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Remarkable Effects of Solvent and Substitution on Photo-dynamics of Cytosine: A Femtosecond Broadband Time-resolved Fluorescence and Transient Absorption Study

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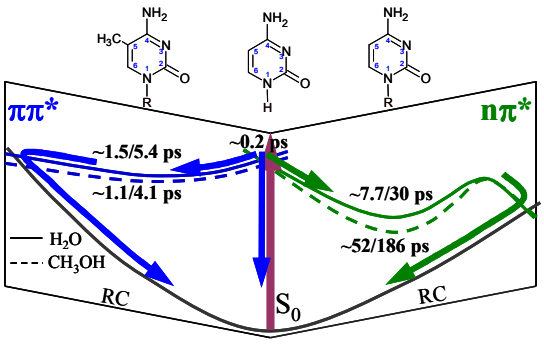
Table of Content Graphic

Solvent alters differently rate of ππ and nπ* decay while substitution enhances or eliminates the nπ* from cytosine nonradiative deactivation.*



ABSTRACT. Cytosine (Cyt) among all the nucleic acid bases features the most complex and least understood nonradiative deactivation, a process that is crucially important to its photostability. Herein, the excited state dynamics of Cyt and a series of its N1- and C5-derivatives, including the full set of Cyt nucleosides and nucleotides in DNA and RNA and the nucleosides of 5-methyl cytosine the 5-methylcytidine and 2'-deoxy-5-methylcytidine, have been investigated in water and in methanol employing femtosecond broadband time-resolved fluorescence coupled with fs transient absorption spectroscopy. The results reveal remarkable state-specific effects of the substitution and solvent in tuning distinctively the timescales and pathways of the nonradiative decays. For Cyt and the N1-derivatives, the nonradiative deactivations occur in a common two-state process through three channels, two from the light-absorbing ππ* state with respectively sub-picosecond (~0.2 ps) and picosecond (~1.5 ps) time constant, and the third is due to an optically dark nπ* state with lifetime ranging from several to hundreds picoseconds depending on solvent and substitutions. Comparing to Cyt, the presence of the ribose or deoxyribose moiety at the N1 position of N1-derivatives facilitates formation of the nπ* at the sub-picosecond timescale and at the same time increases its lifetime by ~4—6 times in both water and methanol. In sharp contrast, the existence of the methyl group at C5 position of the C5-derivatives eliminates completely the sub-picosecond ππ* channel and the channel due to the nπ*, but on the other hand slows down the decay of ππ* state which after relaxation exhibits a single time constant of ~4.1 to ~7.6 ps depending on solvent. Varying solvent from water to methanol accelerates only slightly the decay of ππ* state in all the compounds; while for Cyt and its N1-derivatives, this change of solvent also retards strongly the nπ* channel, prolongs its lifetime from such as ~7.7 ps in water to ~52 ps in methanol for Cyt and from ~30 ps in water to ~186 ps in methanol for deoxycytidine. The spectral signatures we obtained for the ππ* and nπ* state allow unambiguous evidence for clarifying uncertainties on the excited states of Cyt and the derivatives. The results provide

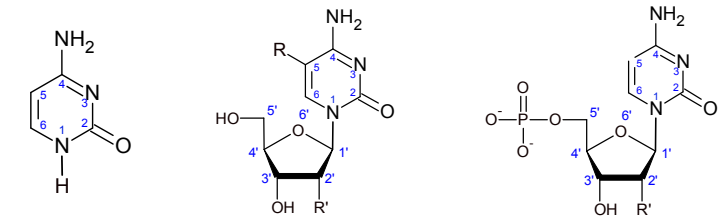
a unifying experimental characterization in an improved level of detail about the photophysics of Cyt and its analogues in biologically relevant conditions and may help understanding the photostability as well as photo-damages of the bases and related DNAs.

INTRODUCTION

All naturally occurring nucleobases, upon UV excitation, have ultrafast decay routes in sub-ps timescale from light-absorbing ππ* state to the ground (S_0) state.[1,2] The ultrafast decays arising from the highly efficient internal conversion are often invoked as the principal mechanism against photo-damage and also regarded the main source of the bases being selected as the building blocks of genetic materials.[1-4] A growing number of recent studies show, however, that the sub-ps decay of ππ* may contribute only partially to the overall nonradiative dynamics, other processes taking place on slower timescales through the ππ* or more importantly through optically dark state such as of nπ* in parentage may play a significant role.[1-5] The complexity is rooted in the intrinsic property of the bases in having peculiar topology of the ππ* potential energy surface (PES) and low lying nπ* states that may couple nonadiabatically with both the ππ* and S_0. However, owing to the short lifetimes and complex dynamics that may vary from subtle changes in factors such as substitution and medium condition, to directly probe the ππ* and at the same time to resolve the nπ* state has remained a challenging task. As a consequence, despite the invaluable insights led by the extensive studies, there are still important questions to answer, including such as whether for a particular base there is indeed a participation of nπ* state in the decay process,[1-9] the precise timescales, pathways, and extents to which the nπ* competes with ππ*, and how these are affected by environmental factor and covalent modification of the bases.[2-5]

With very few exceptions,[1c,4d,4f,5,6i,8a-b] most of the previous studies on the nucleobases in solution were performed in water.[1] In DNA and RNA, the bases are however subject to an interior solvation environments that are much less polar and with hydrogen (H)—bonding configurations different than in water.[1a,1b] Also, the bases exist with substitution of certain hydrogen atom by ribose (in RNA) or deoxyribose (in DNA) and with some other hydrogen substituted by such as the methyl group for the case of

minor bases. To elucidate the effects of solvent and the substitution on the excited state dynamics is therefore not only crucial for a fuller description of the bases in their monomeric form but is also a prerequisite for understanding the photophysics of DNA and RNA assemblies. Herein, as a case study, we present a direct probe of the full deactivation cascades for cytosine (Cyt) and a series of its N1- and C5-substituted derivatives (Scheme 1) in solvents of different polarity and H-bonding capacity.



Scheme 1 Cytosine and its N1 and C5 derivatives studied.

As the only common pyrimidine base between DNA and RNA, Cyt is known for its particularly complex excited state structure[2-4,6-11] which might allow for parallel operation of multiple decay paths from ππ* and nπ* states.[6,7,9-11] The excited state dynamics reported for Cyt are, however, varied depending on the conditions and methods used for the measurements. In water, Cyt and its N1 derivatives including some of its nucleosides and nucleotides were reported to display very rapid decay from the light-absorbing ππ* state. The decay of ππ* was respectively described to be single exponential (with time constant of ~1.0 ps) and bi-exponential (with ~0.2 and ~1.3 ps time constants) by the fs transient absorption (fs-TA)[1a,2a] and fs fluorescence up-conversion (fs-FU) measurement.[4a,4b] In addition to the ππ* state, a longer-lived species (~33-39 ps lifetime) attributed to a dark nπ* state was reported for the N1-derivatives dCyd, CMP, and dCMP in water.[2c,3] Whether the nπ* is present for the parent molecule Cyt is

however a controversial issue. Whilst an earlier fs-TA study based on probe of excited state dynamics at selected absorption wavelengths reported an involvement of the nπ*,[2c] more recent ps time-resolved infrared (ps-TRIR) work stressed an absence of the nπ* in Cyt.[3] On the other hand, ultrafast studies on Cyt in the gas phase reported decay dynamics described by time constants of tens fs to several ps[12] with the nπ* state playing an important role as suggested by a number of sophisticated theoretical computations.[6-11] There is however to the best of our knowledge no time-resolved study on Cyt and any of the derivatives (Scheme 1) in solvent other than water.

The C5 derivatives we investigated (m5Cyd and m5dCyd, Scheme 1) are the nucleosides of 5-methyl cytosine (m5Cyt), a minor base from DNA methylation which is known for its role as the mutational hotspot in skin tumours.[13] Ultrafast studies on m5Cyd and m5dCyd in water reported transient species with ~6—7 ps lifetime[2b,4c] showing complex fluorescence decay.[4c] The cause of the complexity and influence of C5 substitution on the decay dynamics, especially those related to the nπ* state, have so far remained elusive.

The uncertainties and the different dynamics reported for Cyt in water *vs* in the gas phase and between Cyt and its N1 and C5 derivatives raise questions about (i) the detailed pathway of ππ*, (ii) the role of nπ*, and (iii) influences of solvent and N1- and C5-substitution on the nonradiative decays. To help address all these questions, we have performed an integrated spectroscopic investigation which we combined steady state measurements with fs broadband time-resolved fluorescence (fs-TRF), fluorescence anisotropy (fs-TRFA), and transient absorption (fs-TA) on Cyt and all the derivatives in Scheme 1. The study was done in water and for comparison in a less polar protic solvent methanol in order to assess effect of solvent on the dynamics and pathways of the nonradiative deactivation. Featuring simultaneously the wavelength resolution and fs time resolution, our fs broadband spectroscopy provides an important complement to the

literature fs-TA,[2] fs-FU,[4] and ps-TRIR[3] methods which are lack of either the spectral resolution or the time resolution in fs domain. The wavelength resolution of our method allows producing spectral signature for extremely short-lived species,[5c,14,15] a feature crucial for identifying explicitly the ππ* and nπ* state in the deactivation of Cyt and the derivatives. Besides, with the fs-TRF to probe the bright ππ* state and the fs-TA to monitor in addition the optically dark nπ* state as well as the recovery and relaxation of the S_0, the integration of fs-TRF and fs-TA makes possible to determine not only the dynamics of excited state decays but also the correlations in dynamics between the decays and S_0 recovery which enable to unveil the overall cascade of the deactivation processes. The results we obtained provide direct evidence for clarifying the uncertainty on the Cyt deactivation and show unprecedented effects of solvent and the N1- and C5-substitution in modulating distinctively dynamic competition between the ππ* and nπ* for nonradiative decays that occur in largely varied timescales from sub-ps to hundreds ps after the excitation.

RESULTS

Steady state spectroscopy

Figure 1 displays steady state absorption and fluorescence spectra recorded for Cyt, the N1 derivatives Cyd and dCyd, and the C5-derivatives m5Cyd and m5dCyd in water and methanol. Comparison of the spectra in water for Cyt and all of the N1-derivaitves (including CMP and dCMP) is presented in Figure S1 in the supporting information (SI). Due to the low solubility, no spectra (both in the steady state and fs time-resolved measurements) were obtained for CMP and dCMP in methanol. Key spectral parameters including such as the wavelengths of maximum absorption and fluorescence spectra, the maximum molar absorption coefficients and fluorescence quantum yields for all the

systems examined are compiled in Table 1. For compounds that were studied previously in water, the results we obtained from the steady state measurement are consistent with those in the literature.[2b,4a-c]

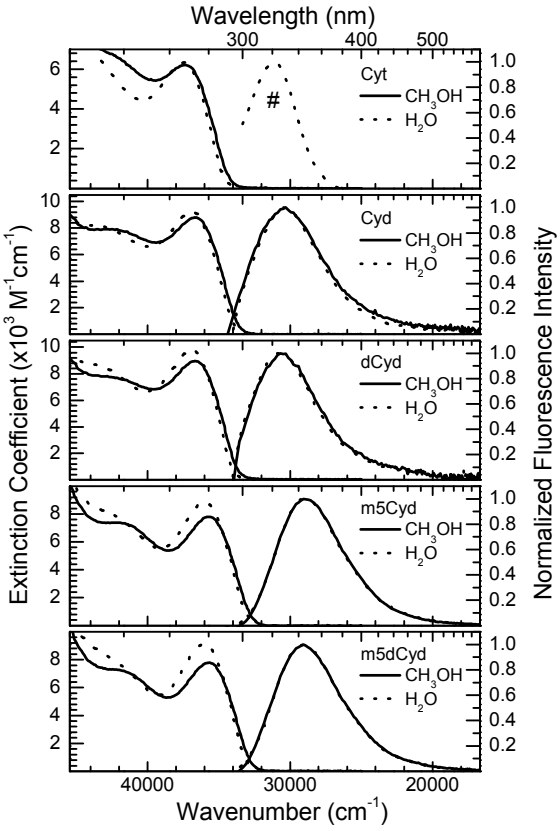


Figure 1. (Left) steady state absorption and (right) normalized fluorescence spectra (λ_{exc} = 267 nm) recorded for Cyt, Cyd, dCyd, m5Cyd, and m5dCyd in water (dotted lines) and in methanol (solid lines). #From [16a].

It can be seen from Figure 1 and Table 1 that varying solvent from methanol to water affects only subtly the steady state spectra, leading to such as small wavelength blue-shifts (by ~2-5 nm) in the absorption for all the compounds and in the fluorescence for Cyt and the N1 derivatives.

Comparison of the spectra between Cyt and the N1-derivatives shows that, regardless of the solvent used, the N1-substitution causes slight wavelength red-shifts (by ~5 nm) both in absorption and fluorescence and increases slightly the absorption coefficient and

the quantum yield of fluorescence. On the other hand, in both water and methanol, the methyl substitution at C5 position leads to substantial red-shifts (by ~15-17 nm) in both absorption and fluorescence and elevates significantly (by ~6-7 folds) the fluorescence quantum yield compared to those of Cyt and the counterpart N1 derivatives. This observation agrees well with those reported before for m5Cyd and m5dCyd in water.[2b,4c]

Table 1 Spectral and dynamic parameters obtained for Cyt and its N1 and C5 derivatives in water and methanol

		$^{a}\lambda_{Abs}$/nm	$^{b}\varepsilon$/Lmol^{-1}cm^{-1}	$^{c}\lambda_{f}$/nm	$^{d}\tau_1$/ps	$^{d}\tau_2$/ps	$^{e}\tau_3$/ps	$a_{\pi\pi^*}$% ($^{f}a_{FC}$%, $^{g}a_{min}$%)	$^{h}a_{n\pi^*}$%	$^{i}\Phi_f$($\times10^{-4}$)
Cyt	H_2O	267	6333	^{k}322	0.2 ± 0.03	1.5 ± 0.1	7.7±0.6	75 (64, 11) ± 2	25 ± 4	^{j}0.82
	CH_3OH	268	6221	^{l}327	0.2 ± 0.03	1.1 ± 0.1	52 ± 3			
Cyd	H_2O	271	9199	325	0.2 ± 0.03	1.8 ± 0.1	35 ± 2	50 (38, 12) ± 4	49 ± 4	1.02 ± 0.06
	CH_3OH	273	8816	326	0.3 ± 0.03	1.3 ± 0.1	144 ± 4			1.08 ± 0.07
dCyd	H_2O	271	9807	324	0.2 ± 0.03	1.1 ± 0.1	30 ± 2	66 (54, 12) ± 3	33 ± 5	0.89 ± 0.06
	CH_3OH	273	8964	326	0.2 ± 0.03	0.8 ± 0.1	186 ± 6			0.83 ± 0.08
CMP	H_2O	271	8597	327	0.3 ± 0.03	1.8 ± 0.1	34 ± 2	60 (42, 18) ± 3	39 ± 5	1.52 ± 0.08
dCMP	H_2O	271	8879	326	0.2 ± 0.03	1.3 ± 0.1	30 ± 2			1.11 ± 0.05
m5Cyd	H_2O	278	8871	341	0.7 ± 0.05	7.6 ± 0.3		99 ± 2		7.53 ± 0.32
	CH_3OH	280	7804	341	0.8 ± 0.05	5.1 ± 0.3				7.46 ± 0.30
m5dCyd	H_2O	278	9153	340	0.6 ± 0.05	5.4 ± 0.3		99 ± 2		5.97 ± 0.25
	CH_3OH	280	7806	340	0.6 ± 0.05	4.1 ± 0.3				5.93 ± 0.28

[a]Wavelength of [b]the maximum extinction coefficient in steady state absorption. [c]Wavelength of the maximum intensity of steady state fluorescence. Decay time constants derived from [d]fs-TRF and [e]fs-TA measurements. Percentage contributions from decay channel [f]$\pi\pi^*_{FC}\rightarrow S_0$, [g]$\pi\pi^*_{FC}\rightarrow\pi\pi^*_{min}\rightarrow S_0$, and [h]$\pi\pi^*_{FC}\rightarrow n\pi^*\rightarrow S_0$. [i] Fluorescence quantum yield from [j][16(b)-(c)]. [k]From [16(a)] and [l]from [16(d)].

Femtosecond broadband time-resolved fluorescence spectroscopy

Figure 2 depicts the temporal evolutions of fs-TRF spectra, the kinetic decay profiles of TRF intensities, and the zero time fluorescence anisotropy spectra (r(0)) recorded with 267 nm excitation of Cyt, Cyd, CMP, and m5Cyd in water. The corresponding data for dCyd, dCMP, and m5dCyd in water and for Cyt, Cyd, dCyd, m5Cyd, and m5dCyd in methanol are given in Figure S2 and S3 in the SI. Figure 3 compares the TRF decay profiles from Cyt, Cyd, and m5Cyd in water *vs* in methanol. Fs-TRFA spectra recorded for

the various compounds in water and in methanol at different time intervals after the excitation are given in Figure S4 in the SI.

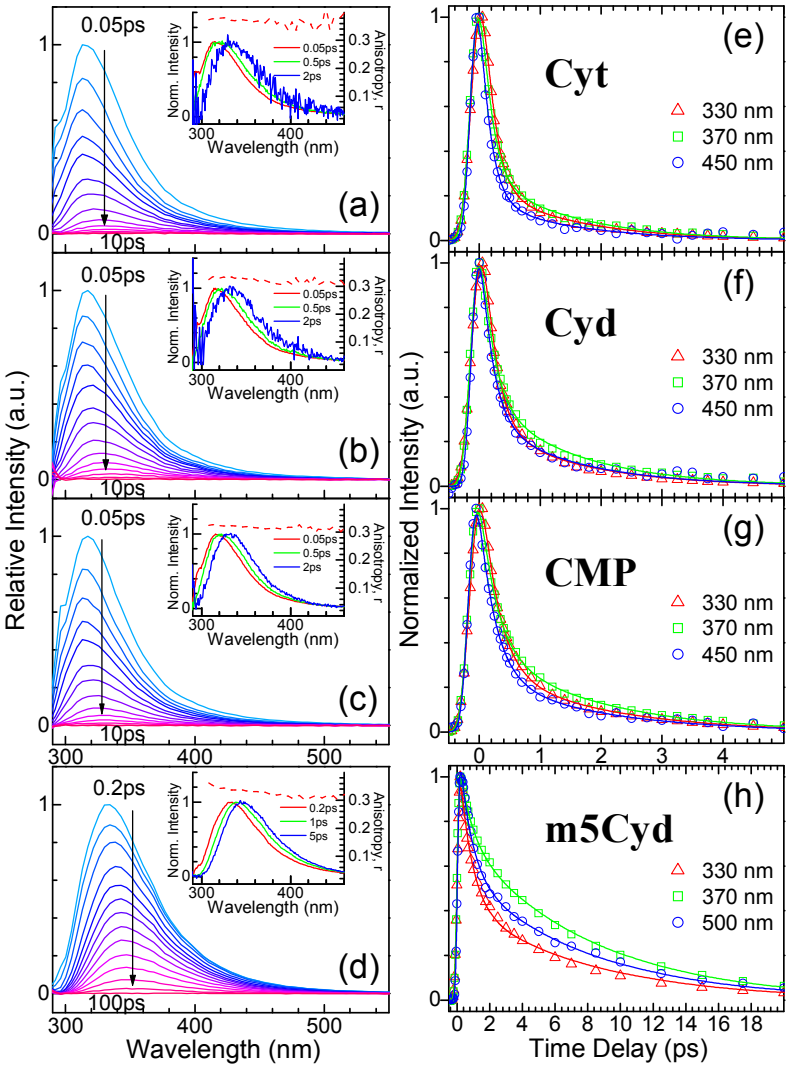


Figure 2. (a-d) fs-TRF spectra and (e-h) experimental (Δ, O, □) and fitted (solid lines) TRF decay profiles obtained with 267 nm excitation at denoted emission wavelength for (a, e) Cyt, (b, f) Cyd, (c, g) CMP and (d, h) m5Cyd in water. The insets show zero time TRF anisotropy spectra and intensity normalized TRF spectra at the denoted time intervals after the photo-excitation. The arrows indicate directions of spectral evolutions.

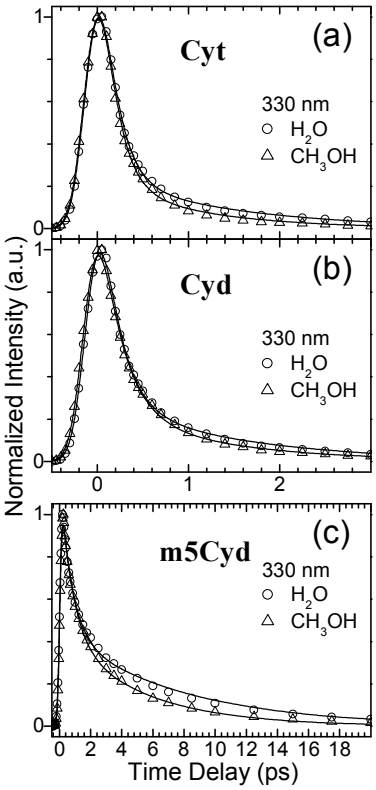


Figure 3. Experimental (O, Δ) and fitted (solid lines) TRF decay profiles obtained with 267 nm excitation at denoted emission wavelength for (a) Cyt, (b) Cyd and (c) m5Cyd in water (O) and in methanol (Δ).

From Figure 2 and Figures S2-S3, it can be seen that, for all the compounds, the fs-TRF spectra in water and in methanol resemble the spectra of the counterpart steady state fluorescence (Figures 1 and S1). Global analysis[14,15] of the TRF intensity decays demonstrated bi-exponential dynamics described by two time constants (τ_1 and τ_2, Table 1) for all the systems examined. The detailed values of the time constants and the related fractional contributions (a_1% for τ_1, a_2% for τ_2) at several selected emission wavelengths as well as the values of r(0) are given in Table S1 in the SI.

The fs-TRF (and the steady state fluorescence) from all the compounds in water and methanol (Figure 2 and Figures 2S-3S) can be attributed with certain to originate from the

photo-populated ππ* state. This is indicated by the data of TRFA (inset in Figures 2 and S2-3, Figure S4) which shows that the anisotropies of transient fluorescence in all systems are nearly independent of the emission wavelength and with the value of time zero anisotropy (~0.32—0.37, Table S1) close to the theoretical value of 0.4 when fluorescence is from the same electronic state for absorption in the photo-excitation. The values of the time zero anisotropy we obtained with Cyt (~0.37) and dCMP (~0.34) in water are nearly identical to those reported in the literature.[2a-b] It is therefore unequivocal that the TRF, albeit its bi-exponential dynamics, is due from one single state, the light-absorbing ππ*, in all the systems examined.

As showed in Table 1 and Figures 2 and S2-S3, for Cyt and N1-derivatives, the time constants (τ_1/τ_2) of the bi-exponential decays are very similar, being ~0.2-0.3/1.1-1.8 ps in water and ~0.2-0.3 /0.8-1.1 ps in methanol. The time constants from C5-derivatives are however relatively much slower, being ~0.6-0.7/5.4-7.6 ps in water and ~0.6-0.7/4.1-5.1 ps in methanol. For the decay times in water, the values we derived coincide with the those reported in the previous fs-FU studies.[4a-c]

A clear trend demonstrated by the fs-TRF data is that, for all the compounds investigated, the decays of fluorescence are consistently slightly faster in methanol than in water (Figure 3 and Table 1). With the time constant of the first component (τ_1) being nearly independent of the solvent, this occurs mainly due to the time constant of the second component (τ_2) is slightly more rapid in the former than later solvent.

Examination of the fluorescence decays at the different emission wavelengths shows that, for Cyt and the N1 derivatives, the decays in both water and methanol varied only marginally with the emission wavelength, which is due to the involvement of small degrees (by ~10 nm) of dynamic Stokes shift (DSS) of the TRF profiles (insets in Figures 2, S2-3) as decays of the TRF intensities. For all these systems, the decays of fluorescence

proceeded primarily in the sub-ps timescale with ~80–90% (a_1%) of the overall fluorescence disappeared at the time constant of τ_1 and the remaining ~10–20% (a_2%) the time constant of τ_2. Unlike this, the TRF decays of the C5-derivatives (in water or methanol) exhibit clear variations with the emission wavelength (Figure 2h), being slower at longer wavelength. This according to the kinetic fitting is due to increase with the wavelength of the relative contribution of the second time component τ_2. Such as for m5Cyd in water, the contribution of τ_2 varies from ~31% at 330 nm to ~67% at 370 nm (Table S1). This is in analogous to the complex fluorescence decays reported earlier for the same system.[4c] To elucidate the deactivation pathway underlying the bi-exponential TRF decays and the cause for the different TRF behaviour between C5-derivatives *vs* Cyt and N1 derivatives, fs-TA measurements were performed and the results are illustrated below.

Femtosecond broadband transient absorption spectroscopy

Figure 4 displays temporal evolutions of fs-TA spectra recorded in water for Cyt, Cyd, CMP, and m5Cyd at various time intervals after the 267 nm excitation. The corresponding full scale spectra and the spectra for dCyd, dCMP, and m5dCyd in water and for Cyt, Cyd, dCyd, and m5Cyd in methanol are presented respectively in Figure S5 and S6 in the SI. It is clear from these data that, for all these systems, the TA spectra feature the positive-going bands across the visible wavelengths due to the excited state absorption (ESA) and negative-going bands at ~340-360 nm due to the stimulated emission (SE) from the $\pi\pi^*$ state and at <~300 nm wavelengths due to the S_0 bleach induced from depletion of the S_0 population by the photo-excitation. Kinetic profiles of the TA intensity decays at representative wavelengths for the ESA (e.g. ~416 and 570 nm) and the S_0 bleach (e.g. ~258 nm) are compared in Figure 5 for Cyt, Cyd, dCyd, and

m5Cyd in water and in methanol and in Figure S7 for the other systems in water. For all the systems examined, the decay time constants derived from global analysis of the TA time profiles are included in Table 1 with the detailed dynamic parameters presented in Table S2 in the SI.

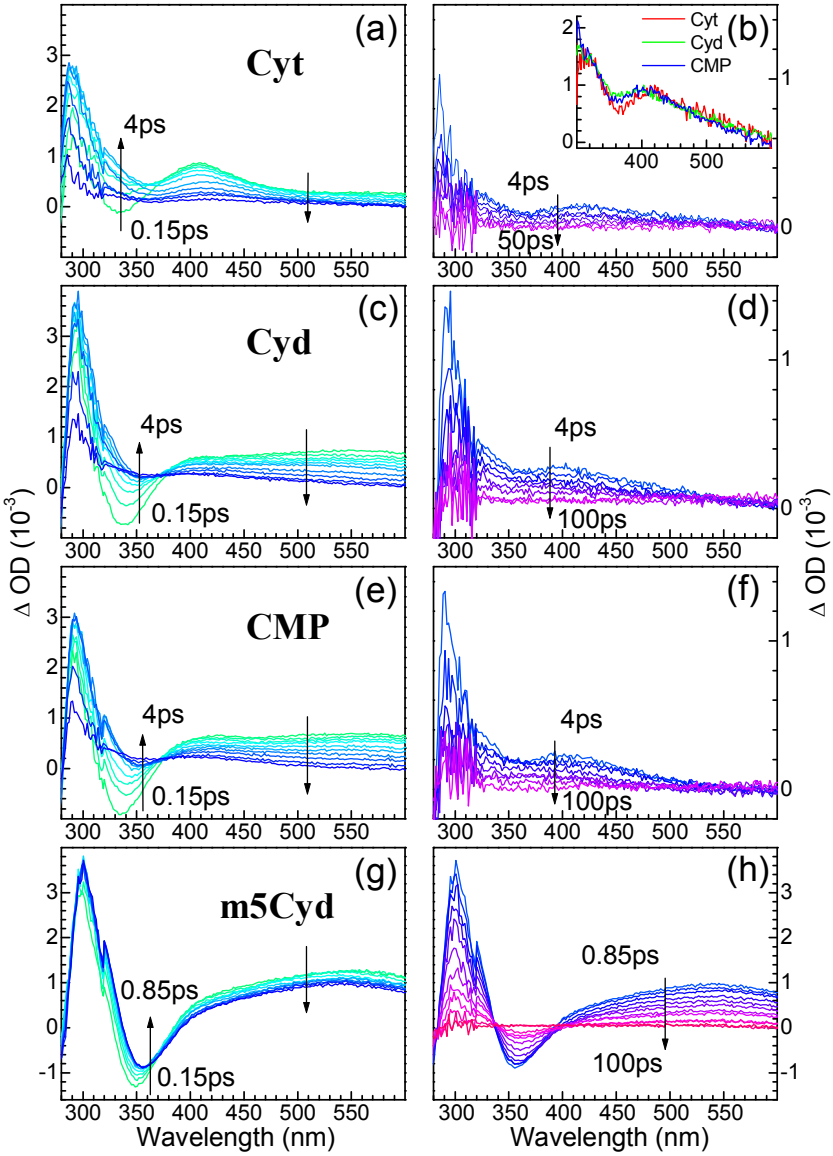


Figure 4. Temporal evolutions of fs-TA spectra recoded at early (left) and late (right) time intervals after 267 nm excitation of (a, b) Cyt, (c, d) Cyd, (c, f) CMP and (g, h) m5Cyd in water. The arrows indicate directions of spectral evolutions. The inset in (b) compares the spectra at ~4 ps after the excitation of Cyt, Cyd and CMP in water.

Femtosecond transient absorption of Cyt and N1 derivatives

Cyt and all its N1-subsituted derivatives exhibit fs-TA (Figures 4(a-f) and S5-7) that are very similar in the spectral profile but different in the decay dynamics depending on the solvent used and the presence or not of the ribose or deoxyribose at the N1 position.

Regardless of the solvent used, all these compounds display initial ESA (at 0.15 ps) having a strong band at ~300 nm and a weaker and diffuse band at the entire visible region. The spectra, as showed in Figure 4 and S5-6, can be considered to evolve in two phases at time before and after ~4 ps after the photo-excitation. In the first phase (<~4 ps), the initial ESA depletes rapidly along with diminishing of the SE from ππ* state. The decay, according to the global analysis (at ~570 nm, Figures 5 and S7), is described by the same set of two time constants (τ_1 and τ_2, Table S2) as that from the decays of TRF in the corresponding system (Table 1 and S1). On the basis of this observation, the early phase decay was attributed to the ππ* state being detected in both fs-TRF and fs-TA and the initial ESA the absorption characteristic of the ππ* in Cyt and all the N1 derivatives in both water and methanol.

After the decay of ππ* state, the TA in the second phase (>~4 ps) features ESA at ~300 nm and with a new band at λ_{max}~410 nm in wavelengths from ~300 to 500 nm (the late time spectra in Figures 4, S5-S6). The ~300/410 nm ESA exhibits decay in different timescales (TA profiles at ~416 nm, Figures 5 and S7) with its lifetime (τ_3) determined to be ~7.7 ps for Cyt and ~30-35 ps for the N1 derivatives in water but much slower in methanol being ~52 ps for Cyt and ~144/186 ps for Cyd/dCyd (Table 1 and S2). Unlike that of the ππ*, the decay of ~300/410 nm ESA has no counterpart in the corresponding fs-TRF (Figures 2 and S2-3), implying its origination from an optically dark species undetectable in the fs-TRF. On the basis of this and that the ~30/34/30 ps lifetimes we

observed with dCyd/CMP/dCMP in water coincide with the lifetimes of nπ* reported for these compounds in the same solvent,[2c,3] the species was attributed to the nπ* and the ~300/410 nm ESA the absorption signature of the nπ* state in the respective compounds.

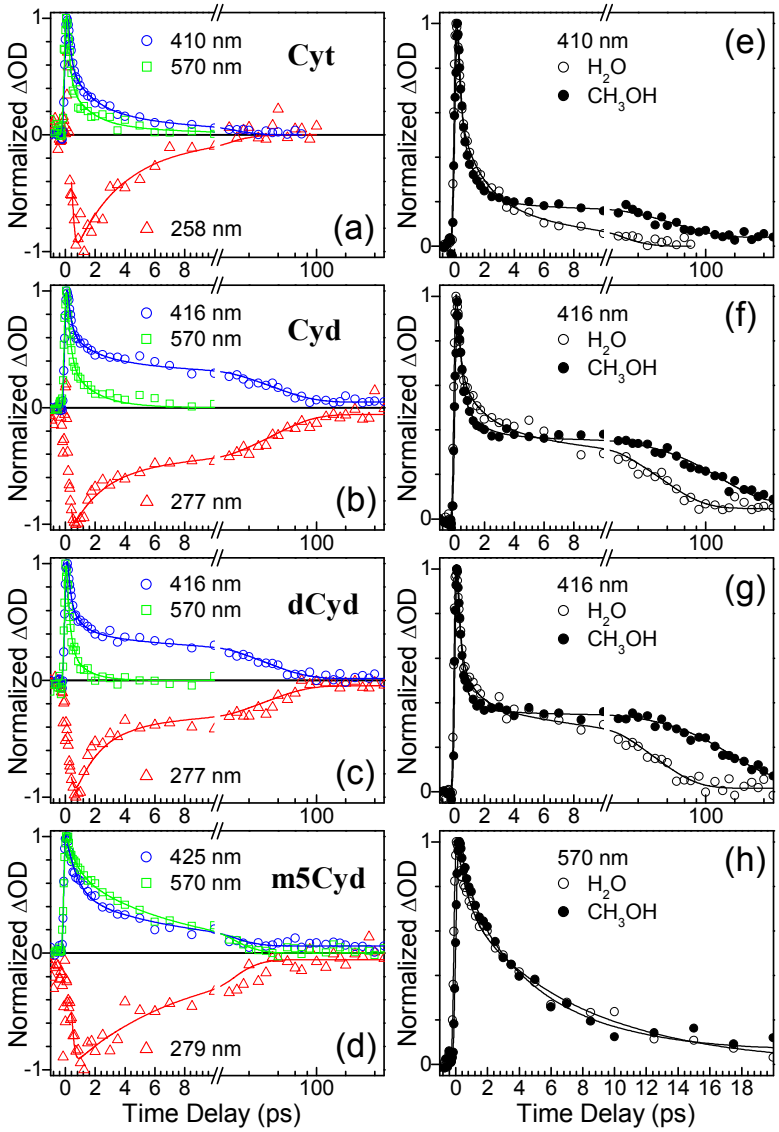


Figure 5. Fs-TA time profiles obtained for (a, e) Cyt, (b, f) Cyd, (c, g) dCyd, and (d, h) m5Cyd in (a-d) water and (e-h) comparison with the corresponding time profiles in methanol.

The observation of nπ* signature absorption in the fs-TA of Cyt (insets in Figure 4(b) and Figure S8) provides direct evidence for participation of the nπ* in Cyt nonradiative

decay both in water and in methanol. For Cyt and the N1 derivatives, the data we observed in methanol afford the first direct identification of the nπ* during the deactivation of these systems in solvent other than water. The large variations in the nπ* lifetime from Cyt to N1 derivatives and upon changing solvent from water to methanol testifies remarkable roles of the N1 substitution and the solvent in altering pronouncedly the dynamics of nπ* state.

Analysis of the TA time profiles (in water) in the S_0 bleach region (Figures 5 and S7) revealed biphasic recovery of the S_0 caused by decays of the ππ* and nπ* state. For Cyt and all the N1 derivatives, the recovery in the first phase demonstrated time constant of ~1.7-2.9 ps (Table S2) which was clearly due to relaxation of the vibrationally excited S_0 produced due to rapid decay of the ππ* to S_0 (ππ*→S_0). The ~1.7-2.9 ps time constants are typical of such process, similar to those reported in the ultrafast studies for the bases in water.[1a,2c,5a,5b] The relaxation taking place in the S_0 is known to proceed at rates slightly slower than the decay of ππ* for it is rate-determined by dissipation of excess vibrational energy from the "hot" S_0 to surrounding solvent molecules.[1a,2,3,5] The percentage recovery due to this process from the decay of ππ* ($a_{\pi\pi^*}$%, Table 1) was estimated to be ~75% in Cyt and ~50/66/60% in Cyd/dCyd/CMP in water. On the other hand, being synchronous with the decay of nπ* state, the second phase recovery of S_0 is obviously due to the decay of nπ* state (nπ*→S_0), which was found to contribute a fraction ($a_{n\pi^*}$%, Table 1) of ~25% for Cyt and ~49/33/39% for Cyd/dCyd/CMP in water. The substantial contributions from the nπ* implies that the nπ* competes strongly with the ππ* for coupling with the S_0 in the deactivation of both Cyt and the N1 derivatives.

A close inspection of the TA spectra (Figures 5 and S7, Table S2) showed that, after the biphasic recovery which altogether amounts ~99% of the overall bleach signal, there was persistence of the remaining ~1% bleach signal over timescale beyond that of current measurement (>~6 ns). This implies presence of very long-lived species after the decays

of ππ* and nπ* state. With no involvement of photochemical reaction as suggested by the nearly identical steady state absorption before and after the TA measurement, the species was most likely due to the electronically excited triplet state which may form through spin orbital coupling with the ππ* or nπ* state as proposed by theoretical studies.[2c,5a,17] This assignment is corroborated by (i) the long-lived nature of the residual signal which according to kinetic analysis is related to a lifetime of >~20 ns (Table S2) under the aerated condition in the TA measurement; (ii) its minute contribution (<~1%) which is close to the yields of triplet reported for Cyt and some of its nucleosides and nucleotides;[18a] and more important (iii) the offset signal was found to correlate dynamically with a weak and broad ESA in the visible wavelengths (Figure S9) which resemble the literature spectra reported for the triplet state of Cyt, Cyd, and dCMP in water.[18b-d]

The above results taken together show clearly that, for Cyt and all the N1 derivatives, the nonradiative decays occur predominantly in the singlet manifold mediated by both the ππ* and nπ* state with the respective contribution of $a_{\pi\pi^*}$% and $a_{n\pi^*}$% (Table 1) and in the timescale of sub-picosecond to picosecond for the decay of ππ* state (τ_1, τ_2) and several to hundreds picoseconds for the nπ* state (τ_3) depending on the solvent. The deactivation, referred to proceed through a two-state pathway, is diagrammed in Scheme 2(a).

Femtosecond transient absorption of C5 derivatives

It can be seen from Figure 4 and S5-S6 that, unlike Cyt and the N1 derivatives, the C5 derivatives (m5Cyd and m5dCyd) in both water and methanol display fs-TA without the ~300/410 nm absorption due to nπ* state. Instead, the fs-TA of the C5-derivatives exhibit ESA and bleach recovery primarily due to the light-absorbing ππ* state.

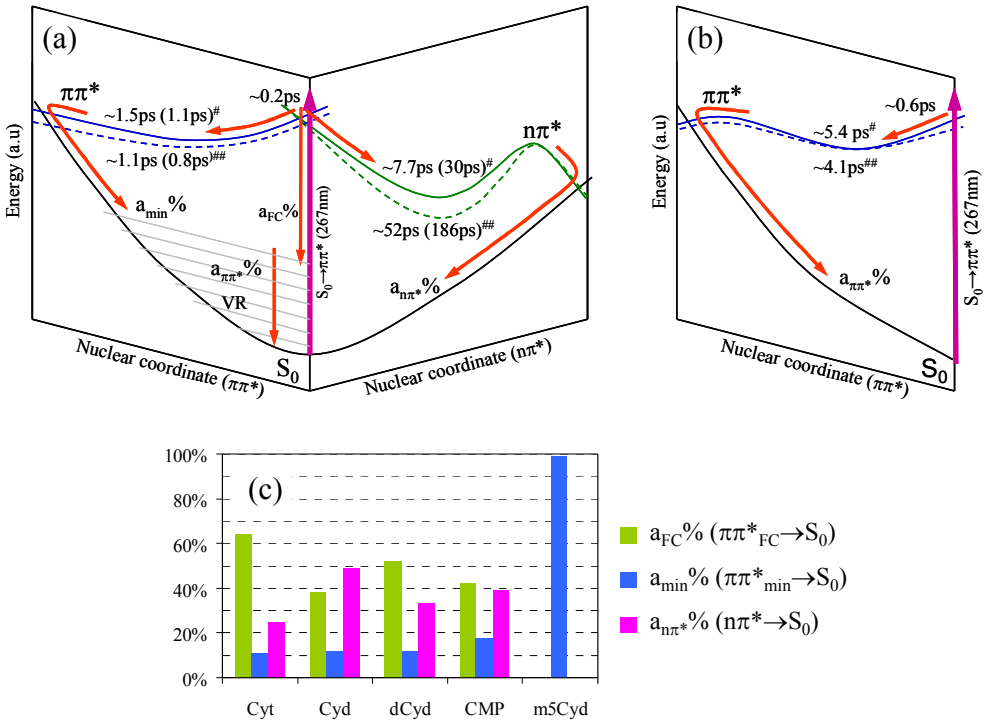
The ESA features the broad absorption characteristic of the ππ* which decays along with depletion of the SE from ππ* state. The decay of ESA demonstrated bi-exponential dynamics with the time constants (τ_1 and τ_2, Table S2) identical to those from the corresponding fs-TRF (Figures 2(h), S2-3, Table S1). The S_0 bleach, on the other hand, exhibits recovery described by mono-exponential dynamics with time constant equivalent to the τ_2 of the excited state decay. After the recovery at τ_2 of most the bleach signal, there is persistence of a small percentage (<~1%) of long-lived residual (Figure 4 and Table S2) which can be attributed to the triplet state of the C5 derivatives by analogy to the case of Cyt and N1 derivatives.

With the minute involvement of the long-lived triplet state, it is straightforward that the decay at τ_2 of the ππ* state is the major process responsible for transferring the excited state population into the S_0. Within this context, the τ_1 related process in both ESA and TRF can be ascribed to relaxation of the ππ*, which as suggested by the nearly solvent-independent DSS (inset in Figures 2(d) and S2-3) can be attributed to the structural relaxation of the initial ππ* ($\pi\pi^*_{FC}$) as it evolving from the FC region to the region where the state crossover occurred. Considering the rather long lifetime of ππ* state (~4.1-7.6 ps, Table 1), the conversion to S_0 is most likely taking place after the ππ* relaxed into the local minimum of its PES ($\pi\pi^*_{min}$).

Therefore, for the C5 derivatives, the nonradiative decays proceeded as a result of the $\pi\pi^*_{FC} \rightarrow \pi\pi^*_{min}$ relaxation at the time constant of τ_1 followed by the $\pi\pi^*_{min} \rightarrow S_0$ crossover at τ_2. The deactivation is therefore referred to occur through a *one-state* pathway ($\pi\pi^*_{FC} \rightarrow \pi\pi^*_{min} \rightarrow S_0$) mediated solely by the ππ* state and is illustrated in Scheme 2(b).

DISCUSSION

The fs-TRF, fs-TRFA, and fs-TA results we obtained show that, in both water and methanol, there are two types of nonradiative cascades, the *two-state* pathway from the ππ* and nπ* for Cyt and its N1 derivatives and the *one-state* channel from the ππ* for the C5-derivatives. The deactivation of ππ* and nπ* is affected distinctively by the solvent. Varying solvent from water to methanol accelerates slightly the decay of ππ* but retards strongly the deactivation of nπ* state (Scheme 2).



Scheme 2. Proposed nonradiative decay channels for (a) Cyd and N1 derivatives and (b) C5 derivatives in water (solid line) and in methanol (dash line). The time constants in (a) are from Cyt(dCyd) and in (b) from m5dCyd in #water and ##methanol. VR = vibrational relaxation. (c) Percentage contribution of the channels from ππ* ($a_{\pi\pi^*}\% = a_{FC}\% + a_{min}\%$) and nπ* ($a_{n\pi^*}\%$) state to the overall deactivation of the denoted compounds in water.

However, for the case of Cyt and N1 derivatives, to ascertain the details of the decay of ππ* and the origin of nπ*, it is instructive to examine any correlation between the fractions of S_0 recovery caused by the ππ* and nπ* ($a_{\pi\pi^*}\%$ and $a_{n\pi^*}\%$) versus those

involved in the bi-exponential decay of photo-prepared ππ* state (a_1% and a_2%) as revealed in the fs-TRF (Figures 2, S2, S3, and Table 1). It is noted in this regard that the $a_{\pi\pi^*}$% and $a_{n\pi^*}$% are both smaller than the fraction of ππ* population (a_1%~80-90%) deactivated at the time constant of τ_1~0.2-0.3 ps but are greater than those (a_2%~10-20%) at the time constant of τ_2~0.8-1.8 ps. This is indicative of two important features. *First*, the $\pi\pi^* \rightarrow S_0$ conversion is most likely occurring in two timescales from two different regions of the ππ* PES: one in the sub-picosecond timescale with the time constant of τ_1 from area close to the Franck-Condon region ($\pi\pi^*_{FC} \rightarrow S_0$), and the other is a sequential process which, as suggested by the DSS in fs-TRF, may proceed after relaxation of the initial ππ* to a local minimum ($\pi\pi^*_{FC} \rightarrow \pi\pi^*_{min}$)[6c] where the conversion ($\pi\pi^*_{min} \rightarrow S_0$) occurred at the time constant of τ_2. With the fraction in the $\pi\pi^*_{min} \rightarrow S_0$ channel (denoted as a_{min}%) having a value corresponding to the a_2% (i.e., a_{min}% = a_2%), the fractional population along the $\pi\pi^*_{FC} \rightarrow S_0$ channel (denoted as a_{FC}%) can be estimated (a_{FC}% = $a_{\pi\pi^*}$% − a_{min}%) to be ~64% for Cyt and ~38/54/42% for Cyd/dCyd/CMP in water (Table 1). The two ππ* channels, masked by the slower rate-determining energy relaxation of the vibrationally excited S_0,[1-2,5] manifested themselves as a single step to account for the first phase S_0 recovery as observed in the fs-TA. The *second* important feature is that, the longer-lived nπ* state, dark and not directly accessible by the photo-excitation, must become involved in the sub-picosecond timescale through coupling with the initial ππ* ($\pi\pi^*_{FC} \rightarrow n\pi^*$). This coupling competes with the channel of $\pi\pi^*_{FC} \rightarrow S_0$ and jointly (a_{FC}% + $a_{n\pi^*}$% ≈ a_1%) they were responsible for the massive decrease of the ππ* population observed at the time constant of τ_1~0.2-0.3 ps. Therefore, for all these compounds, the nonradiative decays, though mediated by the *two-state pathway,* occurred actually through *three-channels* leading from the $\pi\pi^*_{FC}$, $\pi\pi^*_{min}$, and nπ* state with the fractional contribution of a_{FC}%, a_{min}%, and $a_{n\pi^*}$% and the corresponding time constant of τ_1, τ_2, and τ_3, respectively (Scheme 2(a)). On the basis of this, the deactivation of Cyt and N1-derivatives is regarded

as following a *two-state-three-channel* model. Along this line, the deactivation of C5-derivatives is refereed to proceed through a *one-state-one-channel* model ($\pi\pi^*_{FC}\rightarrow \pi\pi^*_{min}\rightarrow S_0$) as diagrammed in the Scheme 2(b) and (c).

Whilst the operation of multiple decay channels from ππ* and nπ* has been accepted widely for Cyt in the gas phase,[7-11] the situation in the solution phase is much less understood. Our study, thanks to the ability to probe nearly all the possibly involved species (bright or dark) in solvents of varied polarity and H-bonding capacity, allows unveiling several unprecedented traits for the evolutions of excited states and roles of solvent and substitution in affecting distinctively the dynamics and pathways of Cyt nonradiative decays.

Nonradiative dynamics of Cyt

For Cyt, the *two-state-three-channel* pathway provides a unifying picture for explaining the bi-exponential dynamics of ππ* and the interplay of ππ* with nπ* to jointly account for the nonradiative decays in solution. The direct observation of nπ* state in the fs-TA (Figures 4-5) provides unambiguous evidence for clarifying the divergence in literature[2c,3] and verifies the involvement of the nπ* in the solution phase nonradiative deactivation. In addition, despite agreeing with earlier studies in regarding the presence of ππ* to S_0 conversion on the ~1 ps timescale,[2,3] our data show that such a process (corresponding to the $\pi\pi^*_{min}\rightarrow S_0$ channel) makes only limited contribution (a_{min}%~11%, Table 1) to the overall decay, while most of the conversion from ππ* to S_0 occurred actually in the sub-ps timescale through the direct channel $\pi\pi^*_{FC}\rightarrow S_0$ (a_{FC}%~64%, τ_1~0.2 ps) competing with the formation of nπ* state ($\pi\pi^*_{FC}\rightarrow n\pi^*$) which deactivated ($n\pi^*\rightarrow S_0$) on the much slower timescale from several to tens ps depending on the solvent.

One determining step in the *two-state-three-channel* model is the splitting in hundreds fs of the photo-prepared ππ* population into the S_0, the nπ*, and the structurally relaxed ππ* state (Scheme 2(a)). This in the other words means coexistence of multiple and nearly barrierless decay paths from the initial ππ* to the nπ* and the S_0, which is in principle consistent with the theoretical studies on Cyt in the gas phase showing the presence of several CIs connecting in nearly barrierless manner between the ππ*, nπ*, and S_0.[7,9-11] In this regard, the two ππ* channels in our model can be associated to the two mostly reported CIs with the out-of-plane twisting of C5-C6 bond and the semi-planar stretching of C5-C6 bond for the crossover from ππ* to S_0.[6,8,9]

On the other hand, between the two possible nπ* states having the lone pair electrons from the N3 ($n_N\pi^*$) and O7 ($n_O\pi^*$),[6c,d,h,7,9,16a] the state we observed is mostly likely corresponding to the $n_O\pi^*$. According to the theoretical studies,[9] the $n_O\pi^*$ at its energy minimum geometry is the lowest energy excited state in the singlet manifold and plays an important role in the deactivation of Cyt in the gas phase. The $n_N\pi^*$ state, lying already at higher energy (than both ππ* and $n_O\pi^*$) in the gas phase, is less probable to be involved in the decays in solution for the state is expected to be further destabilized by interaction with solvent in the solution phase.[1,4d-f,8a-b,14a,19] Pertinent to this, it is noted that, although originally ascribed to the $n_N\pi^*$,[3a] the nπ* state observed in the ps-TRIR for dCyd and dCMP in water was later re-assigned to the state of $n_O\pi^*$.[3b]

Occurring in the gas phase[7,9,12c] and also in the solution as demonstrated here, the multiple path nonradiative decay through ππ* and nπ* is clearly *an intrinsic property of Cyt regardless of the environmental condition*. Given that the ππ* and nπ* usually response very differently to solvent and covalent substitution, the data we obtained for Cyt in water provides a benchmark for assessing effects of solvent and the N1 and C5 substitution on the dynamics and pathways of the Cyt deactivation.

Effects of N1-substitution

An obvious result from comparing the data between Cyt and the N1 derivatives is that the substitution at N1 alone by ribose or deoxy-ribose with or without the phosphate moiety *makes no qualitative difference to* the overall pattern of Cyt nonradiative decay. All the examined N1 derivatives share with Cyt the *two-state-three-channel* pathway (Scheme 2). This is in contrast with ps-TRIR studies[3] which reported deactivation through both the ππ* and nπ* for the N1 derivatives (dCyt and dCMP) but through only the ππ* state for the case of Cyt. According to our data, the N1 substitution causes important *but only quantitative* changes to the deactivation of Cyt.

The most significant change arising due to the N1 substitution is that it affected the ultrafast (in ~0.2 ps) branching of the initial ππ* population, favouring the channel of nπ* state. This is manifested by the increase in both the efficiency ($a_{n\pi^*}$%) and the lifetime (τ_3) of the nπ* channel from such as ~25% and 7.7 ps in Cyt to ~33-49% and ~30-35 ps in the N1 derivatives. This observation is consistent with the general trend reported for the pyrimidine bases in water.[2c] However, as a novel feature, our data shows that the increase of nπ* efficiency occurs since the N1 substitution suppresses effectively the ultrafast $\pi\pi^*_{FC} \rightarrow S_0$ channel (a_{FC}% ~64% in Cyt to 38-52% in N1 derivatives, Table 1) allowing enhanced formation of the nπ* in as early as ~200 fs after the excitation. The long lifetime of nπ* implies the nπ* possessing a well defined energy minimum separated by a sizeable energy barrier from the S_0. The lengthening of nπ* lifetime reflects that the N1 substitution raised the barrier height and slowed down the barrier crossing to the S_0. With the N1 substitution to strengthen its the ultrafast coupling with the initial ππ* and at the same time to retard it from deactivated into the S_0, the nπ* of N1-derivatives is clearly a more effective trap to the excitation than that of Cyt, implying an important role of the nπ* in the photostability and photochemistry of DNA and RNA.

In contrast to the nπ* state, the ππ* state exhibited decays with the rates fairly similar between Cyt and the N1 derivatives. This suggests very limited influence of the N1 substitution on the ππ*/S_0 crossover.

The distinctive influence of N1 substitution on the nπ* *vs* ππ* state suggests that the decays into S_0 from the two states must proceed through different nuclear coordinates especially in terms of the atoms involved in the ribose or deoxyribose moiety. In spite of the large number of sophisticated theoretical studies on the nonradiative dynamics of Cyt,[6-9,17] there has been very limited study on its N1 derivatives[20] and no known comparable work on the nucleosides and nucleotides of Cyt in either the gas phase or solution to the best of our knowledge.

Effects of C5-substitution

According to our results, the presence of an extra methyl at the C5 position removes completely both the ultrafast ππ* channel ($\pi\pi^*_{FC}\rightarrow S_0$) and the channel due to the nπ* state ($n\pi^*\rightarrow S_0$). The substitution, on the other hand, prolongs substantially (by ~4-5 times) the decay of the stepwise ππ* channel ($\pi\pi^*_{FC}\rightarrow\pi\pi^*_{min}\rightarrow S_0$), making it the sole path for transferring the population from excited state into the S_0 (Scheme 2(b)). Making correlation of the dynamics between fs-TRF and fs-TA shows explicitly that the complex fluorescence decays reported previously for m5dCyd in water,[4c] which is similarly observed in our measurement for both m5dCyd in methanol and m5Cyd in both water and methanol, is actually occurring due to relaxation of the excited state population within the ππ* state, bearing no relevance to the state of nπ*. In this regard, instead of the substitution at N1, it is the C5 substitution that governs the participation or not of the nπ* state in the nonradiative decays. The *one-state-one-channel* pathway adopted by m5Cyd and m5dCyd can therefore be considered a special case of the *two-state-three-channel*

pathway by Cyt and N1 derivatives, emphasizing a key role of the C5 substitution in altering the fundamental pattern of the nonradiative decay.

The slower decay of ππ* in C5 derivatives provides explanation to the higher fluorescence quantum yield of the C5 derivatives than those of Cyt and the N1 derivatives (Table 1) and is supported by theoretical studies showing the involvement of the C5 atom in the ππ*/S_0 conversion. Indeed, as mentioned above, the C5 involved out-of-planar C5-C6 twisting or semi-planar C5-C6 stretching is among the most relevant reaction coordinates for achieving the switch from ππ* to S_0. The methyl at C5, presumably through the hyper-conjugation with the pyrimidine ring π system,[4d,4f,21] may impose restraint to the required change in the C5-C6 bond and slow down the access to related CIs(ππ*/S_0) for the state conversion.

As for the elimination of nπ* channel in the C5 derivatives, while this may likewise be ascribed to the C5 atom being also involved significantly in the nuclear coordinates for the ππ*/nπ* coupling, an alternative explanation is that the methyl at C5 may cause a preferential stabilization of the ππ* to nπ*, which may in turn shift the relative energy or even reverse the energetic ordering of the two states, leaving the nπ* at relatively high energy, unavailable for the decay process. Indeed, the steady state absorption and fluorescence of the C5 derivatives are substantially red-shifted from those of Cyt and the N1-derivatives (Figure 1 and Table 1), suggesting energy stabilization of the ππ* by the methyl at C5 position. Further study in particular at the theoretical perspective would help in assessing the nuclear coordinates for the lack of nπ* state in the deactivation of C5 derivatives.

It is remarkable to note that the wavelength red-shift in absorption of C5-derivaitves in relative to Cyt and the N1 derivatives has been invoked responsible for the increased mutation rate in the m5dCyd containing DNAs, a phenomenon relevant to the DNA

methylation and the mutation hotspot in skin tumors.[2b,4c,13] A recent study suggests, however, that the presence of extra methyl at the C5 position may alter the conformation of DNA and as a result affecting the reactivity of ππ* which may lead to an increased yield of cyclobutane dimers (CPDs),[22] a major photoproducts for inducing the skin tumors.[13] Given the absence of the nπ* and the ππ* being the intimate precursor to the CPDs formation, the longer ππ* lifetime we observed with the C5 derivatives could be another factor contributing to the greater yield of CPDs and the elevated mutation rate in the methylated DNA.

Effects of solvent

The variations in the decay dynamics we observed for Cyt and the derivatives in water *vs* in methanol exemplify a striking *state specific effect* of the solvent on nonradiative decays. For all the compounds investigated, without affecting the overall deactivation pattern, changing solvent from water to methanol accelerates slightly the rate of ππ* channel ($\pi\pi^*_{min} \rightarrow S_0$, τ_2). For Cyt and the N1 derivatives, this change of solvent also retards strongly the decay of nπ*, extending its lifetime by ~4-6 folds, from e.g. ~7.7 ps (in water) to ~52 ps (in methanol) for Cyt and ~30 ps (in water) to ~186 ps (in methanol) for dCyd.

The effect of solvent we observed with the ππ* state suggests that the $\pi\pi^*_{min} \rightarrow S_0$ conversion, albeit being a feasible low or nearly barrierless process as indicated by its rapid rate,[1a,c,6-9] is less favoured to a subtle but definite degree in the highly polar water (dielectric constant ε = 78.3) than in methanol (ε = 32.7). This may originate from a different influence of the solvent on the energy of ππ* in relative to the S_0. According to the steady state spectra (Figures 1 and S1, Table 1), the absorption (of all the compounds) and fluorescence (of Cyt and the N1 derivatives) of the ππ* state both exhibit bathchromic

shifts in solvent from water to methanol. This implies that the ππ* is energetically better stabilized than the S_0 upon decreasing the solvent polarity, which is indicative of the ππ* possessing a dipole monument smaller than the S_0,[19] supported by the theoretical calculation on solvatochromic shift of Cyt.[8b] The better stabilization of ππ* in the less polar methanol may not only occur in the FC region to account for the lower optical transition energy to or from the S_0, it may also affect the location and topography of the CI (ππ*/S_0), contributing to the slightly faster conversion rate than that in water (Scheme 2). The fact that all the compounds show similar effect of solvent on the sequential ππ* decay suggests that the related CI (ππ*/S_0) may feature similar electronic and conformation structure irrespective of the N1 and C5 substitution.

It is relevant to note that, for all the compounds in both water and methanol, the fs-TRF (Figures 2 and S2-3) exhibited DSS in time scale associated mainly with the τ_1 (~0.2 for Cyt and the N1-derivaitves and ~0.6 ps for the C5-derivaitves). According to the literatures,[14,15,23] the time-dependent shifts of fluorescence in such time regimes may arise from an ensemble of processes including such as the intramolecular vibrational relaxation, structural relaxation, and dynamic solvation due to reorientation of solvent molecules towards the newly formed excited state (the ππ* in the current case). However, in view of that the extents of DSS are independent of the solvent (being ~10 cm^{-1} in both water and methanol) and the timescales of DDS are either much slower or much faster than the time of solvation (~50 fs for water and ~13 ps for methanol),[23] the dynamic solvation is considered playing a minute role in contributing to the time-dependent fluorescence red-shifts exhibited by Cyt and all the derivative. On the other hand, as mentioned above and supported by theoretical studies,[6-11] the DSS we observed may arise mainly due to the structural changes of ππ* state upon evolving from the FC region towards the local energy minimum of its PES. This is consistent with the recent fs fluorescence investigation on the nucleosides and nucleotides of guanine which reported

similar time-dependent fluorescence red-shifts attributed to conformation relaxation and diffusion of excited state population along the PES of the ππ* state.[4g,5c]

Unlike the ππ* state which is affected mainly by the electrostatic interaction with solvent, the state of nπ* is energetically sensitive to both the nonspecific electrostatic interaction and more importantly the specific H-bonding with solvent molecules which may typically destabilize the nπ* in relative to S_0.[4d,4e,4f,19,24] Compared to water, methanol is much less polar and with weaker capacity of H-bonding. The nπ* state at its energy minimum is thus expected to be better stabilized and thereby associate with a greater barrier height for the nπ*/S_0 crossover in methanol than in water, explaining the longer lifetimes of the nπ* in the former than latter solvent (Scheme 2).

It is worth noticing that, for Cyt, the change of solvent (from water to methanol) and the presence of N1 substitution appear to have similar effects on the dynamics of nπ*, both extending the lifetime of nπ* and to comparable degrees. Instead of being just a coincidence, this might suggest a certain interplay between the solvent and the N1 substitution in affecting the nπ*/S_0 coupling. Indeed, the ribose or deoxyribose group, which is sterically bulky, can itself interact extensively with the solvent through both the electrostatic and H-binding interactions.[25] The extra attachment of this group at the N1 position may thus disturb the microscopic solvation configuration of the pyrimidine moiety, reducing its exposure and weakening its interaction to the solvent. As a result, even in the same solvent, the local solvation experienced by the nπ* in the N1 derivatives could be less intense than that in Cyt, and thus the nπ* in the former compounds is better stabilized, contributing to its longer lifetime than in Cyt. Note that, although the involvement of low lying CI(nπ*/S_0) has been suggested in many theoretical studies for Cyt in the gas phase,[7,9,6] the feasibility of the nπ*/S_0 coupling for Cyt in solution of varied properties and how this is affected by the N1-substitution have remained largely unknown before this work.

CONCLUSION

The excited states of Cyt and the full set of its DNA and RNA nucleosides and nucleotides as well as the C5 methylated nucleosides were studied using fs-TRF, fs-TRFA, and fs-TA in conjunction with steady state absorption and fluorescence in water and methanol. The results clarified the uncertainty on the nπ* state and revealed a common *two-state-three-channel* pathway from the ππ* and nπ* for nonradiative decays of Cyt and its N1 derivative in both water and methanol. A *one-state-one-channel* pathway from the ππ* state was identified for the decays of C5 derivatives. Comparison of the data obtained for Cyt and the derivatives in the different solvents demonstrated remarkable effects of solvent and the N1 and C5 substitution in shaping distinctively the nonadiabatic couplings between the ππ*, the nπ*, and the S_0 for deactivation in timescale varying from sub-ps to hundreds ps after the excitation. The data we obtained allows improved characterization of the photophysics and provide direct experimental parameters for development of theoretical modelling on influences of solvent and substitution on the topographies of the excited state PESs and dynamics of the nonradiative decays. The results may also help understanding the photostability and photodamage of Cyt and the derivatives. In particular, given the widely documented sub-ps to ps excited state lifetimes of the nucleobases, the ~186/144 ps lifetime we observed with the nπ* state of dCyd/Cyd in methanol is an unusual feature. While such a long lifetime may certainly increase the propensity towards photochemical modification, the nπ* state might play a minimal role in the photochemistry but on the other hand may contribute importantly to the photostability of DNA in its canonical form. This is inferred since the canonical DNA is generically much less prone to photodamage than their methylated counterpart where the contained m5dCyd component, according to our result, possesses a longer-lived ππ* state but with no involvement of the nπ* state during the course of excited state decay.

Supporting Information

Details of the materials and the time-resolved measurements and spectral correction for fs-TRF, fs-TRFA, and fs-TA; steady state absorption and fluorescence spectra of Cyt and its derivatives in water; fs-TRF spectra and TRF decay profiles for dCyd, dCMP and m5dCyd in water and for Cyt, Cyd, dCyd, m5Cyd, and m5dCyd in methanol; fs-TRFA spectra for Cyt, Cyd, dCyd, CMP, dCMP, m5Cyd, and m5dCyd in water and methanol; fs-TA spectra and TA time profiles of Cyt, Cyd, dCyd and m5Cyd in water and methanol and of dCyd, dCMP and m5dCyd in water; detailed spectral and dynamic parameters obtained from the fs-TRF, fs-TRFA and fs-TA measurements for Cyt and its N1- and C5-substituted derivatives in water and methanol. This material is available free of charge via the Internet at http://dx.doi.org.

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