Long-lived fluorescence of homopolymeric guanine–cytosine DNA duplexes

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Guanine-cytosine pairs in DNA duplexes: protectors or blackguards against UV radiation?

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The fluorescence spectrum of the homopolymeric double helix poly(dG)·poly(dC) is dominated by emission decaying on the nanosecond time-scale, as previously reported for the alternating homologue poly(dGdC)·poly(dGdC). Thus, energy trapping over long times is a common feature of GC duplexes which contrast with AT duplexes. The impact of such behaviour on the DNA photodamage needs to be evaluated.

Watson-Crick guanine-cytosine (GC) pairs have been considered as protectors of the genetic code against UV radiation.[1-4] Their specificity was attributed to an exceptionally short lifetime of the excited states directly populated upon photon absorption, explained by a relaxation mechanism involving an electron driven inter-base proton-transfer. Such a hypothesis, in line with the lifetimes of the bright excited states determined for model helices by fluorescence upconversion,[5, 6] was questioned recently.[7, 8] Although the lifetime of the bright excited states of poly(dGdC)·poly(dGdC) (denoted as A) is 200 fs,[5] its steady-state fluorescence spectrum is dominated by emission from "dark" states peaking at higher energy than that of bright states and decaying on the nanosecond time-scale.[8] Here we show that the emission spectrum of the homopolymeric duplex poly(dG)·poly(dC) (denoted as H) contains a high energy band and 93% of the emitted photons are associated with nanosecond time-constants. This behavior contrasts with that of adenine-thymine duplexes whose average lifetime is shorter[9, 10] than that of GC duplexes even when exciplex emission is clearly detected.[11] Trapping of the excitation energy by GC pairs over long times could play an important role in the DNA photochemistry.

This is the first spectroscopic study performed for a homopolymeric GC duplex with well defined structure. In previous attempts, such duplexes were obtained by annealing of single strands[6, 7] which are known to lead to quadruplex formation.[12] In the present work, H (*ca.* 1200 base pairs) was prepared and characterized by biochemical techniques;[13] characterizations using circular dichroism spectroscopy, atomic force microscopy and scanning tunneling microscopy were performed as described previously.[13-15] Its properties are compared to those of the commercially available alternating analog A (*ca.* 1000 base pairs; Amersham Biosciences). GC duplexes were dissolved in 0.1 M phosphate buffer containing 0.25 M NaCl.

Time-resolved measurements were carried out by time-correlated single photon counting, the excitation source being the third harmonic of a Titanium:Sapphire laser (150 fs, 267 nm). In order to avoid detecting emission from damaged helices,[16] the laser peak intensity was kept lower than 3 kWcm^{-2} and 3.5 ml of solution were continuously stirred.

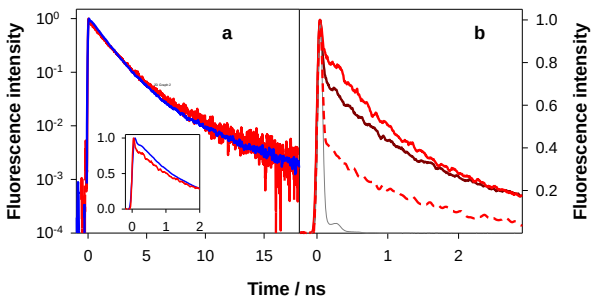

Fig. 1. Comparison of normalized fluorescence decays. (a) poly(dG)·poly(dC) (red) and poly(dGdC)·poly(dGdC) at 310 nm (blue), the inset representing the signals on a linear scale; (b) poly(dG)·poly(dC) at 310 nm (red) and 340 nm (dark red). For clarity, the signals in (b) were smoothed. Solid lines: 0.25 M NaCl; dashed line: 0.1 M NaCl. The instrumental response function is shown in grey.

The fluorescence decays recorded at 310 nm for H and A look quite similar (Fig. 1a). However, numerical fitting with three-exponential functions reveals subtle differences (Table 1 and SI). The main contribution to the fluorescence decays, in terms of amplitude, corresponds in both cases to the fastest component τ_1. For A, this time-constant amounts to 0.2 ps, as determined by fluorescence upconversion,[5] while for H it is much longer (30 ps). Interestingly, a 22 ± 6 ps component was detected by transient absorption for a d(C_4G_4) octamer in aqueous solution[7] in which duplex - quadruplex equilibrium is likely to occur.[12]

The main contribution to the total fluorescence intensity of both H and A corresponds to a 1.4 ± 0.1 ns component, whose weight, defined as $p_i = \alpha_i\tau_i / (\alpha_1\tau_1 + \alpha_2\tau_2 + \alpha_3\tau_3)$, is larger for the alternating sequence compared to the homopolymeric one. The longer component ($\square$ $\square\tau_3$), characterized by a time constant of a

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few ns, has a much lower amplitude. As reported previously for A,[8] upon increasing the emission wavelength, the contribution of τ_1 to H decays increases whereas that of τ_2 decreases (Fig. 1b).

The fluorescence decays of GC duplexes are very sensitive to conformational motions. An increase in the amplitude of these motions, achieved by lowering the ionic strength (Table 1 and SI) or reducing the size of the duplex[8] makes the overall decays faster. Still, the 1.4 ns component is always present, although its amplitude diminishes considerably. At low ionic strength (0.1 M NaCl), the longer nanosecond component of H vanishes and a time-constant of 0.21 ± 0.05 ns is found instead (Table 1 and SI).

Table 1. Parameters derived from the fits of the decays with three-exponential functions: $\Sigma a_i \exp(-t/\tau_i)$; $p_i = \alpha_i\tau_i / (\alpha_1\tau_1 + \alpha_2\tau_2 + \alpha_3\tau_3)$ represents the weight of each time constant τ_i (in ns).

decay	τ_1	τ_2	τ_3	α_2	α_3	p_2	p_3
H, 310 nm, 0.25 M NaCl	0.03	*1.3*	2.9	0.32	0.12	0.53	0.45
H, 340 nm 0.25 M NaCl	0.03	*1.3*	4.2	0.23	0.09	0.43	0.54
H, 310 nm 0.1 M NaCl	0.01 [a]	0.21	*1.4*	0.01	0.06	0.03	0.88
A, 310 nm 0.25 M NaCl	2×10^{-4} [b]	*1.4*	4.7	0.21	0.02	0.78	0.22

[a] fixed to 0.01 ns, which is the time-resolution of the setup after deconvolution; [b] fixed value, derived from fluorescence upconversion experiments[5]

Conformational motions play a crucial role in the properties of the excited states of GC duplexes as revealed by two theoretical studies.[17, 18] One of them, performed in the frame of the exciton theory for the alternating decamer $(dGdC)_5{\cdot}(dGdC)_5$, showed that conformational dynamics reduce the extent of exciton states.[17] The other, using semi-empirical quantum chemistry methods for smaller homopolymeric duplexes ($(dG)_3{\cdot}(dC)_3$ and $(dG)_4{\cdot}(dC)_4$)), showed that conformational motions intervene in the coupling between Frenkel and charge transfer excitons.[18]

The fingerprint of exciton states is visible in the absorption spectra of A and H duplexes which differ substantially from that of an equimolar mixture of dGMP and dCMP (Fig. 2a). The absorption maximum of H (253 nm) is located at a shorter wavelength compared to that of A (255 nm). A similar trend was also found for homopolymeric and alternating AT duplexes and was explained by the existence of exciton states built on different geometrical arrangements of the dipolar transitions of the monomeric chromophores.[19] The fluorescence spectrum of H differs significantly from that of A (Fig. 2b). Its maximum is located at 340 nm but a sharp peak is clearly distinguishable around 300 nm. The fluorescence quantum yields of the duplexes, $(2.5 \pm 0.3)\times10^{-4}$ for H and $(2.0 \pm 0.3)\times10^{-4}$ for A, are higher than that of the nucleotide mixture, $(1.1 \pm 0.1)\times10^{-4}$ whose emission peaks at 330 nm. When the NaCl concentration is reduced from 0.25 to 0.10 M, the fluorescence quantum yield of H decreases by about 60% and the maximum of the fluorescence spectrum shifts to 325 nm (Fig. 2b).

We propose the following explanation regarding the long-lived fluorescence of GC duplexes. The sharp, high energy peak, associated with the 1.4 ns time constant arises essentially from an excited state with a well-defined potential energy minimum. Considering its narrow width (fwhm: 2700 cm^{-1}, versus 7300 cm^{-1} for the bright ππ* states)[8],we assign it to an excited state delocalized on a single GC base pair (J-aggregate like), present in both H and A duplexes. At longer wavelengths, a longer-lived emission from a second type of excited state, very sensitive to base stacking, dominates the spectrum of H while being less important for A.[8] We correlate this second type with charge transfer states formed by vertical stacking.

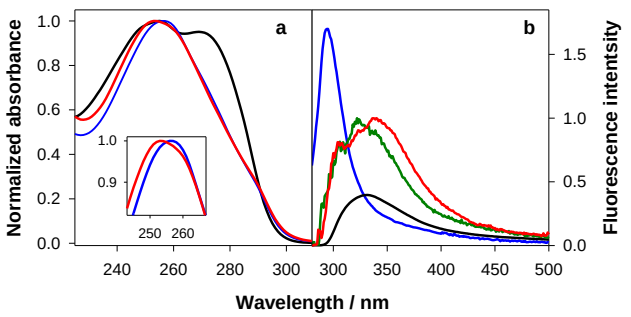

Fig. 2. Comparison of normalized absorption (a) and fluorescence (b) spectra of poly(dG)·poly(dC) (red: 0.25 M NaCl; green: 0.1 M NaCl), poly(dGdC)·poly(dGdC) (blue: 0.25 M NaCl) and an equimolar mixture of dGMP and dCMP (black). (b) Excitation wavelength: 267 nm. The spectra are representative of the quantum yields with the exception of the green spectrum whose intensity was multiplied by a factor of three.

The relative contribution of the two types of "dark" long-lived excited states to the duplex emission depends on the base sequence. This is not surprising because the orbital overlap between bases, which governs the properties of charge transfer states, is specific for each sequence and conformation.[20] Consequently, the energetic ordering between the various excited states may also change. Thus, following the initial energy transfer,[5, 8] one or the other type of "dark states" will be populated.

Finally, we associate the shortest time constant of H (30 ps) to bright excited states possibly resulting from a mixing between Frenkel and charge transfer excitons.[18] The mixing is expected to diminish with increasing structural disorder, which is in line with the shortening of τ_1 at low ionic strength (<10 ps). The presence of even faster components, as in the case of A, can only be revealed by femtosecond spectroscopy.

Theoretical calculations performed by the same method for GC duplexes with both alternating and homopolymeric sequence, including orbital overlap and taking into account conformational dynamics are blatantly needed for better understanding of their peculiar behavior. Will GC base pairs, after having been considered as guards defending DNA against UV radiation, instead be found guilty for the formation of DNA photolesions?

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