectra for the poly(A) monomer, AMP. In this figure, the absorption spectra have been normalized to the maximum of the long wavelength absorption peak. The emission spectra were recorded from solutions having identical absorbance at the excitation wavelength of 260 nm. The absorption spectra of poly(A) and poly(dA) are identical. The AMP absorption spectrum is similar to the polymer spectra, but subtle differences exist. The absorption maximum of both homopolymers is blue-shifted by several hundred wavenumbers and the low energy band edge is red-shifted with respect to AMP. These spectral shifts mimic ones observed at 77 K.¹³

The absolute fluorescence quantum yield ϕ_f of poly(A) was previously reported to be 3×10^{-4} .¹⁴ Although the absorption spectra of both polymers are indistinguishable between 200 and 320 nm, ϕ_f of poly(dA) is nearly 2.5 times higher than for poly(A). From the ratio of the areas under the emission spectra in Figure 1 and the fluorescence quantum yield of 3×10^{-4} for poly(A),¹⁴ we estimate ϕ_f of poly(dA) to be 7.3×10^{-4} . To the best of our knowledge, this is the first reported fluorescence quantum yield for poly(dA) at room temperature. Using the same

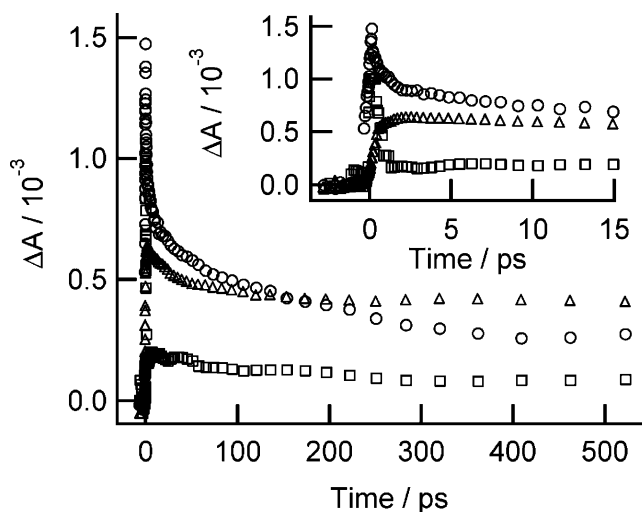


Figure 2. Transient absorption signals from poly(A) (circles), AMP (squares), and buffer (triangles) at 570 nm in back-to-back experiments at neutral pH. The inset provides a short time view of the signals.

ratio procedure, ϕ_f of AMP is estimated to be 0.7×10^{-4} . This value is in excellent agreement with the value of $(0.68 \pm 0.10) \times 10^{-4}$ recently reported for the 2'-deoxymononucleotide of adenine, dAMP.¹⁵ Interestingly, the situation is reversed at 77 K, and a greater ϕ_f value is observed for the ribopolymer than for the deoxyribopolymer.¹³ The room-temperature emission spectra are red-shifted compared to that of the monomer. This red-shifted emission band has been attributed in the past to excimer states.^{13,16}

3.2. Femtosecond Transient Absorption Experiments. Figure 2 shows the pump–probe signal for poly(A) measured at a probe wavelength of 570 nm. Also shown in this figure are the pump–probe decays of AMP and the buffer solution, together with the signals at short times (Figure 2, inset). The buffer-only signal results from two-photon ionization of water.² The transient absorption signal from the buffer solution is nearly constant for delay times below 100 ps (Figure 2, inset). At longer times, and up to the time window of our apparatus (≈ 0.6 ns), the buffer-only signal decays because of geminate recombination of the hydrated electron with OH^\bullet and H_3O^+ .¹⁷ The signal from poly(A) displays a long-lived decay that at first glance resembles absorption by hydrated electrons seen in previous experiments on single nucleosides.^{1,2} For this reason, the signal power dependence was studied in detail.

3.3. Signal Power Dependence. In pump–probe experiments such as these, the signal is the absorbance change (ΔA) induced by the pump pulse at various probe wavelengths. In our previous studies of monomeric bases, the signals contained contributions from excited-state absorption (ESA) by the S_1 states of the bases and from solvated electrons produced by two-photon ionization of the solvent.^{1,2} These contributions can be distinguished by their different pump power dependence. The ESA signal varies linearly with the average power (or, equivalently, intensity) of the pump beam since the excited states responsible for this signal are populated monophotonically. On the other hand, absorption of two photons is required to ionize the solvent or the solute. As a result, the signal contribution from hydrated electrons varies quadratically with pump power.

The dependence of the ESA signals on pump intensity was investigated at several fixed time delays for poly(A) and AMP. The results are shown in Figure 3. For AMP, a plot of ΔA measured at 10 fs time delay as a function of the pump power gives a straight line with zero intercept (Figure 3a, inset), as

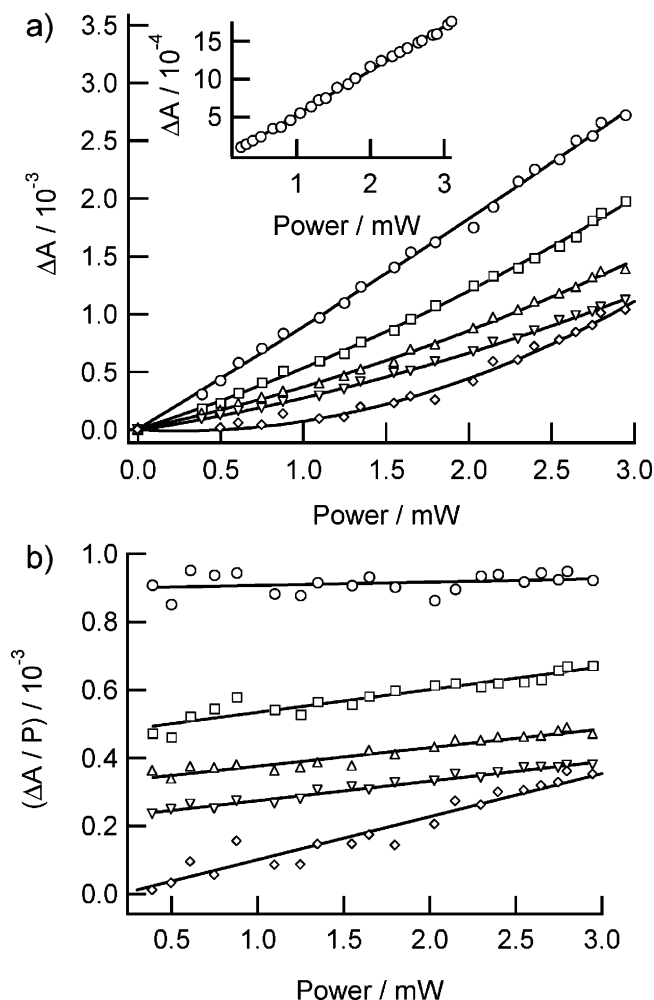


Figure 3. (a) Transient absorption signals and (b) transient absorption signals divided by the pump power of poly(A) as a function of the pump power at time delays of 400 fs (circles), 10 ps (squares), 100 ps (up triangles), and 400 ps (down triangles). The buffer signals (diamonds) are shown at time delays of 2 ps for comparison. The inset to panel (a) shows the power dependence of AMP at a time delay of 10 fs.

expected for a one-photon process. Linear plots were also obtained at delays up to 400 fs. The situation is more complex for poly(A), as shown in Figure 3a. From time zero up to 400 fs, a plot of ΔA as a function of the pump power gives a straight line with intercept equal to zero, indicating that the signal in this time window is due to a one-photon process. However, as seen in Figure 3a, a deviation from linearity is observed at time delays greater than 1 ps. This behavior arises from the temporal overlap of the two-photon ionization of water and the ESA signal of the polymer at these time delays. At times less than or equal to 400 fs, the contribution of the water ionization signal is sufficiently small in comparison to the stronger ESA signal that only a linear variation is observed (see Figure 2, inset, and Figure 3a). The data in Figure 3a were further analyzed using the empirical function

$$\Delta A = aP + bP^2 \quad (1)$$

where a and b are fitting parameters related to the absorption cross sections and quantum yields of the states involved, and P is the pump power. This equation assumes that the pump–probe signal has contributions from two processes: one-photon ESA by the polymer and absorption by hydrated electrons produced by two-photon photoionization of the solvent. In this case, a

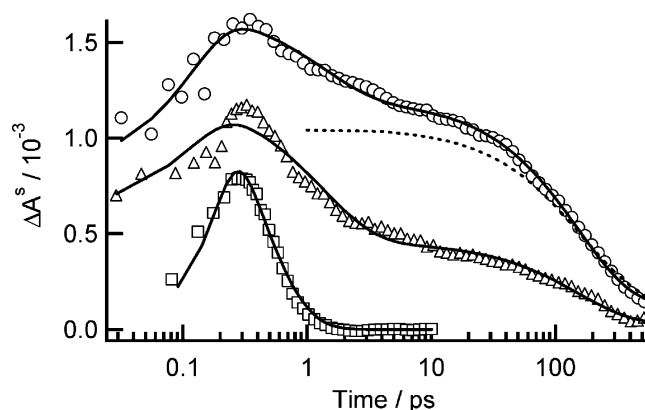


Figure 4. Transient absorption signals from AMP (squares), poly(A) (triangles), and poly(dA) (circles) at 570 nm in back-to-back experiments at neutral pH. Solid curves are from fits, while the dashed curve is the fit to the emission decay at 340 nm reported in ref 19 for the oligonucleotide (dA)₁₅.

plot of ΔA divided by the pump power must give a straight line with a nonzero intercept when plotted versus pump power. Figure 3b depicts such a plot for poly(A), showing that a one-photon component is indeed present in the ESA decay of poly(A) even 400 ps after the pump pulse. Similar behavior was observed for poly(dA) (data not shown). At less than 400 fs time delay, $\Delta A/P$ is fit by a line with zero slope within experimental uncertainty (Figure 3b), as expected for a strictly monophotonic process. For the sake of completeness, Figures 3 also shows the power dependence of the pump–probe signals from the buffer-only solution at a time delay of 2 ps. In this case, a plot of $\Delta A/P$ gives a straight line with zero intercept and a nonzero slope, consistent with two-photon ionization of water.

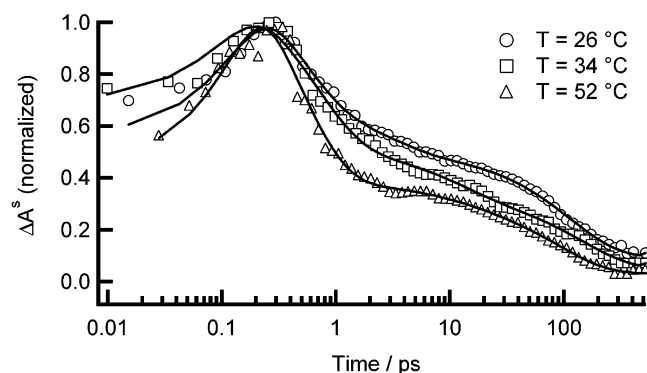
3.4. Subtraction Procedure. Since the signals contain a contribution from solvated electrons produced by two-photon ionization, a procedure was developed for isolating the monophotonic ESA signal due to the polymer from the raw pump–probe signal. First, back-to-back scans were recorded on a solution of the polymer under study, and on an AMP solution. Both solutions were prepared to have identical absorbance at the pump wavelength in the low-intensity limit. Next, the signal from a buffer-only solution at the same probe wavelength was scaled to agree with the AMP signal at long times. Finally, the scaled buffer-only signal was subtracted from the raw signal recorded from the polymer of interest to obtain the corrected or “solvent-subtracted” signal (ΔA^8 in Figures 4–6). As a result of its short S_1 lifetime, the ESA signal from AMP has completely decayed away 2 ps after the pump pulse. Thus, the signal recorded at times greater than 2 ps in AMP arises entirely from the solvated electrons produced by two-photon ionization of the solvent. Since the same amount of two-photon excitation is expected in the equal absorbance solutions of AMP and the polymer, the long-time signal from AMP can be used to predict the solvated electron signal contribution at all times. The power dependence of the solvent-subtracted signals from poly(A) and poly(dA) were found to be linear, validating the correction procedure.

Solvent-subtracted transient absorption signals for both adenine homopolymers and for AMP are shown in Figure 4. A logarithmic time axis is used in Figure 4 in order to best depict the decays, which occur over a wide dynamic range. Figure S1 in the Supporting Information shows the same data with a conventional, linear time axis. The signals were recorded in back-to-back experiments without any adjustments to the laser

TABLE 1: Global Fit Parameters at Room Temperature and Neutral pH^a

polymer	τ_1/ps	A_1	τ_2/ps	A_2	τ_3/ns	A_3
poly(A)	1.33 ± 0.13	0.46	154 ± 14	0.25	∞	0.018
poly(dA)	1.33 ± 0.13	0.29	154 ± 14	0.64	∞	0.075
AMP	0.37 ± 0.04^b	1				

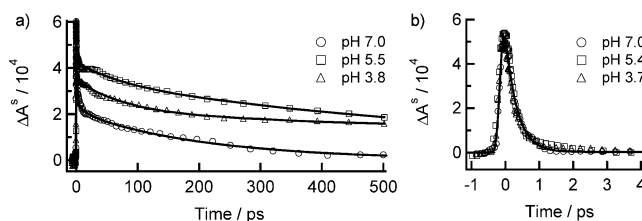
^a Stated uncertainties are twice the standard error and relative amplitudes are reported. ^b Globally fit at 570, 600, and 630 nm probe wavelengths.

**Figure 5.** Transient absorption at 570 nm following UV excitation of poly(A) at the indicated temperatures. The signals have been normalized.

apparatus. Transients for AMP and the two homopolymers show an ultrafast decay component, but the polymers also show signal components that decay on much slower time scales. The signals from poly(A) and poly(dA) have not reached the baseline at the longest delay time probed in our experiments, and three decay components are required to adequately describe the transients. The transients were globally fit to two exponentials plus a constant offset because the longest time constant was poorly determined as a result of our limited delay range of ≈ 600 ps. Allowing the lifetimes to vary independently for each polymer did not significantly improve the quality of the fits, and a more complex dynamic model is unwarranted at this time. Parameter values are summarized in Table 1. Transients recorded for poly(A) and poly(dA) at probe wavelengths of 600 and 630 nm are similar to those seen at 570 nm (data not shown).

3.5. Effect of Temperature on the Excited-State Dynamics of Poly(A). The poly(A) solution shows a systematic decrease in the excited-state population of the long-lived components with an increase in temperature from 25 to 70 °C. Solvent-subtracted signals for poly(A) are shown in Figure 5 at three different temperatures on a logarithmic time axis. Figure S2 in the Supporting Information shows the same data with a linear time axis. As described in section 3.4, back-to-back ESA experiments of the buffer, AMP, and poly(A) solutions were performed at each relevant temperature to correct the signals for the two-photon ionization of water.

3.6. Acid-Induced Transitions of Poly(A). Since poly(A) is double-stranded in acidified aqueous solution,^{10,11} it is a convenient model system for studying how base pairing affects the excited-state dynamics of the adenine chromophore. Transient decays for poly(A) and AMP solutions at the probe wavelength of 570 nm are shown in Figure 6 at three different pH values. All signals at the relevant pH conditions have been corrected for the contribution of the two-photon ionization of water as described above. The reported pK_a values of poly(A) and AMP are 5.87¹⁰ and 3.80,¹⁸ respectively. A dramatic increase of the relative fraction of the long-time signal of poly(A) is observed when $\text{pH} \approx pK_a$ (Figure 6a). Although the amplitudes of the slower components decrease somewhat at pH values below the pK_a , they are still larger than at pH 7.

**Figure 6.** Transient absorption signals from (a) poly(A) (normalized) and (b) AMP at 570 nm in back-to-back experiments at different pHs. The solid curves are fits.

4. Discussion

4.1. Assignment of the Signals to Singlet Excited States.

The origin of the monophotonic signals observed in the adenine systems is discussed first. Triplet excited states can be ruled out by the fact that similar pico- and nanosecond decay components were observed in previous time-resolved emission experiments and assigned to singlet states.^{16,19–21} Indeed, the quantum yield of intersystem crossing for AMP is the lowest of all the DNA bases (<0.01),¹⁴ and the triplet lifetimes of the monomer bases are on the order of 1 μs in aqueous solution at room temperature,¹⁴ making it very unlikely that triplet states could account for the mostly subnanosecond dynamics observed here. It has been suggested that monophotonic ionization is possible for nucleic acid components in aqueous solution.²² Although we cannot completely rule out one-photon ionization in stacked base regions, the similarity between the time-resolved emission signal observed by Plessow et al.¹⁹ for the closely related oligonucleotide (dA)₁₅ and our solvent-subtracted signal for poly(dA) (see Figure 4) strongly suggests that we are observing the dynamics of singlet excited states. Additional evidence in support of the assignment to singlet states is the observation that the ratio of the steady-state fluorescence quantum yield of poly(A) and poly(dA) in Figure 1 is almost identical to that of the integrated ESA decays of the polymers at 570 nm.

Remarkably, the adenine homopolymers exhibit two long lifetime components (τ_2 and τ_3), even though AMP shows only ultrafast electronic energy relaxation at room temperature. We assign the long-lived decays to excitations in segments of two or more stacked bases. This assignment is supported by the observed decrease in the fractional contribution of the long-time signal with increasing temperature (Figure 5). The fastest decay component (τ_1) is assigned to excitations in unstacked regions of the polymer. This is consistent with the known distribution of bases in single-stranded polymers in either stacked (helical) or unstacked (random-coil) segments.²³ Raising the temperature increases the fraction of unstacked bases, increasing the fast, monomer-like signal and decreasing the long-lived components assigned to excitations in stacked bases. Dewey and Turner used laser temperature-jump experiments to estimate that 34% of the bases in poly(A) are not base stacked at room temperature.²⁵ In contrast, they found that just 21% of the bases in poly(dA) are unstacked or in random-coil-like regions. The ratio of these two numbers ($34\%/21\% = 1.6$) is in excellent agreement with the ratio of the A_1 amplitudes (0.46/

0.29 = 1.6) in Table 1. This supports our assignment of the τ_1 lifetime to excited states formed in random-coil regions.

The only other study of an adenine multimer with time resolution comparable to ours is a recent fluorescence upconversion study of (dA)₂₀ by Markovitsi et al.²⁶ Surprisingly, these authors did not observe the long-lived components that are so prominent in Figure 4, and in the lower time resolution study of Plessow et al.,¹⁹ although Markovitsi et al. did observe a weak offset with an amplitude of 2% of the deconvoluted signal maximum. In contrast, the amplitude of the τ_2 component measured in our poly(dA) experiment is 64% as large as the maximum deconvoluted signal. This indicates that the transition cross sections for the short- (monomer-like) and long-lived (base-stacked) excited states may be more similar in excited-state absorption than in emission. The transient absorption technique is therefore particularly well suited to studying long-lived excitations in oligo- and polynucleotides since less dynamic range is required to detect the various signal components. This is further supported by preliminary femtosecond transient absorption experiments on 18-mer homo-oligomers of adenine and thymine,²⁴ which reveal long-lived components similar to those observed by Plessow et al.¹⁹ and by Georgiou, Beechem, and co-workers.²⁷

Previous time-resolved studies of adenine oligo- and polynucleotides have detected pico- and nanosecond decay components.^{16,19} For example, the dashed line in Figure 4 shows the biexponential fit to the emission decay at 340 nm reported for (dA)₁₅ by Plessow et al.¹⁹ This signal is in excellent agreement with our transient absorption signal for poly(dA) except at times below 5 ps, where our higher time resolution is able to detect an ≈ 1 ps decay. This indicates that the long-lived, excited states are emissive in character. The long-time signal characterized by τ_2 and τ_3 could arise from two distinct excited-state populations or result from a single excited-state population that undergoes a complex dynamic evolution. We favor the latter interpretation since both the time constants and relative amplitudes from the time-resolved emission study of Plessow et al.¹⁹ agree well with the ones found in our study. If the signals were the result of two distinct singlet excited states, then the agreement between transient absorption and emission would require that the ratio of the cross sections for the two states be the same in both experiments.

The longest decay component (τ_3) observed for both homopolymers cannot be accurately characterized because of the limited delay range (0.6 ns) of our apparatus, but can be estimated to be >1 ns. Compared with the AMP lifetime of 0.37 ps, this indicates that some excited states formed in the polymers decay over 2700 times more slowly than in AMP. Despite the long lifetimes of these states, the fluorescence quantum yields of the adenine homopolymers increase by less than a factor of 10 compared to AMP. This suggests that fluorescence from the long-lived polymer excited states may be relatively forbidden. However, we wish to stress the importance of determining the actual quantum yield for forming the long-lived states. A small population of an optically bright state (in emission or excited-state absorption) can produce the same signal as a much larger population of a state with a lower radiative transition probability. Since the long-lived excitations are formed exclusively in base-stacked regions, we anticipate that these states are delocalized over two or more bases. Our expectation is that delocalization will lead to decreased radiative transition probabilities, suggesting that the yield of long-lived excited states may be quite high. Regardless of whether the quantum yield for formation of the long-lived states is large or

small, the present results indicate that femtosecond transient absorption is an excellent technique for following their dynamics.

4.2. Photochemical and Photophysical Differences between Poly(A) and Poly(dA). The amplitudes associated with the two slowest decay components, A_2 and A_3 , are greater in poly(dA) than in poly(A), as seen in Figure 4 and Table 1. This is consistent with the greater steady-state fluorescence observed for poly(dA) (Figure 1). The ratio of the areas under the transient absorption signals in Figure 4 compares well with the ratio of the areas under the emission spectra in Figure 1. This is further confirmation that the long-lived signals arise from emissive excited states and thus cannot be due to solvated electrons produced by ionization. However, the reason for the stronger transient absorption and fluorescence signals of the deoxyribose polymer is unknown at present. It is unlikely to be the result of a difference in the fraction of stacked bases since thermodynamic measurements indicate only a 13% increase in the extent of base stacking for poly(dA) compared to poly(A).²⁵ One possibility is that the long-lived excited states have significantly higher absorption (and emission) cross sections in poly(dA) than in poly(A). We propose instead that the excited states are the same in both polymers, but are formed in higher yield in poly(dA). The fact that the long-time signals from both polymers can be fit to the same time constants is consistent with our proposal, but further experiments are needed.

The lifetimes of the singlet states in the adenine homopolymers are orders of magnitude longer than the subpicosecond lifetime of the singlet excited state in the adenine monomer. This raises the possibility of significant nuclear motions on the excited-state potential energy surface of the polymers. We suggest that these motions may control the branching between the initial excited state created by light absorption and the long-lived states. The initial bright state could be largely localized on one base as suggested by the similar absorption spectra of AMP and the two homopolymers (Figure 1). However, recent calculations indicate that spectral similarities between a monomer and its associated polymer are not inconsistent with excited states that are delocalized over several bases.²⁸ The initial bright states in base-stacked polymer regions could either decay nonradiatively to the electronic ground state or internally convert to a new, long-lived singlet state that is delocalized over two or more stacked bases. The branching between these two decay channels governs the yield, and is likely to be sensitive to the ground-state secondary structure, which is determined largely by backbone conformation. Thus, as a result of the different sugar-phosphate conformations, the ground-state base stacking morphology in poly(A) differs from that found in poly(dA). The adenine bases are arranged in a more oblique manner in the ribose polymer than in the deoxyribose one, which are arranged in a more parallel manner.²⁹

The long-lived singlet states described here likely play an important role in polynucleotide photochemistry. Interestingly, pronounced photochemical differences are observed between poly(A) and poly(dA) despite the fact that they contain identical chromophores. Single-stranded poly(dA) forms dimeric photo-products nearly as readily as poly(dT) and poly(dC), whereas poly(A) is nearly photochemically inert.^{30–33} It is believed that the singlet excited state is the precursor of the adenine photodimer.³² As first discussed by Pörschke^{30,31} and more recently by Davies,^{32,34,35} the photochemical differences found between poly(A) and poly(dA) at neutral pH probably reflect their different stacking geometries. Our steady-state and time-resolved experiments suggest that the different photoreactivity

is a result of the greater yield of long-lived singlet states in the former polymer. The observation of an increased yield of long-lived singlet states in duplex poly(A) suggests that this form should be more photoreactive. In fact, the photochemical behavior of poly(A) and that of poly(dA) are much more similar under acidified conditions.³⁰

4.3. Effect of Base Pairing on Singlet Excited-State Dynamics in Adenine Homopolymers. Additional evidence that the long-time components are associated with excitations in base-stacked regions comes from our results on acidified poly(A). As seen in Figure 6a, the amplitudes of the long-lived decays are larger in the double-stranded forms, which are present at low pH. On the other hand, the transient absorption signals from AMP are essentially pH-independent and protonated adenine exhibits only a subpicosecond decay time (Figure 6b). Thus, the long-lived decay components seen in acidified solutions of poly(A) cannot be due to slower excited-state relaxation in protonated adenine, but must instead arise from the secondary structure of the duplex polymer.

It is well-known that poly(A) adopts a double-stranded helix when $\text{pH} = \text{pK}_a$, which is destabilized somewhat at $\text{pH} < \text{pK}_a$.^{10,11} Base pairing between two adenine bases at low pH is the result of hydrogen bond formation between the N7 position of one adenine base and the exocyclic amino group of a second one.^{11,36} Protonation at the N1 position of the adenine bases stabilizes the base pairs by electrostatic attraction between the protonated base and the unshielded phosphates.^{11,36} When the pH is approximately equal to the pK_a , poly(A) forms a partially protonated double helix with 10 nucleotides per turn and 3.6 Å rise per base pair. When the pH is lowered below the pK_a , the duplex adopts a more fully protonated state in which the chains are more extended with 8.4 nucleotides per turn and 3.8 Å rise per base pair.³⁷ Both double-stranded forms are thought to maintain the same base pairing motif between adenine bases.^{11,37} The greater interbase separation in the more extended duplex formed at $\text{pH} < \text{pK}_a$ is likely to decrease electronic coupling between adjacent bases. This could account for the reduced signal level at pH 3.8 compared to pH 5.5 (Figure 6a).

The results in Figure 6a also demonstrate that base pairing at room temperature does not quench the long-lived states seen in the single-stranded forms. Instead, the signal amplitudes are even larger in the duplex than in single-stranded poly(A). This may be due in part to a higher fraction of stacked bases in the duplex. On the other hand, it could indicate that the base stacking conformation found in the acid forms increases the branching to the long-lived states. In either case, the data indicate that base pairing does not inhibit the formation of long-lived excitations. Interestingly, in contrast to our room-temperature results, base pairing was previously observed to dramatically quench excited states of poly(A) in ethylene glycol:water glasses at 77 K.^{38,39} Further experiments are needed to resolve this apparent discrepancy.

5. Conclusion

We have reported the first transient absorption study of excited-state dynamics in nucleic acid polymers with femto-second time resolution. The results show that electronic energy relaxation in adenine homopolymers is multiexponential, taking place on time scales ranging from femtoseconds to nanoseconds. No evidence for triplet states was found in these transient absorption measurements. The results show further that secondary structure affects the dynamics of singlet excitations in poly(A) and poly(dA). In particular, the base-stacking geometries found in DNA-like single-stranded poly(dA) at neutral pH

appear to favor the yields of long-lived excited states compared with the RNA-like single-stranded poly(A). These long-lived signals are the signature of singlet excitations in helical base-stacked regions. The higher yield of long-lived states in poly(dA) may explain its greater propensity for photodimer formation.^{31,32} This work also demonstrates that femtosecond pump-probe spectroscopy is a conformationally sensitive technique for studying nucleic acids—a technique that provides new insights into the relationship between macromolecular structure and dynamics.

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Supporting Information Available: Plots of the signals in Figures 4 and 5 on a linear time axis. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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