Internal conversion to the electronic ground state occurs via two distinct pathways for pyrimidine bases in aqueous solution

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The femtosecond transient absorption technique was used to study the relaxation of excited electronic states created by absorption of 267-nm light in all of the naturally occurring pyrimidine DNA and RNA bases in aqueous solution. The results reveal a surprising bifurcation of the initial excited-state population in <1 ps to two nonradiative decay channels within the manifold of singlet states. The first is the subpicosecond internal conversion channel first characterized in 2000. The second channel involves passage through a dark intermediate state assigned to a lowest-energy 1nπ* state. Approximately 10–50% of all photoexcited pyrimidine bases decay via the 1nπ* state, which has a lifetime of 10–150 ps. Three- to 6-fold-longer lifetimes are seen for pyrimidine nucleotides and nucleosides than for the corresponding free bases, revealing an unprecedented effect of ribosyl substitution on electronic energy relaxation. A small fraction of the 1nπ* population is proposed to undergo intersystem crossing to the lowest triplet state in competition with vibrational cooling, explaining the higher triplet yields observed for pyrimidine versus purine bases at room temperature. Some simple correlations exist between yields of the 1nπ* state and yields of some pyrimidine photoproducts, but more work is needed before the photochemical consequences of this state can be definitively determined. These findings lead to a dramatically different picture of electronic energy relaxation in single pyrimidine bases with important ramifications for understanding DNA photostability.

conical intersection | DNA photophysics | excited-state dynamics

There has been interest for many years in excited electronic states of DNA and RNA bases because of their role in the UV photodamage of nucleic acids (1). These excited states sometimes decay to deleterious photoproducts, which can cause mutations and interfere with the normal cellular processing of DNA. It has been argued that damage is greatly inhibited by their intrinsic photochemical properties. In particular, the ultrafast decay of excited electronic states to the electronic ground state (S0) involves passage through a dark intermediate state (2). Approximately 10–50% of all photoexcited pyrimidine bases decay via the 1nπ* state, which has a lifetime of 10–150 ps. Three- to 6-fold-longer lifetimes are seen for pyrimidine nucleotides and nucleosides than for the corresponding free bases, revealing an unprecedented effect of ribosyl substitution on electronic energy relaxation. A small fraction of the 1nπ* population is proposed to undergo intersystem crossing to the lowest triplet state in competition with vibrational cooling, explaining the higher triplet yields observed for pyrimidine versus purine bases at room temperature. Some simple correlations exist between yields of the 1nπ* state and yields of some pyrimidine photoproducts, but more work is needed before the photochemical consequences of this state can be definitively determined. These findings lead to a dramatically different picture of electronic energy relaxation in single pyrimidine bases with important ramifications for understanding DNA photostability.

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Abbreviations: Ura, uracil; Thy, thymine; Cyt, cytosine; DMU, 1,3-dimethyluracil; dTMP, thymidine 5’-monophosphate; TCHU, 1-cyclohexeylaracil; CI, conical intersection; 1nπ*, lowest-energy excited singlet state; S0, electronic ground state.

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signals, which allow the repopulation of S0 to be observed. Signals of the ground electronic state (S0) and the lowest excited electronic state (S1) as a function of two representative coordinates, q1 and q2, chosen from a nuclear configuration space of much higher dimensionality. The decay paths are shown by dashed lines on the surfaces and by solid lines projected onto the q1, q2 plane. The photophysical pathway in A moves from the Franck–Condon region reached by photon absorption (black arrow) to a CI with S0 and finally back to the starting point at the minimum of S0. Photophysical decay in B takes the excited-state wavepacket from a reactant geometry on S0 (point R) to the geometry of a new chemical product (P).

Fig. 1. Nonradiative photophysical (A) and photochemical (B) decay pathways on hypothetical potential-energy surfaces. The surfaces denote the energies of the ground electronic state (S0) and the lowest excited electronic state (S1) as a function of two representative coordinates, q1 and q2, chosen from a nuclear configuration space of much higher dimensionality. The surfaces denote the geometry on S0 (point R) to the geometry of a new chemical product (P).

of channels, but there is no experimental evidence that IC actually occurs via more than one channel in solution. Here we present femtosecond transient absorption results showing decay via two distinct channels for the hydrated pyrimidine bases Ura, thymine (Thy), and cytosine (Cyt). The first channel is direct IC from the 1ππ* state to S0. The second channel, which accounts for 10–50% of all deexcitation events, involves decay via a comparatively long-lived intermediate electronic state, which we assign to the lowest-energy 1nππ* state. These results reveal a more complex picture for pyrimidine base photophysics and DNA photostability.

Results

Fig. 2 compares transient absorption signals from several 5’ mononucleotides in aqueous solution at 250 and 340 nm after excitation at 267 nm. These probe wavelengths provide a representative view of the dynamics of the various electronic states, which absorb broadly in this spectral region. At 250 nm, both pump and probe wavelengths fall within the strong, lowest-energy π→π* transition of each nucleotide, leading to negative induced absorbance (ΔA) signals, which allow the repopulation of S0 to be observed. Signals were recorded at a probe wavelength of 340 nm because a number of intermediates potentially absorb here, including vibrationally hot S0 molecules (<360 nm; refs. 30 and 31), pyrimidine triplet states (300–550 nm; ref. 32), and a dark singlet state recently detected in 1-cyclohexyluracil (1CHU) (300–450 nm; ref. 31).

For each of the pyrimidine mononucleotides, cytidine 5’-monophosphate (CMP), thymidine 5’-monophosphate (dTMP), and uridine 5’-monophosphate (UMP), the signal at 340 nm is positive and approximately mirrors the 250-nm bleach signal at delay times greater than ~10 ps. Parameters from a global fit to the signals at 250 and 340 nm using two exponentials plus an offset are summarized in Table 1. At both probe wavelengths a positive spike is present at short delay times when pump and probe pulses overlap. This feature arises from coherent two-photon absorption by solvent molecules and was excluded from the fits. No long-lived decay components are observed from the purine ribonucleotide adenosine 5’-monophosphate (AMP) at the same two wavelengths (Fig. 2).

The slow-decay component (τ2 in Table 1) is seen in all pyrimidine bases and base derivatives studied but is not present in signals recorded at 570 nm (Fig. 3). It is known that transient absorption signals at visible wavelengths from pyrimidine and purine nucleobases decay on a subpicosecond time scale (1, 6, 30).

Among the unmodified pyrimidine bases, τ2 increases in the following order: Cyt < Ura < Thy. The same order is observed for the 5’ mononucleotides of these compounds, but τ2 is three to six times longer (Fig. 4 and Table 1). The same lifetimes are observed, within experimental uncertainty, for the 5’ mononucleotides and nucleosides (data not shown) of Cyt, Thy, and Ura. Lifetimes of both 1,3-dimethyluracil (DMU) and 1CHU are more similar to the free base Ura than to the 5’ mononucleotide UMP (Table 1).

Discussion

Signal Assignments. The pump pulse at 267 nm abruptly depletes the ground-state population by exciting molecules to the 1ππ* state. This results in the negative absorbance changes (ΔA < 0) seen immediately after time 0 at 250 nm (Fig. 2), a wavelength absorbed
strongly by ground-state molecules. Steady-state absorption spectra are shown for all molecules studied in supporting information (SI) Fig. 6. The recovery of this signal to ΔA = 0 measures the time needed for the excited population to return to S₀, as discussed elsewhere (33). The pronounced biphasic recovery at 250 nm indicates unambiguously that S₀ is repopulated on two distinct time scales defined by τ₁ and τ₂. In the following discussion we refer to these as the fast and slow channels for IC.

Assignment of the fast channel to direct IC from the initial 1ππ* state to S₀ is supported by signals at several probe wavelengths. At 570 nm, where the 1ππ* states of the nucleobases show excited-state absorption (30), subpicosecond decays are observed (Fig. 3). Non-radiative decay to S₀ creates vibrationally hot ground-state molecules. As a result, the S₀ absorption spectrum is transiently shifted to longer wavelengths until vibrational cooling can reestablish thermal equilibrium with the surrounding solvent molecules (6, 30, 31, 33). The hot S₀ absorption spectrum changes most rapidly at thermal equilibrium with the surrounding solvent molecules (6, 30, 31, 33). The pronounced biphasic recovery at 250 nm indicates unambiguously that S₀ is repopulated on two distinct time scales defined by τ₁ and τ₂. In the following discussion we refer to these as the fast and slow channels for IC.

Each parameter marked with a dash was globally linked to the row above in fitting. Uncertainty intervals are 95% confidence estimates.

*Data are from ref. 31.

signal at 250 nm of between 2 and 4 ps indicates that vibrational cooling is complete on this time scale (6, 30, 31, 33). The observed values for τ₁ are in good agreement with previous observations for AMP and dTMP in aqueous solution (33).

The slow channel is the central discovery communicated in this work and will be the focus of subsequent discussion. It is observed in all pyrimidine bases in aqueous solution but is conspicuously

<table>
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<th>Nucleobase</th>
<th>λ, nm</th>
<th>τ₁, ps</th>
<th>A₁, %</th>
<th>τ₂, ps</th>
<th>A₂, %</th>
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Fig. 3. Solvent-corrected transient absorption traces of CMP, dTMP, UMP, and AMP in pH 7 buffer pumped at 267 nm and probed at 570 nm. Structures of the bases are shown with R representing a ribose-phosphate group. Solid lines are from global fits to the data.
absent in purine bases like AMP (Fig. 2) and guanosine 5’-monophosphate (data not shown). The slow lifetime ($\tau_2$ in Table 1) is assigned to relaxation of an intermediate electronic state that absorbs at 340 nm (Fig. 2) but not at visible wavelengths (Fig. 3). The approximate mirror symmetry of the 250- and 340-nm transients indicates that population in the intermediate state relaxes directly to $S_0$. The electronic state absorbing at 340 nm cannot be the $^1\pi\pi^*$ state because transients at visible probe wavelengths (Fig. 3) and previous transient absorption and fluorescence-upconversion measurements (1) indicate that the latter state has a lifetime of $<1$ ps. The long-lived population at 340 nm is in an intermediate electronic state along the decay pathway from the $^1\pi\pi^*$ state to $S_0$. Assignment of the intermediate state to a minor tautomers or a species produced by photoionization was considered and dismissed (see SI Discussion). Because emission from pyrimidine nucleobases is not observed on the $\tau_2$ time scale (15, 34, 35), the intermediate state is a “dark” state with a negligible radiative transition rate to $S_0$. The nature of this dark state is discussed below in more detail.

Residual negative and positive offsets seen at 250 and 340 nm, respectively ($A_1$ in Table 1), are assigned to absorption by long-lived triplet states (31). These states have absorption maxima between 350 and 450 nm but do not absorb beyond 550 nm (36), consistent with the weak offset seen at 340 but not at 570 nm (Figs. 2 and 3). Triplet yields for pyrimidine bases and their nucleosides and nucleotides are $<$2% in aqueous solution (37, 38). The amplitude $A_3$ has a large uncertainty because of the weak signals but is consistent with triplet state formation by no more than a few percent of excited molecules. This assignment is further supported by our prior study of ICHU in a variety of solvents, including less polar ones where intersystem crossing (ISC) yields are large enough to be accurately measured (31). Some of the long-time bleach signal at 250 nm may also arise from photohydrates. These photoaddition products with water are formed in CMP and UMP with quantum yields of no more than 2% (38).

Nonradiative Decay Yields. For the pyrimidine bases, at least 98% of all excited molecules decay nonradiatively to $S_0$ in aqueous solution at room temperature (38). Until now this decay was thought to occur solely via ultrafast IC to $S_0$ (the fast channel) (1). The additional slow channel reported here shows that the 98% IC yield must contain contributions from two parallel decay pathways.

The fraction of excited molecules that decay via the fast and slow channels was evaluated from the relative amplitudes $A_1$, $A_2$, and $A_3$ (Table 1) of the bleach recovery signals at 250 nm (Figs. 2 and 4). The yield for the fast direct IC is $A_1$, whereas $A_2$ is the yield of molecules that decay via IC from the dark state to $S_0$. These estimates are accurate provided that $S_0$ is the only state with significant absorption at this wavelength (31, 33). There is no stimulated emission at 250 nm because this wavelength lies above the electronic origin of the emissive $^1\pi\pi^*$ state of each compound. Hence, all states other than $S_0$ can contribute only positive \( \Delta A \) changes. The overall negative sign of the signals at 250 nm indicates that $S_0$ is the dominant ambor at this wavelength. If the intermediate electronic state were to absorb at 250 nm, then the relative amplitude of the $\tau_2$ decay ($A_2$) would decrease. $A_3$ is thus a lower bound for the true yield (33).

From the $A_2$ values in Table 1, the dark-state yield is $42 \pm 6%$ in UMP, $41 \pm 19%$ in CMP, and $14 \pm 4%$ in dTMP. Because ISC to the triplet state is proposed to occur from the dark state (see below), the yield of molecules entering the slow channel ($1 - A_2$) is likely somewhat higher. The free bases have somewhat smaller yields of nonradiative decay via the dark state compared with their nucleotides (Table 1 and Fig. 4). Interestingly, a larger yield is not simply due to substitution at N1 of the pyrimidines because similar yields are found for Ura (28 $\pm 5\%$), 1CHU (32 $\pm 5\%$), and DMU (28 $\pm 14\%$). Collectively, these observations show that decay via the slow channel accounts for between 10% and 50% of all deexcitation events.

Assignment of the Dark State. Next, we propose an assignment for the dark state populated in the slow-decay channel and discuss its dynamics. The main criteria used in making our assignment are the accessibility and stability of this state. The dark state must be energetically accessible from the $^1\pi\pi^*$ state in an aqueous environment because only this state is significantly excited. In addition, the dark state requires a stable minimum, which is separated from CIs with lower energy states by sufficiently high barriers to explain the many-picosecond lifetimes. *Ab initio* calculations provide essential guidance. Numerous calculations have shown that there are several low-lying singlet excited states classified by their $^1\pi\pi^*$ and $^1\pi^*$ character.

The number of candidate singlet states is limited (see SI Discussion for why triplet states can be ruled out). A singlet biradical state reachable from the $^1\pi\pi^*$ state was proposed by Zgierski and colleagues (13, 14) that is a key intermediate in the nonradiative decay of Cyt and Ura. This state is reached by a barrierless pathway involving torsional motion about the C5,C6 double bond reminiscent of dynamics seen in the $^1\pi\pi^*$ state of ethene (39). Zgierski and colleagues (13, 14) suggest that a state switch occurs from the $^1\pi\pi^*$ state to the biradical state, but calculations by others (12, 20, 22) find an essentially identical pathway, which does not appear to depart from the $^1\pi\pi^*$ surface. This fact and the apparent absence of a stable minimum before the CI with $S_0$ lead us to reject assignment to a biradical state.

We assign the dark state with absorption at 340 nm and lifetime $\tau_2$ to a lowest-energy $^1\pi^*$ state. Calculations on Cyt and Ura have shown that the $^1\pi^*$ state is the lowest-energy excited singlet state at its energy-minimized geometry (10, 22, 28). The existence of a stable $^1\pi^*$ minimum on the excited-state potential-energy surface is suggestive of conditions necessary for the slow relaxation seen here. Lifetimes of more than a few picoseconds are rarely observed in the condensed phase for excited states that are not the lowest-energy excited state at their minimum energy geometry (Kasha’s rule). However, the stability of this state is difficult to predict, because we know of no calculations of energetic barriers separating the minimum of the $^1\pi^*$ state of each pyrimidine from a CI with, for example, $S_0$. Studies of the energy-minimized $^1\pi^*$ state of 9H-adenine have indicated that barriers of just 0.1 eV separate its minimum from CIs with other states, including $S_0$ (18, 19, 23). Thus, even if the $^1\pi^*$ state were accessible from the $^1\pi\pi^*$ state of 9H-adenine, this low barrier suggests that it would decay to $S_0$ on an ultrafast time scale, explaining the absence of slow channel decay in AMP (Fig. 2). Marian (23) has suggested that the small barrier in 9H-adenine would vanish in solution because of blue shifting of the $^1\pi^*$ state.

According to calculations, both Ura (12, 15, 40) and Thy (15, 40) have a lowest-energy $^1\pi^*$ state in the gas phase at the equilibrium ground-state geometry. On the other hand, the $^1\pi^*$ state of isolated Cyt lies slightly above the $^1\pi\pi^*$ state (22). When aqueous solvation effects are included, the lowest $^1\pi^*$ state shifts to be essentially isoenergetic with the $^1\pi\pi^*$ state of Ura and moves above the $^1\pi\pi^*$ state for Thy (15). However, the energetic state ordering at the ground-state geometry (the vertical state ordering) is not predictive of the order found at other geometries as demonstrated in many recent calculations (21, 22, 28). Thus, a $^1\pi^*$ state that lies at higher energy in the Franck–Condon region can become accessible at another geometry reached by nuclear motions in the $^1\pi\pi^*$ state. Fig. 5 is a qualitative illustration of the two nonradiative decay pathways after bifurcation of the initial excited-state population. It will be important to investigate in the future how solvation affects these state crossings, which to our knowledge have only been studied computationally in the gas phase. Finally, we note that there is experimental precedent for a $^1\pi^*$ state with a picosecond lifetime in a closely related compound in the condensed phase (41). Chachisvillis and Zewail (41) measured a lifetime of 9–23 ps, depending on solvent, for the $^1\pi\pi^*$ state of pyridine, pyrimidine, and triazine in femtosecond transient absorption experiments.
Sequential decay via a $^1n\pi^*$ state ($^1\pi\pi^*$ → $^1n\pi^*$ → $S_0$) has been frequently discussed as the main deactivation channel for isolated nucleobases in the gas phase (2, 27, 29, 42–44), although there is considerable disagreement about the precise kinetics. Reported lifetimes from photoelectron measurements for the $^1n\pi^*$ state are on the order of several hundred femtoseconds to several picoseconds for all of the pyrimidines (29, 42, 43). In contrast to these reports, Kong and coworkers (45, 46) detected a state with a many-nanosecond lifetime upon electronic excitation of isolated Thy and Ura. They assigned this state to a $^1n\pi^*$ state, which they suggested would be undetectable in solution (46). Our results show that this state is clearly not quenched by hydration. Additionally, the observation that branching yields for ICHU are essentially the same in polar and nonpolar solvents (31) suggests that both fast and slow channels could be observable in the isolated molecules.

**Mechanism of Dark-State Decay.** The nuclear motions that lead to decay of the dark state cannot be determined from our transient absorption measurements. Nonetheless, the experimental results suggest some general aspects of the decay mechanism, which we now discuss. The lack of emission on the picosecond time scale for any pyrimidine base (15, 35, 47) indicates that population in the Franck–Condon excited state does not control the $^1n\pi^*$ lifetime. While ISC always occurs along the $^1n\pi^*$ state, it is important to consider whether the latter states are responsible for any of the triplet yields for CMP and UMP compared with dTMP.

**Photochemical Possibilities.** Although the photoproducts formed in DNA by UV light have been known for decades in some cases, it is still unknown what excited states lead to reaction. Because this work shows that decay of the initial $^1\pi\pi^*$ population creates large numbers of relatively long-lived $^1n\pi^*$ states, it is important to consider whether the latter states are responsible for any of the pyrimidine photoproducations known to form from singlet precursors. This includes the pyrimidine photohydrates, which cannot be formed via triplet sensitization, and the pyrimidine (6–4) pyrimidine photoproducations (38). Pyrimidine photohydrates are formed in monomeric bases and in DNA when a water molecule adds across the C5,C6 double bond. Quantum yields of hydration are similar for CMP and UMP but are vanishingly small for dTMP and other 5-substituted pyrimidines on account of steric hindrance (49). Likewise, our results indicate higher $^1n\pi^*$ yields for CMP and UMP compared with dTMP. Additionally, increased photohydrate yields are observed in nucleotides relative to the free bases (38) in agreement with the experimental $^1n\pi^*$ yields. However, there is one trend that we cannot explain: the photohydrate yield of DMU is reported to be one order of magnitude higher than Ura (49), yet similar $^1n\pi^*$ yields are seen for both.

The first step in the reaction to form a pyrimidine (6–4) photoproduct is formation of either an oxetane or azetidine intermediate (38). This is a $[2+2]$ photocycloaddition reaction between the carbonyl group of Thy or the imino group of Cyt and the C5,C6 specific result of ribosyl substitution because two other N1-substituted derivatives, 1CHU and DMU, have shorter lifetimes that match the free base Ura (Table 1).

The free base and its nucleotide have very similar electronic structure because excitation is localized on the aromatic base moiety. The absorption spectra of nucleosides and nucleotides of pyrimidine bases are slightly red-shifted compared with the free bases, but similar red shifts are observed for ICHU and DMU (SI Fig. 6), which do not show increased lifetimes compared with Ura (Table 1). Additionally, preliminary experiments in which the excitation wavelength was varied from 260 to 285 nm show no significant change in either dark-state yield or lifetime for ICHU. These findings indicate that the amount of excess energy in the Franck–Condon excited state does not control the $^1n\pi^*$ lifetime.

We propose that the rate of excess vibrational energy transfer to the solvent is responsible for the lifetime differences between the free bases and their nucleotides. It has been shown previously that solute-solvent hydrogen bonds strongly enhance vibrational cooling in nucleobase monomers (30). The additional hydrogen bonding sites of the ribosyl group thus allow the nucleotides to dissipate excess vibrational energy to the solvent more rapidly than in the free bases, provided that vibrational energy flow from the base to the sugar is unrestricted. As a result of their greater energy content, the free bases undergo more rapid barrier crossing to $S_0$ and therefore have shorter lifetimes than the nucleotides.

**Mediation of ISC by the $^1n\pi^*$ State.** Although a minor decay channel in pyrimidines, ISC to the triplet state is important because of the role that these long-lived states may play as precursors to various DNA photoproducts (38). We propose that the $^3\pi\pi^*$ state is populated in pyrimidine bases via the intermediate $^1n\pi^*$ state (31), a transition favored over direct $^1\pi\pi^*$ to $^3\pi\pi^*$ ISC by classical propensity rules (48). ISC is proposed to occur efficiently only when the $^1n\pi^*$ state has sufficient excess energy. Once molecules in the $^1n\pi^*$ state have cooled, ISC is no longer possible and the only accessible decay channel is IC to $S_0$. In support of this model, no growth in transient signals was observed in ICHU at wavelengths where the triplet state absorbs (31). In support of the notion that ISC occurs along the $^1n\pi^*$ decay pathway, we note that the purine bases, which lack observable $^1n\pi^*$ population, have much lower triplet yields in room temperature aqueous solution (38).

Fig. 5. Schematic of proposed nonradiative decay mechanism for the pyrimidines shown in two arbitrary coordinates as a function of energy. After excitation to the $^1\pi\pi^*$ state (green), population moves toward the minimum of this state ($^1\pi\pi^*_{\text{min}}$, encountering a CI with the $^1n\pi^*$ state (blue) along the way. Upon leaving this CI it can either go toward $^1\pi\pi^*_{\text{min}}$, leading to fluorescence (blue arrow) or the $^1n\pi^*_{S_0,CI}$ (right path), or it can go toward the minimum of the $^1n\pi^*$ state (left path). After the $^1n\pi^*_{S_0,CI}$, the molecule relaxes back to the $S_0$ minimum (gray). To reach $S_0$ from the $^1n\pi^*$ state, a significant barrier must be overcome. Not shown are the $^1n\pi^*$ to $^3\pi\pi^*$ crossing and any photochemical paths that may occur from any of the three states depicted.
double bond of a neighboring pyrimidine. It has thus been suggested that a $^1\pi\pi^*$ state is the reactive excited state (50). Higher yields are found for doublets where the 3rd base is Cyt and not Thy (51). This matches the higher yield we see for the $^1\pi\pi^*$ state of Cyt compared with Thy.

An open question is whether the $^1\pi\pi^*$ state detected here in base monomers is present in base multimers, including DNA and polynucleotides. Based on the findings described here, we are now confident that the long-lived singlet state seen in our earlier experiments on (dT)$_{18}$ (33) is the same state seen in dTMP. However, it has been argued that there is little stacking in (dT)$_n$ (52), and further work is needed to determine whether base stacking, which strongly influences the electronic structure of DNA (33), inhibits the formation or alters the decay rate of the $^1\pi\pi^*$ state seen in the monomeric bases. This clearly has important consequences for pyrimidine (6–4) pyrimidine formation because these photoproducts are formed only in base-stacked doublets. In summary, much more work is needed before any photoproducts can be definitively associated with $^1\pi\pi^*$ states.

Conclusions

We have shown that the $^1\pi\pi^*$ population created by UV excitation of pyrimidine bases in room temperature aqueous solution decays via two very different nonradiative decay channels shown schematically in Fig. 5: ultrafast IC to S$_0$ and decay to a dark state. The latter state, which has an $\approx 100$-fold-longer lifetime than the $^1\pi\pi^*$ state, is tentatively assigned to a $^1\pi\pi^*$ state and is proposed to be a gateway state to the triplet state. The photochemical consequences of this state are poorly understood at present, but there are some suggestive correlations with yields for photohydrols and pyrimidine (6–4) pyrimidine photoproducts. Future computational models of excited states of pyrimidine bases must account for the unusual branching in the singlet manifold, which appears to also take place in the gas phase. Dynamical calculations will be essential for estimating branching yields and for testing the hypothesis that vibrational excess energy in the $^1\pi\pi^*$ state is an important variable controlling its lifetime.

This work shows the power of the transient absorption method for following the evolution of dark excited states, which are undetectable in emission measurements. High yields of long-lived singlet states have been observed in DNA oligomers (33), polynucleotides (53), and now in monomeric bases in aqueous solution. This shows that it is an oversimplification to equate the photostability of DNA or its building blocks with subpicosecond IC dynamics. Although ultrafast nonradiative decay is still operative in all of our samples, large numbers of singlet excitations are formed in each, which decay orders of magnitude more slowly than the singlet state. Much more experimental and theoretical work is needed to understand the nature of these long-lived states and their potential for photochemical damage.

Methods

The experimental setup has been described previously (30, 33). Briefly, signals were recorded by using a Ti:sapphire-based transient absorption spectrometer. Pump pulses were provided by the third harmonic of the laser fundamental. Probe pulses were obtained from a tunable optical parametric amplifier (Coherent, Santa Clara, CA). Pump pulse intensities were typically in the range of 0.2–2 GW cm$^{-2}$. Probe pulses were always attenuated to have at most 1/10th of the intensity of the pump pulses. The transmitted probe pulse was detected with a photomultiplier tube after spectral filtering with a double grating monochromator. Pump and probe pulse polarizations were set to magic angle to eliminate reorientation signals. Transient absorption signals were recorded by using a lock-in amplifier referenced to a chopper placed in the pump path. The instrument response function was $\approx 200$ fs as determined by the FWHM of the coherent two-photon absorption seen in solvent-only scans. Approximately 0.4 ml of the solution under study was held in a 1.2-mm-path-length cell between CaF$_2$ windows. The cell was spun at several hundred rpm about its axis to avoid reexcitation of the pumped volume by successive laser pulses. Signals were carefully monitored for photodegradation by UV absorption spectroscopy during the experiments, and solutions were replaced with fresh ones as needed. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) and used as received. Samples were prepared to have an absorbance between 1 and 2 at the pump wavelength in pH 7 phosphate buffer.

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