Femtosecond fluorescence studies of DNA/RNA constituents

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Abstract. In this overview, femtosecond fluorescence studies of various DNA constituents are presented, ranging from the monomeric chromophores to different model helices. In order to interpret the experimental results in terms of fundamental processes on the molecular scale they are discussed in the light of recent theoretical calculations. The ultrafast fluorescence decay observed for the monomers is explained by the involvement of highly efficient conical intersections (CI) between the first singlet excited state and the ground state. For the model helices, the picture is more complex, but fluorescence anisotropy data reveal collective effects.

1. Introduction

The interaction between irradiation and nucleic acids (DNA/RNA) is currently a subject of intense research. Direct irradiation-triggered as well as indirect radical-induced chemical alterations of the double helix may lead to the formation of mutations and ultimately cancer. A central but badly elucidated role in the chain of events leading up to the biological consequences is played by the initially electronically excited states of nucleic acids. These transient states have the hard task to handle the excess energy in such a way to minimize damage.

Since a few years we focus our research on the directly UV-excited electronic states in nucleic acids in order to characterize their structure and dynamics with regards to their reactivity. To probe the initially populated states, we have developed specific time-resolved fluorescence spectroscopic techniques covering a very large temporal domain, from ≈100 femtoseconds to the ≈100 nanoseconds.

Time-resolved fluorescence is a powerful tool to study molecular excited state dynamics. In principle, when the fluorescence arises from a well defined excited state, its intensity reflects directly the excited state population. In most molecules, and for nucleic acids in particular, the excited state population decay is ruled by non-radiative deactivation processes, so by measuring the fluorescence decay one characterizes the non-radiative mechanisms. In addition, valuable information can be obtained from the fluorescence anisotropy decays, which not only reflect the molecular reorientational dynamics, but also informs on ultrafast changes of the electronic structure. Comparison of the experimental results with theoretical models is necessary for the understanding of the underlying non-radiative processes.

However, DNA and RNA are very complex organized molecular systems even though composed of only four basic monomeric chromophores; two purines (adenine, A, and guanine, G) and two pyrimidines (cytosine, C and thymine/uracil, T/U), see figure 1. A huge amount of experimental and computational studies has thus been devoted during the last few years to the monomeric chromophores; the nucleic acid bases, nucleosides and nucleotides (NABs) of DNA/RNA. An

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important, but less abundant, number of studies treat simple model helices. Instructive overviews can be found in refs. [1-6].

In this article we will briefly overview recent time-resolved fluorescence studies of NABs in room-temperature solution performed with femtosecond time-resolution, either by fluorescence upconversion (FU) or by optical Kerr gating (OKG). We examine in more detail the behavior of the monomeric building blocks, whose fluorescence lifetime is extremely short and we tackle that of model helices. As a matter of fact, the fluorescence of model helices contains important nanosecond components and femtosecond measurements need to be combined with data obtained by other techniques, for example time-correlated single photon counting (TCSPC) [7-10]. However, this longer-lived fluorescence is outside the scope of the present article.

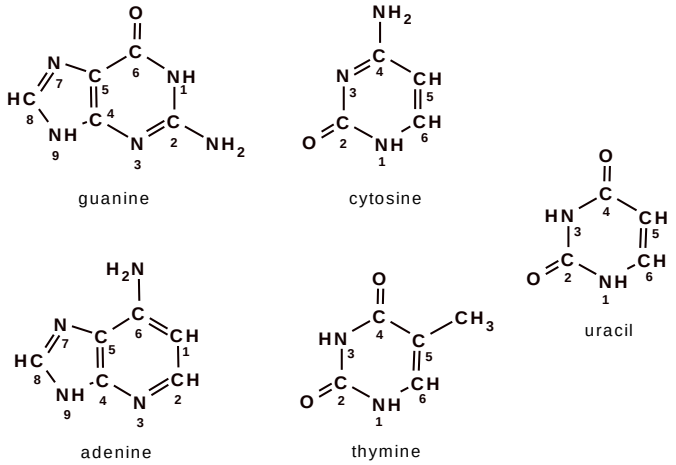

Figure 1. Structures of the most common naturally occurring nucleic acid bases.

2. Monomeric chromophores

Information regarding the initially excited Franck-Condon states can be deduced already from the absorption spectra which peak close to 260 nm for all the monomeric NABs. Analysis of the absorption spectra in combination with theoretical calculations indicate that for the pyrimidines there is only one strong $\pi\pi^*$ transition in the first UV band, while for the purines there are two close-lying $\pi\pi^*$ states, (L_b and L_a), which is clearly visible for guanine [1]. For all these heterocyclic molecules the possible presence of low-lying "dark" $n\pi^*$ states has played an important role in the discussions regarding the excited state dynamics.

Relying on the steady-state quantum yield measurements it was deduced already back in the 70's that the fluorescence lifetimes of the monomeric NABs in room-temperature water solution were on the order of a picosecond or less [11], implying extremely efficient internal conversion processes. Such short times eluded direct measurements for many years until femtosecond spectroscopic techniques became available.

During the last ten years, numerous femtosecond fluorescence studies have been performed on the monomeric chromophores in room-temperature water solution. These have been performed either by FU [12-20] or by OKG [21,22]. They all agree in assigning sub-picosecond lifetimes to the bright excited states. A comparison of the fluorescence decays for the four mononucleotides is given in Figure 1.

We would like to stress the fact that the fluorescence decays of all NABs are strongly non-exponential, which can be seen in figure 2. This non-exponentiality indicates the presence of complex and/or multiple excited state relaxation processes. Moreover, there is a marked difference in the decay between the nucleoside and the nucleotide for the pyrimidines while it is negligible for the purines

[15]. This should be considered in view of the fact that the steady-state fluorescence spectra of nucleosides and nucleotides are identical. In the following we will rapidly look through the available experimental data without going into details, which will be further discussed below when reviewing the theoretical works.

The fluorescence decay of uracil is by far the fastest of the natural NABs [18]. In practice, its determination is limited by the experimental time-resolution, which is about 100 fs, meaning that it could be substantially faster. The fluorescence decays of the two other pyrimidines, thymine and cytosine, are substantially longer.

Concerning thymine [13,15,18,22], bi-exponential fitting gives an ultrafast component, ranging between 150 and 300 femtoseconds, followed by a slower component around one picosecond having much lower amplitude. In their broadband femtosecond Kerr-gate study of thymidine, Kwok *et al.* [22] assigned the origin of the bi-exponential decays to the existence of an intermediate state favoring the excited state deactivation. This so-called "doorway" state ("doorway" since it leads either to the ground state or the triplet state) could be the dark $^1n\pi^*$ state mixing with the close-lying $^1\pi\pi^*$ state.

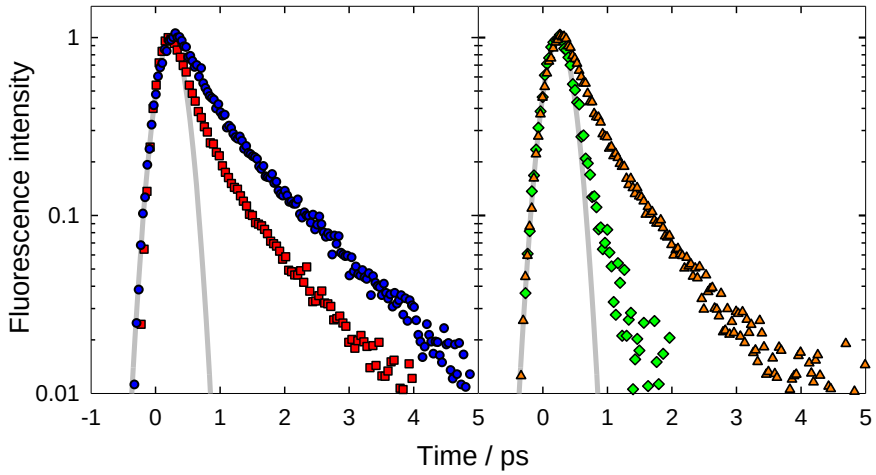

Figure 2. Total fluorescence decays at 330 nm for the four nucleotides, dCMP (red squares), TMP (blue circles) dAMP (green diamonds) and dGMP (orange triangles), after excitation at 267 nm. Also shown is the 330 fs (fwhm) instrument response function (gray).

Likewise, for cytosine, the fluorescence decays show an ultrafast component of 0.2-0.4 ps and a slower one [15,16,19]. This slower component is about one picosecond for the nucleoside and 1.4-1.7 ps for the nucleotide. Schwalb *et al.* [19] evoked different internal conversion pathways as the origin for the two decay times, the slower involving a dark $^1n\pi^*$ state.

The fluorescence decays of adenosine and adenosine monophosphate, recorded either by FU or OKG, are nearly as fast as that of uracil [12,14,15,21]. Actually, a bi-exponential fit gives a fast component of about 100 fs and longer one of a few hundred femtoseconds [15,21]. Kwok *et al.* associated the bi-exponentially with the L_a and L_b $^1\pi\pi^*$ excited states, respectively. The fast component should correspond to the L_a - L_b internal conversion. It is worth mentioning that the fluorescence decay of adenine is much slower than those of dA and dAMP, with a long component of about 8 ps and a faster one of a few hundred femtoseconds [14,17]. The fast component was assigned to the canonical 9H tautomer while the long one was assigned to the 7H tautomer. Adenine is the only NAB for which different tautomeric forms have been shown to be present in aqueous solution [23].

Regarding the guanine chromophore, the base itself (guanine) is not soluble enough in water so there are only data for the nucleoside and the nucleotide. It is characterized by a very broad steady-

state fluorescence spectrum, extending beyond 700 nm [15]. Contrary to the other monomeric NABs, the fluorescence decays depend strongly on the wavelength, indicating important spectral shifts [20]. For dGMP, the fast component varies between and the slow one between 1 and 4 ps going from 310 to 600 nm. This cannot be explained by solvation dynamics, but rather as due to diffusive wave packet dynamics on a flat excited state surface, in accordance with recent theoretical calculations [24]. (See also below.)

One may resume the results on the NABs as follows. The fluorescence decays are clearly non-exponential but can be approximately described by an ultrafast component (≈100 fs) and a slower one (ranging from about 0.4 ps for 2'-deoxyadenosine up to 1.7 ps in the case of cytidine monophosphate [19]). It is worthwhile to mention that there are no "long" components (> 3 ps) in the fluorescence decays of monomeric NABs in water solution. As described below, much longer fluorescence decays have been observed in chloroform solution. [25] A brief review of our results on NAB fluorescence lifetimes in water is given in Table 1.

Table 1. Fluorescence lifetimes (ps) of NABs measured at 330 nm in aqueous solution. They were obtained from fits with bi-exponential functions; $\alpha\exp(-t/\tau_1) + (1-\alpha)\exp(-t/\tau_2)$

compound	τ_1	τ_2	α	$\langle\tau\rangle$ [a]	ref.
Ura	0.10±0.01[b]				[18]
Thy	0.20±0.02	0.63±0.02	0.56±0.02	0.39 ± 0.02	[18]
dT	0.15±0.02	0.72±0.03	0.70±0.02	0.32 ± 0.01	[15]
TMP	0.21±0.03	1.07±0.06	0.67±0.02	0.50 ± 0.02	[15]
Cyt	0.20±0.02	1.30±0.07	0.85±0.02	0.37 ± 0.01	[16]
dC	0.18±0.02	0.92±0.06	0.83±0.02	0.30 ± 0.01	[15]
dCMP	0.27±0.02	1.38±0.11	0.84±0.02	0.45 ± 0.02	[15]
Ade	0.23±0.05	8.0±0.3	0.65 ± 0.05	2.95	[14]
dA	0.10±0.01[b]	0.42 ± 0.10	0.91 ± 0.01	0.13 ± 0.01	[15]
dAMP	0.10±0.01[b]	0.52 ± 0.10	0.94 ± 0.02	0.13 ± 0.01	[15]
dG	0.16±0.02	0.78±0.05	0.73±0.02	0.33 ± 0.01	[15]
dGMP	0.16±0.02 - 0.29±0.08[c]	0.94±0.09 - 4.0±1.0[c]			[20]

[a] $\langle\tau\rangle = \alpha\tau_1 + (1 - \alpha)\tau_2$,
[b] limited by the time-resolution after deconvolution,
[c] varying with the emission wavelength (310 - 600 nm).

The central question is of course what mechanism is responsible for the ultrafast internal conversion from the bright $^1\pi\pi^*$ state to the ground state for each of the monomeric NABs. Are there common features or are the processes involved unique for each molecule? In order to elucidate these questions, experimental studies of substituent and solvent effects in combination with high-level quantum chemistry calculations have proven to be essential.

2.1. Substitution effects

In addition to the studies of the naturally occurring RNA/DNA bases described above, various substituted bases have been characterized by femtosecond fluorescence spectroscopy. Among all the possible side group substitutions, methylation is particularly important since some types of

methylation occur naturally. In this respect, thymine is a 5-methylated form of the RNA base uracil. Replacing the hydrogen in position 5 on uracil by a methyl group, as is the case of thymine, increases the fluorescence lifetime from less than 100 fs to about 400 fs [18]. This increase may seem minor, but substituent effects are of course very complex, not only the local chemical bond is changed but the electronic structure of the excited state may differ. Another and very particular case of methylation is 5-methyl-2'-deoxycytidine (5MdC), which is known to play a key role in biological functions [26]. Compared to natural cytosine, methylation causes a huge increase in fluorescence lifetime. The mean decay time for the 5MdC excited state is about one order of magnitude longer than for dC [27]. Interestingly, the fluorescence decays of 5MdC show strong wavelength dependence, which indicates complex excited state dynamics involving multiple states and/or species.

The effect of methylation is illustrated in figure 3 where the fluorescence decays at 330 nm of uracil and thymine as well as dC and 5MdC are shown. The decay of uracil is limited by the instrument response function while the two traces of thymine and dC are practically identical. The fluorescence decay of 5MdC is by far the longest.

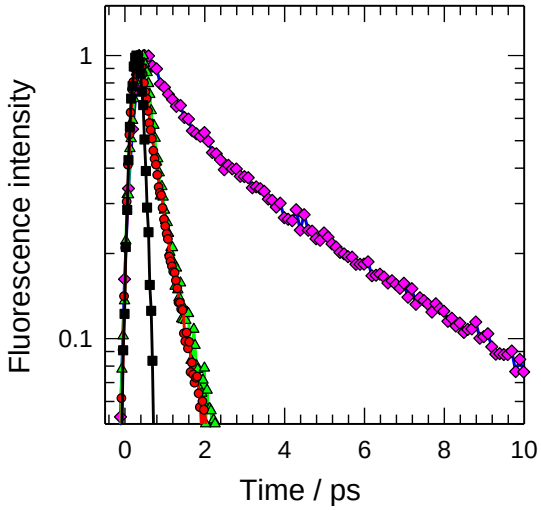

Figure 3. Methylation effect; uracil (black squares) and thymine (red circles), dC (green triangles) and 5MdC (pink diamonds) at 330 nm.

Performing systematic studies of substituent effects is therefore a very powerful tool to gather information about the electronic structure of the excited state that can be compared with predictions from quantum chemistry calculations. (see below)

We have performed such a systematic study of substituent effects in uracil [18,28-30], by using methyled, halogenated uracils and other modifications. Methylation at positions 1, 3 or 6 has no observable effect on the fluorescence lifetime; the fluorescence decays for these compounds are as fast as for uracil itself. Only methylation at position 5 (5-methyluracil = thymine) has significant effect, increasing the average lifetime by a factor of four. Moreover, it was found that the nature of the substituent in the 5-position influences strongly the fluorescence lifetime. For 5-fluorouracil, a mean lifetime of 1.3 ps is observed, more than ten times longer than that of uracil itself. Based on this study, it was shown that the excited state deactivation mechanism of uracil involves an important displacement of the 5-substituent. In parallel, a picosecond fluorescence study of a "blocked" uracil, 5,6-trimethyleneuracil, showed that such geometrical constraints has a dramatic effect, leading to a substantially longer fluorescence lifetime [31,32]. The authors proposed that the reaction path involves motion of both the 5- and the 6-substituent.

As in the case of uracils, the nature and position of substituents may strongly influence the electronic structure of the excited states and thus the fluorescence properties also for other NABs. In this context, the amino group has a particular role in the structure and dynamics of the NABs. It is

naturally present in the cytosine, adenine and guanine chromophores, but not in uracil/thymine. Still all these chromophores are characterized by very short fluorescence lifetimes. What is more important is that the actual position of the amino group strongly influences the excited state properties as shown in the case of two aminouracils [33]. Both 5- and 6-aminouracil have much red-shifted absorption and fluorescence spectra, indicating that the amino-group influences the electronic structure. Interestingly, amino substitution on the 6-position does no affect the lifetime, which remains as short as for uracil, while amino substitution on the 5-position leads to a significant increase in lifetime. Moreover, the fluorescence decays of 5AU depend strongly on the emission wavelength, indicating a complex excited state relaxation mechanism[34].

As described above, the adenine chromophore (6-aminopurine) has a very short fluorescence lifetime while in the case of N,N-dimethyladenine the fluorescence is much longer-lived and extends into the visible region. A global analysis of fluorescence upconversion data produced 5 different time constants ranging between 100 fs and 62 ps [35,36]. These very complex dynamics were interpreted in terms of a 4-state model including $^1\pi\pi^*$ (L_a), $^1\pi\pi^*$ (L_b), $^1n\pi^*$ as well as an intramolecular charge-transfer state.

2-aminopurine (2AP), which is a structural analog to adenine, is highly fluorescent with a 11.8 ns lifetime [37]. 2AP was studied in water and in ethanol by FU, indicating important dynamical spectral shifts which were assigned to solvation dynamics [38]. More interestingly, 2-aminopurine can form a Watson-Crick base pair with thymine and thus be incorporated naturally in a DNA double helix and is used as a fluorescent probe to follow its conformational dynamics on longer times [39]. However, ultrafast charge transfer in 2AP modified DNA sequences has been observed by FU [40]. The 2AP fluorescence and fluorescence anisotropy have also been used to follow rapid RNA conformational dynamics [41].

2.2. Solvent effects

We now turn to the issue of solvent effects. With this we understand local environmental effects on the fluorescence properties of the monomeric NABs. This issue is not limited to the understanding of the photophysics of individual NABs but is related to a more fundamental question; to what extent the electronic structure of UV excited DNA can be considered "monomeric". For a given monomeric chromophore, the surrounding bases in the DNA helix may constitute a "solvent". Of course, this local environment is very different from water, the solvent in which the large majority of spectroscopic investigations of NABs have been performed. For these reasons, it is of great interest to characterize the NAB fluorescence in different solvents of different polarity, hydrogen bonding capacity and viscosity.

Solvent effects may be "static", in the sense that the solvent influences the energetic ordering of the molecular excited states, but they may also be dynamic, i.e. how the dipolar solute-solvent interaction evolves with solvent rearrangement. Based on the fact that the NABs excited state lifetimes are probably faster than the solvent response, the dynamic effect of the solvent on the singlet excited state relaxation processes was up to recently thought to be "quite modest" [1]. This may be true for "slower" solvents such as alcohols, while for water the solvation dynamics are probably too fast for the time-resolution available with presently used experimental setups. Solvation dynamics in water is dominated by an ultrafast "inertial" component of only a few tens of femtoseconds, although it also contains a slower picosecond component of low amplitude [42]. Indeed, spectral shifts due to solvation dynamics have not been reported for any of the natural NABs in water.

For some modified bases, for example 5MdC [27] and 5AU [33], which have much longer excited state lifetimes, important dynamical spectral shifts have been observed in water, but it is today not clear to what extent these may be linked to the solvent response. It is rather believed that they are due to intramolecular processes. A clear example is the case of 2-aminopurine [38], for which dynamical spectral shifts (0.2 and 0.9 ps in water) were observed and assigned to solvation dynamics.

However, the solvent does not only influence the excited state dynamics but may also directly affect their relative energy ordering (static effect), and thus radically change the photophysical properties of the chromophore.

In our group, we have studied how the fluorescence properties of chosen uracils depend on the solvent [28-30,43]. An example is given in figure 4, where the fluorescence decays of thymine and 5-fluorouracil in acetonitrile, methanol and water are compared.

As can be seen in figure 4, there are significant differences in decay times for a given chromophore in different solvents. The decays observed in aprotic acetonitrile are the fastest while those in protic water are the slowest. This phenomenon cannot be explained in simple terms of macroscopic solvent properties such as polarity or viscosity, but is instead related to how the solute-solvent interaction (dipolar, H-bonding) fine-tunes the energetic ordering of the chromophore's excited states. Briefly, the relative positioning of a "dark" nπ* state with regards to the bright ππ* state may vary with the solvent. If it is situated below the ππ* state it will accelerate the apparent deactivation rate for which the internal conversion rate is already very fast.

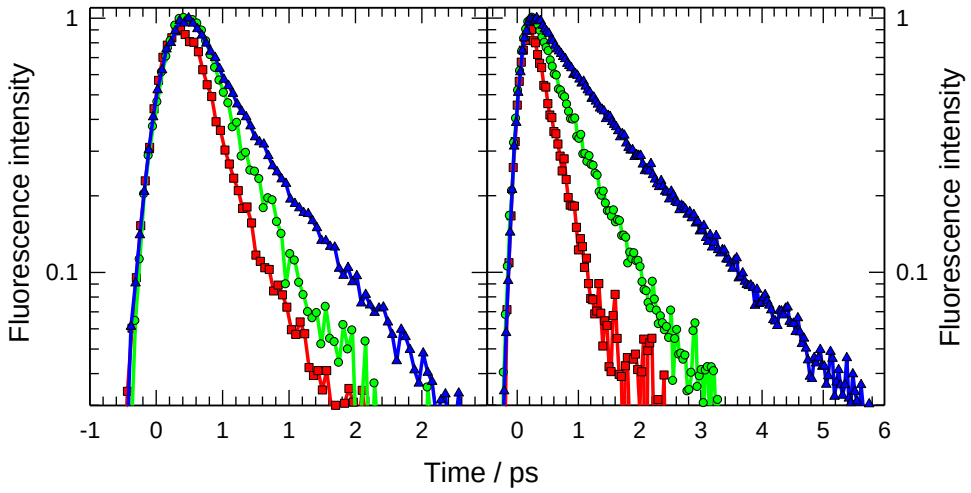

Figure 4. Fluorescence decays at 330 nm of thymine (left) and 5-fluorouracil (right) in acetonitrile (red squares), methanol (green circles) and water (blue triangles) after excitation at 267 nm.

In a fluorescence upconversion study of modified cytidine and guanosine in chloroform solution after excitation at 283 nm, Schwalb and Temps measured much more complex decays than what was observed in water [25]. For both compounds, ultrafast components comparable to those in water, were observed but also much longer ones; for cytidine 21 ps and for guanosine 7 ps as well as low amplitude component of hundreds of picoseconds. The long-lived components were assigned to nπ* states, since these are expected to be more stable than the directly excited ππ* state in chloroform. [25] As these molecules were subtituted with a tert-butyldimethylsilyl group instead of the deoxyribose group to render them soluble in chloroform, it is not straightforward to compare these results with those obtained in aqueous solution using natural cytidine and guanosine.

In their studies of N,N-dimethyladenine, Schwalb and Temps compared the excited state charge transfer dynamics in water to those in dioxane [35,36]. The much longer lifetime of the ICT state in dioxane (1.4 ns) than that in water (62 ps) was taken as an indication that the deactivation mechanism is strongly affected by the solvent.

The very low fluorescence quantum yields and the ultrafast fluorescence decays of the NABs imply the presence of highly efficient nonradiative deactivation processes taking place in the first excited singlet state. A deeper understanding of these processes can only be obtained through high-level theoretical calculations. Consequently, many theoretical studies based on quantum chemistry

calculations have been dedicated to uracil/thymine [18,31,44-55], cytosine [32,47,56-67], adenine [46,51,68-78] and guanine [24,79-86].

2.3. The non-radiative deactivation mechanism

The picture emerging is that the ultrafast decay of the excited state is due to highly efficient conical intersections (CI) between the first singlet excited state and the ground state. These CIs are related to conformational changes taking place in the "bright" $^1\pi\pi^*$ state. Obviously, the geometrical changes involved in the CIs differ from one molecule to the other. As indicated above, a complication is the fact that the deactivation processes may involve near-lying "dark" state(s), for example of $^1n\pi^*$ character, which are basically inaccessible by fluorescence spectroscopy. Moreover, most theoretical work has been performed in vacuum, while this article focuses on solution phase fluorescence. Since the relative energy ordering of the various excited states is very sensitive to the environment, a direct comparison between theory and experiment is not always pertinent.

From a theoretical point of view, an instructive comparison of the nonradiative processes involved in the excited state deactivation for the NABs has recently been given by Serrano-Andres and Merchan [87].

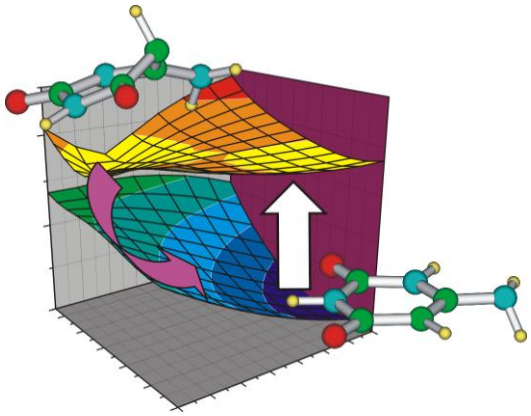

Figure 5. Proposed deactivation mechanism for uracil derivatives. The figure depicts actually 6-aminouracil, for which more details can be found in ref. [34]

Generally speaking, one may say that for the pyrimidines, the internal conversion through the CI involves a pyramidalization of C_5 and a torsion of the C_5C_6 bond associated with an out-of-plane motion of the 5-substituent. This is valid both for uracil/thymine [18,48] and cytosine [65]. In figure 5 this reaction is schematically shown for 6-aminouracil. However, alternative quantum chemistry calculations indicate that not only the 5-substituent but also the 6-substituent are moving out of the molecular plane forming a biradical state [31,32].

For the purines, on the other hand, the internal conversion from the first excited $^1\pi\pi^*$ state of the ground state involves a twisting around the C_2N_3 bond provoking an out-of-plane bending of the 2-substituent. For guanine the CI to the ground state can be described as an out-of-plane distortion of the C_2 center [83]. The observed bi-exponential excited state dynamics has been attributed to the wavepacket motion along a barrierless pathway on a "flat" $^1\pi\pi^*$ state surface towards the CI [24]. Also for adenine calculations indicate that the CI is reached via a relatively flat energy region before the hydrogen atom in the 2-position moves out of the molecular plane [78].

Such a general description is bound to be oversimplified, and a very active debate exists regarding the details of the decay mechanism for each individual base. It is important to underline that eventhough the main features seem to be well understood, many aspects still need further theoretical considerations, such as the actual shape of the excited state potential energy surface along the reaction

coordinate towards the CI. Needless to say, a correct description can only be obtained by correctly incorporating solvent effects. Finally, to describe the excited state evolution quantitatively and thus enable comparisons with measured lifetimes, quantum dynamics calculations will be required. Such computations are beginning to appear only now [49,55,66,88,89].

3. DNA helices

The time-evolution of the fluorescence of DNA model helices [7-10,90-94] as well as that of natural DNA [95] is different from those of their monomeric constituents. This concerns in particular the fluorescence lifetimes but in many cases also the fluorescence spectra. At this point, let us rapidly side-step a bit to note that already from steady-state absorption spectra there is strong evidence that the first UV band corresponds to collective excitations, i.e. excitonic states that are populated directly by photon absorption [6,96]. The processes involved in the time evolution of these delocalized states and the nature of the emitting state(s) are the subjects of a very intense debate today [5,97]. It is not our purpose here to make a complete "synthesis" of the time-resolved fluorescence properties of the various model helices studied to this date. The main findings are that the fluorescence decays, and thus non-radiative relaxation mechanisms active in the excited state, are strongly sequence dependent.

Already from the first femtosecond fluorescence studies it was obvious that the fluorescence lifetimes, and thus the excited state lifetimes, were very different for the organized systems with regards to the monomeric NABs. For the adenine and thymine containing single and double strands $(dA)_{20}$, $(dT)_{20}$ and $(dA)_{20}\cdot(dT)_{20}$, fluorescence decays at 330 nm showed picosecond lifetimes [7,92], which is much longer than those of the monomers. Interestingly, for the alternating oligomer $(dAdT)_{10}\cdot(dAdT)_{10}$ a much shorter lifetime was observed at this wavelength compared to that of the homopolymeric duplex [93]. Such sequence effects are also observed for polymeric duplexes [97], as illustrated in figure 6.

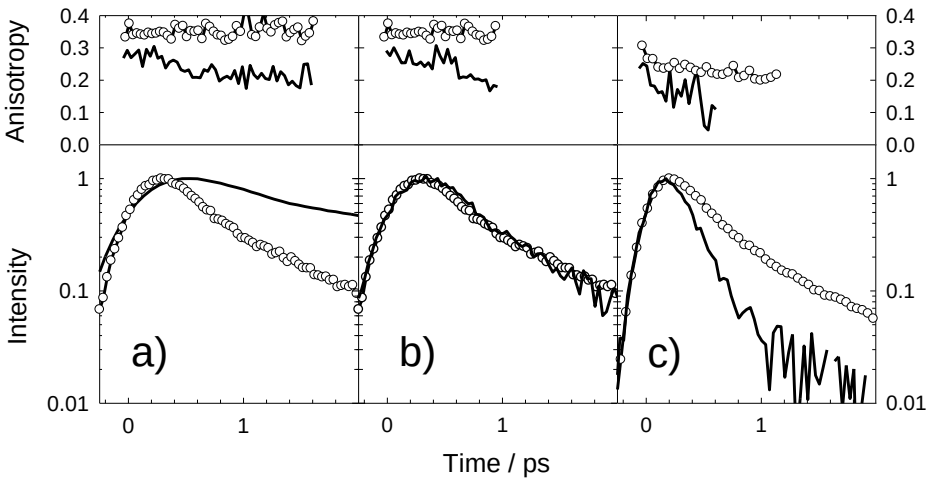

Figure 6. Fluorescence anisotropies (upper) and fluorescence decays (lower) obtained at 330 nm for a) poly(dA)•poly(dT), b) poly(dAdT)•poly(dAdT) and c) poly(dCdG)•poly(dCdG) in phosphate buffer. Also shown are the corresponding traces for an equi-molar mixture of monomers (circles).

The fluorescence decays recorded at 330 nm of poly(dGdC)·poly(dGdC), poly(dAdT)·poly(dAdT) and poly(dA)·poly(dT) are successively shorter, equal and longer than those of equimolar mixtures of the constitutive nucleotides. The extraordinary fast decay observed for poly(dGdC)·poly(dGdC) [98] is in line with experiments on isolated Watson-Crick guanine-cytosine pairs dissolved in chloroform [25]. More generally speaking, the presence of guanine-cytosine pairs in homopolymeric purine-pyrimidine oligomeric duplexes leads to a decrease of the FU lifetimes [99]. Theoretical calculations

emphasized the role of interbase (interstrand) proton transfer in the internal conversion mechanism involving the formation of an excited charge transfer state [100-102].

However, it is worth noticing that fluorescence lifetimes measured by TCSPC are longer than those obtained by transient absorption spectroscopy [1,2,8,9,91,94,95].

Sequence effects are thus clearly established, but of course there is no straightforward explanation of the observed fluorescence lifetimes in terms of this single variable. The excited state non-radiative processes active in helices are very complex and depend on many other factors as well. However, additional information on the excited states has been obtained from fluorescence anisotropy measurements. For all model helices discussed here a sub-picosecond decay of the anisotropy is observed, as shown in figure 6. Such an ultrafast decay cannot be due to a physical rotation of the helix and is instead interpreted in terms of energy transfer within the excitonic band which is initially delocalized over several base pairs [5,6].

Single strands containing guanine runs have the propensity to self-associate into four-stranded structure called quadruplexes. To this date, only one system characterized by a well-defined quadruplex structure, formed by four thymine-capped single strands d(TGGGGT), has been studied with femtosecond resolution [103]. The fluorescence lifetime of these quadruplexes were found to be much longer than that of the corresponding monomeric chromophores. Although sequence effects on the lifetimes of the bright $\pi\pi^*$ of model DNA helices have been clearly established many questions remain open. These have been outlined in ref. [10].

4. Conclusion

To conclude, we have shown how femtosecond spectroscopic techniques have contributed to elucidate the mechanisms involved in the very efficient excited state deactivation of nucleic acids, both monomers and model helices. In order to truly understand the underlying mechanisms, experimental results need to be combined with theoretical calculations, which are today only feasible on the monomeric level. Finding a correct theoretical description of the excited states of model helices constitutes a major challenge in the years to come.

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