MOLECULAR SPECTROSCOPY

Complexity of excited-state dynamics in DNA

Arising from: C. E. Crespo-Hernández, B. Cohen & B. Kohler *Nature* 436, 1141–1144 (2005).

Absorption of ultraviolet light by DNA is known to lead to carcinogenic mutations, but the processes between photon absorption and the photochemical reactions are poorly understood. In their study of the excited-stated dynamics of model DNA helices using femtosecond transient absorption spectroscopy[1], Crespo-Hernández *et al.* observe that the picosecond component of the transient signals recorded for the adenine–thymine oligonucleotide $(dA)_{18}\bullet(dT)_{18}$ is close to that for $(dA)_{18}$, but quite different from that for $(dAdT)_9\bullet(dAdT)_9$; from this observation, they conclude that excimer formation limits excitation energy to one strand at a time. Here we use time-resolved fluorescence spectroscopy to probe the excited-state dynamics, which reveals the complexity of these systems and indicates that the interpretation of Crespo-Hernández *et al.* is an oversimplification. We also comment on the pertinence of separating base stacking and base pairing in excited-state dynamics of double helices and question the authors' assignment of the long-lived signal component found for $(dA)_{18}\bullet(dT)_{18}$ to adenine excimers.

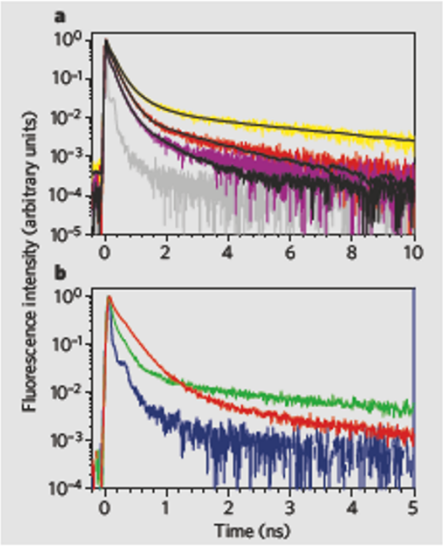

Figure 1 | Fluorescence decays recorded by time-correlated single-photon counting. **a**, $(dA)_{20}$ at 330 nm (magenta), 360 nm (red) and 420 nm (yellow). Black lines correspond to fits with multi-exponential functions, yielding the following sets of time constants (3.2, 37.5, 186 and 748 ps), (11.6, 101, 253 and 1,830 ps) and (39, 198, 551 and 5,050 ps), respectively. The instrumental response function (grey) is represented by the sub-picosecond fluorescence decay at 330 nm of thymidine-5′-monophosphate. **b**, $(dA)_{20}$ at 360 nm (red), $(dA)_{20}\bullet(dT)_{20}$ at 330 nm (blue), $(dAdT)_{10}\bullet(dAdT)_{10}$ at 420 nm (green). **Methods.** DNA oligomers (from Eurogentec) dissolved in phosphate buffer (pH 6.8) were excited by femtosecond pulses (100 fs, 267 nm). All decay curves correspond to the total fluorescence $F(t)$ constructed from the parallel (I_{par}) and perpendicular (I_{perp}) components according to: $F(t) = I_{par}(t) + 2GI_{perp}(t)$, where G accounts for the polarization-dependent sensitivity of the detection system. For further experimental details, see ref. 3.

Figure 1a shows the fluorescence decays of $(dA)_{20}$ at three different wavelengths. Combining these results with our previous measurements obtained for $(dA)_{20}$ by femtosecond fluorescence upconversion[2], at least five exponentials are needed to fit the decays over the 100 femtoseconds to 20 nanoseconds time range. A crucial point is that all time constants vary strongly with the emission wavelength. The same effect is encountered for $(dAdT)_{10}\bullet(dAdT)_{10}$ and has been reported previously[3] for poly(dA)•poly(dT).

We interpret this complex behaviour in terms of a model in which a large number of excited states are formed that are delocalized over several bases, which may be located both on the same strand and on opposite strands, with subsequent energy transfer[3]. This model is based on calculations made in the frame of exciton theory and combines quantum chemistry data and molecular dynamics simulations[4–7], and accounts not only for the fluorescence decays but also the steady-state absorption and fluorescence spectra. Delocalization of the excitation energy is governed by the electronic coupling, which depends on the oligomer's conformation. Conformational changes occurring on pico- and nanosecond timescales are controlled by an ensemble of interactions involving not only the bases but also the backbone, counterions and water molecules[5,8]. In this sense, both base stacking and base pairing determine excited-state dynamics.

For these reasons, the time constants provide a phenomenological description of the decays and do not correspond to specific excited states. However, it is possible to make a rough comparison of the overall excited-state dynamics by considering the decays recorded at the maxima of the fluorescence spectra of the three oligomers (Fig. 1b). In the case of $(dAdT)_{10}\bullet(dAdT)_{10}$ and $(dA)_{20}$, the fluorescence maxima have been assigned to excimer emission[9,10]. We observe that, in contrast to the transient absorption signals, the fluorescence decay obtained for $(dA)_{20}\bullet(dT)_{20}$ on the sub-nanosecond timescale is much shorter than that for $(dA)_{20}$. The same observation is valid when comparing the decays of these single and double strands at identical wavelengths.

Neither in fluorescence nor in transient absorption experiments is the amplitude of the detected signals proportional to the excited-state population. The transient absorption signals depend on the difference between the molar extinction coefficients of the $S_0 \rightarrow S_1$ and $S_1 \rightarrow S_n$ transitions (where S_0 and S_1 denote the ground and first excited electronic states and S_n denotes different, higher excited electronic states) at the probed wavelengths. As the steady-state absorption spectra of these oligomers correspond to a large number of transitions[6] and nothing is known about the $S_1 \rightarrow S_n$ spectra of their various excited states, the percentage of the 'excimer' population reported by Crespo-Hernández *et al.* is not necessarily correct.

The important difference between the transient absorption and fluorescence decays of $(dA)_n\bullet(dT)_n$ indicates the formation of dark transient species, as also discussed by Crespo-Hernández and colleagues[1,10]. If these dark species are adenine 'excimers', they must have a different electronic structure from the fluorescent 'excimers' of $(dA)_n$ and, therefore, different lifetimes. Consequently, the similar time constants observed by transient absorption for $(dA)_{18}\bullet(dT)_{18}$ and $(dA)_{18}$ may be fortuitous. The species observed by transient absorption in $(dA)_{18}\bullet(dT)_{18}$ could as well be interstrand A–T charge-transfer states, as suggested by theoretical calculations[11]. The behaviour of such states in deuterated water, as examined by Crespo-Hernández *et al.*, is not readily predictable because water molecules form a variety of interstrand and intrastrand bridges between bases[12].

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Crespo-Hernández et al.

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We have shown[1] that long-lived excited electronic states known as excimers[2], which arise from base stacking[3], are formed in high yields in a variety of synthetic DNA oligonucleotides. Markovitsi *et al.*[4] question our interpretation, and claim that these states can be accounted for by their exciton theory. However, neither this nor their emission data contradict our finding that in single- and double-stranded A•T (adenine–thymine-paired) DNA, excited states decay through long-lived intermediate states in which excitation is shared by stacked bases.

Time-resolved fluorescence and transient absorption signals from nucleic acids can differ in their dynamics[1,3] and result from different populations. Emission experiments are sensitive to states with significant radiative transition probability, whereas transient absorption can detect states that are dark in emission, such as the long-lived states in DNA[1,3]. Fluorescence provides an important but narrow view of the decay pathways taken by excess electronic energy in DNA. Less than 0.1% of all excitations in DNA decay by photon emission[2] and transient absorption[1], and various time-resolved ionization spectroscopies[5–7] provide information about decay pathways that involve dark states.

The different fluorescence decays of Markovitsi *et al.* for $(dA)_{18}$•$(dT)_{18}$ versus $(dA)_{18}$ disagree with a time-resolved emission study in which long-lived decays were identical for analogous 15-mer oligonucleotides[8]. Both studies had similar time resolution, which is adequate for characterizing the components with lifetimes of more than 100 picoseconds that dominate the transient absorption signals. It is difficult to address this discrepancy as Markovitsi *et al.* compare signals at different wavelengths in their Fig. 1b, and provide lifetimes for $(dA)_{20}$ but not for $(dA)_{20}$•$(dT)_{20}$. They also report faster emission decays for $(dA)_{20}$•$(dT)_{20}$ than for $(dA)_{20}$ on the subnanosecond and nanosecond timescale (their Fig. 1b), but the reverse ordering is reported by the same group on the picosecond timescale (Fig. 3 of ref. 9). These uncertainties must be resolved before further discussion is warranted.

Markovitsi *et al.*[4] claim that modelling of DNA (their refs 4–7) accounts for decays, steady-state absorption and fluorescence spectra, where exciton states are calculated from dipolar couplings among the $^1\pi\pi^*$ states of each constituent base. Although a first step towards modelling steady-state absorption spectra, none of the studies uses this theory to account for trends in steady-state emission spectra or lifetimes, as suggested[4]. The theory omits orbital overlap interactions, which are important for chromophores separated by small distances[10], and cannot describe the charge-transfer states that arise in DNA[1,11,12]. In our paradigm[1], charge-transfer states are the intermediates that the initial bright states pass through to reach the electronic ground state.

No evidence is provided by Markovitsi *et al.* that their emission signals represent the principal decay pathway. In contrast, our transient absorption signals at 250 nm indicate that most excitations in stacked bases decay to long-lived states[1]. We dispute their argument that the amplitude of fluorescence and transient absorption signals are not proportional to excited-state populations: such signals are proportional to a sum over the population of each state present, multiplied by its transition cross-section. Target analysis can be used[13] to recover time-dependent populations and yields from these signals with a simple kinetic model. Bleach recovery directly probes ground-state repopulation, and the sum of amplitudes of the slower exponentials provides a yield of the long-lived excimer states: if excimers absorb at 250 nm, then this yield is a lower bound[1]. In any case, similar transient absorption decays are observed for $(dA)_{18}$ and $(dA)_{18}$•$(dT)_{18}$ (Fig. 4a of ref. 1). The same long-lived states seen in the adenine single strand are seen in the duplex, indicating that base pairing does not introduce ultrafast quenching pathways. This is why vertical base stacking, and not base pairing, plays the primary role in DNA excited-state dynamics.

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