Picosecond Study of the Luminescence and Transient Absorption of Methylene Blue–Polynucleotide Complexes

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Transient absorption signals observed after picosecond laser excitation of Methylene Blue (MB^+) bound to double-stranded polydeoxyguanylic-deoxycytidylic acid {poly[d(G–C)]·poly[d(G–C)]} are assigned to the dye's singlet excited state reacting with a lifetime of 4 ps to form the electron-transfer product ($MB^{\cdot}\cdot G^{\cdot+}$) which itself decays with a lifetime of 130 ps.

Base- or sequence-specific photoreactions of dyes or other small molecules with DNA are a topic of considerable interest with potential applications in DNA-sequencing and DNA-modification.[1] For some dyes it is found that there is extensive fluorescence quenching when the dye is bound to DNA.[2] This is the case with Methylene Blue (MB^+) for which it is also known that the fluorescence is quenched when the dye is bound close to G–C centres, but not at A–T sites.[3] This quenching has been attributed to rapid electron transfer to the singlet excited state from guanine.[3,4] Although nanosecond laser flash photolysis studies showed that as expected the triplet yield was reduced in the presence of poly[d(G–C)]·poly[d(G–C)], no evidence was adduced for electron transfer products with lifetimes greater than 50 ns.[3,5] In this paper we report picosecond transient absorption and luminescence experiments which reveal that the decay of the MB^+ singlet state is greatly increased when bound to poly[d(G–C)]·poly[d(G–C)] but not in the presence of polydeoxyadenylic-deoxythymidylic acid {poly[d(A–T)]·poly[d(A–T)]}. The behaviour in the guanine-containing polymer is explained by the kinetic scheme given in Scheme 1.

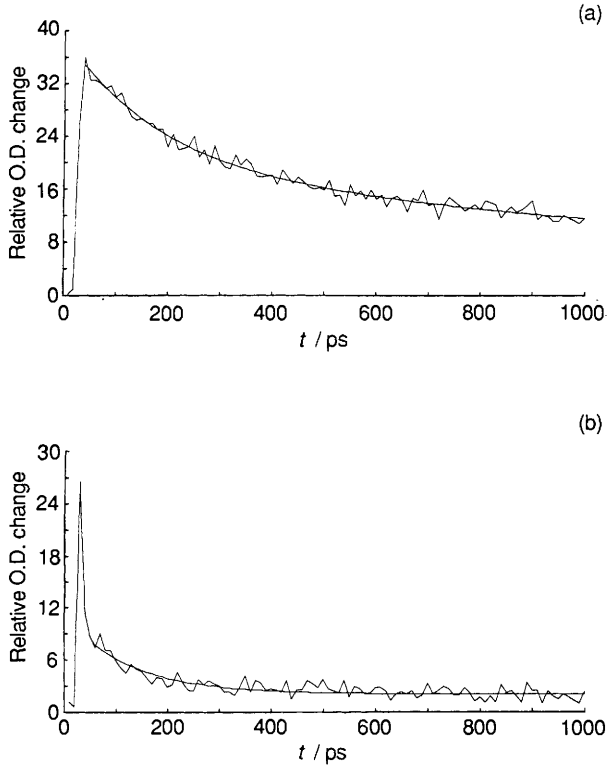
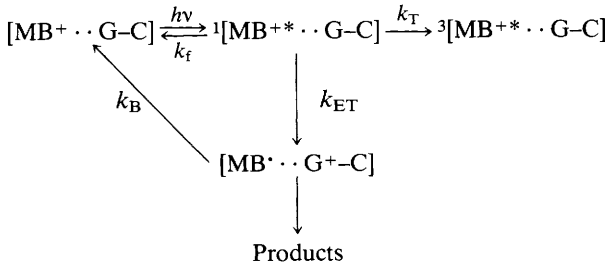


Figure 1. Transient optical density (O.D.) change observed at 670 nm from Methylene Blue (6.5×10^{-5} M) bound to poly[d(A–T)]·poly[d(A–T)] (7.8×10^{-4} M Nucl.) (Figure 1a) and poly[d(G–C)]·poly[d(G–C)] (1.62×10^{-3} M Nucl.) (Figure 1b) in 10 mM pH 6.9 phosphate buffer. The data were fitted from the peak by a non-linear least-squares method with a one or two exponential function plus background.

Fluorescence lifetime measurements[6]† on a sample of Methylene Blue (1.4×10^{-5} M) (excited either at 580 or 640 nm) gives a lifetime of 380 ps in either water or 10 mM phosphate buffer. Consistent with this, transient bleaching[7]† of a 6.5×10^{-5} M solution measured at 670 nm following 580 nm excitation reveals a lifetime of 370 ps for recovery of the ground state.

The transient bleaching and absorption in the presence of polynucleotides poly[d(A–T)]·poly[d(A–T)] and poly[d(G–C)]·poly[d(G–C)] were recorded following excitation at 580 nm. In both cases depletion of the ground state is observed between *ca.* 630 and 740 nm and transient absorption between 380 and 510 nm. Figure 1 shows the bleaching recovery signals observed at 670 nm. In the case of poly[d(A–T)]·poly[d(A–T)] (Figure 1a) the decay can be analysed as a lifetime of 290 ps accompanied by a species with a lifetime greater than 1 ns. Transient absorption measurements at 430 nm show a decay of 330 ps. The shorter-lived species may be assigned to the singlet state and the longer-lived signal to the triplet state (lifetime expected to be 11 μs).[3,5] These observations are in agreement with earlier findings that neither the fluorescence quantum yields nor the triplet state yields are strongly affected by binding to poly[d(A–T)]·poly[d(A–T)].[3]

The behaviour observed with poly[d(G–C)]·poly[d(G–C)] is strikingly different (Figure 1b). The trace indicates a very rapidly decaying transient (lifetime 4 ps), a weak intermediate transient of lifetime 130 ps, and a long-lived signal (triplet state) markedly reduced in amplitude compared with that found for poly[d(A–T)]·poly[d(A–T)]. The short-lived signal



Scheme 1

† Fluorescence lifetimes were measured by time-correlated single photon counting using a mode-locked dye laser.[6] (Time resolution 40 ps.) Transient absorption measurements were carried out by a pump-probe technique using amplified dye laser pulses (duration 1 ps) on the apparatus described earlier.[7] Uncertainty in lifetime is 10% or larger for $\tau > 10$ ps and 20% for $\tau < 10$ ps.

(4 ps), which is not observed with poly[d(A–T)]·poly[d(A–T)] is attributable to the singlet state being quenched by guanine (k_{ET} in Scheme 1). Taking E^0 for $MB^{+}*/MB^{\bullet}$ as 1.89 V[3] and E^0 for G^{+}/G as 1.54 V[4] gives ΔG^0 of −0.35 eV (−33.7 kJ mol^{-1}) for this reaction. (The value should be taken as approximate as the E^0 values are determined for free dye and for guanine.) The dye is expected to be intercalated between the base pairs of the double-stranded polynucleotide.[8] Given the short distance between the dye excited state and the guanine base (3.4 Å), Marcus theory predicts a rapid reaction rate for this photo-induced electron transfer. Single photon counting measurements recorded with samples with nucleotide/dye molar ratio (P/D) of 18 and 53 showed a short (*ca.* 40 ps) as well as a longer decay (500 ps). The longer fluorescence signal observed, which has a lower amplitude (<20%) originates either from unbound dye or more probably from Methylene Blue which is surface-bound to the polynucleotide. The very short-lived species observed in transient absorption/depletion is too short-lived to be resolved by the single photon counting equipment and, therefore, is expected to appear as an instrument-limited decay of 40 ps.

We propose that the intermediate species (lifetime of 130 ps) observed in transient absorption and depletion, when the dye is bound to poly[d(G–C)]·poly[d(G–C)], is $MB^{\bullet}\cdot G^{\bullet+}$ (Scheme 1). The fact that this species is not observed by single photon counting confirms that it is not an electronically excited state. It is noteworthy that its rate of reaction to reform the ground states (k_b) is less than that of the charge separation step of its formation (k_{ET}) even though it is more exergonic (ΔG^0 −1.48 eV; −143 kJ mol^{-1}). This is consistent with it being in the inverted region for electron-transfer reactions as is observed for other species.[7]

In conclusion the present study indicates that the singlet state of Methylene Blue is very rapidly quenched when bound to poly[d(G–C)]·poly[d(G–C)], and that the electron transfer products so formed also react very rapidly. This rapid back-reaction may be one of the reasons for the rather low yield for the photo-sensitised cleavage of DNA which is proposed to proceed *via* the guanine radical cation.[8,9]

The authors are grateful to the SERC, the Royal Society, and the EEC SCIENCE programme for financial support.

Received, 28th February 1990; Com. 0/00927J

References

1 C. Helene, *Photobiochem. Photobiophys. Suppl.*, 1987, 3.
2 G. Löber, *J. Lumin.*, 1981, **22**, 221.
3 J. M. Kelly, W. J. M. van der Putten, and D. J. McConnell, *Photochem. Photobiol.*, 1987, **45**, 167.
4 L. Kittler, G. Löber, F. A. Gollmick, and H. Berg, *J. Electroanal. Chem.*, 1980, **116**, 503.
5 W. J. M. van der Putten and J. M. Kelly, *Photochem. Photobiol.*, 1989, **49**, 145.
6 C. D. Tran and G. S. Beddard, *Eur. Biophys. J.*, 1985, **13**, 59.
7 R. J. Harrison, B. Pearce, G. S. Beddard, J. A. Cowan, and J. K. M. Sanders, *Chem. Phys.*, 1987, **116**, 429.
8 C. OhUigin, D. J. McConnell, J. M. Kelly, and W. J. M. van der Putten, *Nucleic Acids Res.*, 1987, **15**, 7411.
9 W. Blau, D. T. Croke, J. M. Kelly, D. J. McConnell, and C. OhUigin, *J. Chem. Soc., Chem. Commun.*, 1987, 751.