in the magnetic exchange parameters can determine large variations in the ground states and, therefore, the limiting values of the effective magnetic moments at low temperature. Thus, these simplified calculations have satisfactorily shown that with reasonable values of the parameters, close proximity of excited levels of different spin multiplicity is possible.

In the calculations, we have assumed tetragonal symmetry and T_d symmetry as far as the Mn^{IV}···Mn^{IV} interactions are concerned. While complex **4** is, indeed, tetragonal in the crystal, complex **3** is not, so differences in the ground states between the two could be expected.

Concluding Comments

The preparation and X-ray structure of the new $Mn^{IV}_4Mn^{III}_8$ complex, $[Mn_{12}O_{12}(O_2CPh)_{16}(H_2O)_4]$ (**3**), are reported. It is shown that this new complex and the analogous complex $[Mn_{12}O_{12}(O_2CCH)_{16}(H_2O)_4]\cdot MeCOOH\cdot 4H_2O$ (**4**) have interesting properties. Complex **4** has a $S = 10$ ground state both in zero applied fields as well as in large (20 T) magnetic fields. The nonsolvated complex **3** appears to have a $S = 9$ ground state in zero applied field, but with the introduction of a magnetic field the ground state becomes the $M_S = -10$ component of a $S = 10$ state.

High-field EPR experiments with a CO_2 laser spectrometer show that there are appreciable zero-field interactions for both of the complexes. High magnetic anisotropy is present.

Perhaps the most interesting observation made for the complexes **3** and **4** is the presence of a nonzero imaginary component of the AC susceptibility. Thus, even though we are dealing with $Mn^{IV}_4Mn^{III}_8$ molecular species, there are magnetization relaxation effects seen in zero applied field. The origin of this relaxation is not clear. Either these complexes are large enough to exhibit relaxation effects, or there are as yet uncharacterized *intermolecular* interactions present in these solids. Considerable additional experiments are needed to identify the origin of these unusual relaxation effects.

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Supplementary Material Available: Complete listings of bond lengths and angles, positional parameters, and tables of magnetic susceptibility data for complex **3** (22 pages). Ordering information is given on any current masthead page.

Photochemistry of Intercalated Methylene Blue: Photoinduced Hydrogen Atom Abstraction from Guanine and Adenine

Stephen J. Atherton and Anthony Harriman*

Contribution from the Center for Fast Kinetics Research, The University of Texas at Austin, Austin, Texas 78712. *Received July 28, 1992*

Abstract: Methylene blue intercalates more effectively into poly[dGdC] ($K = (1.6 \pm 0.4) \times 10^6\ M^{-1}$) than poly[dAdT] ($K = (1.6 \pm 0.5) \times 10^4\ M^{-1}$); in the latter case, intercalation competes with surface binding ($K = (2.3 \pm 0.6) \times 10^4\ M^{-1}$). Intercalation is accompanied by a dramatic reduction in the lifetime of the first excited singlet state, causing decreases in quantum yields for both fluorescence and formation of the triplet state. In the case of methylene blue intercalated into poly[dGdC], transient absorption spectroscopic measurements have shown that the excited singlet state of the dye abstracts a hydrogen atom (or an electron) from an adjacent nucleic acid base, presumed to be guanine on thermodynamic grounds. The rate constants for forward and reverse transfers, respectively, are $(2.5 \pm 0.3) \times 10^{11}$ and $(3.3 \pm 0.4) \times 10^{10}\ s^{-1}$. The rate constant for the forward transfer in poly[dAdT] is $(1.4 \pm 0.5) \times 10^{10}\ s^{-1}$ and is presumed to be slower than that of the reverse transfer. Using thermodynamic arguments, it is concluded that the quenching processes involve hydrogen atom transfer from guanine or adenine to the excited singlet state of methylene blue.

The fluorescence of certain polycyclic cationic dyes is quenched upon intercalation between base pairs in polynucleotides such as DNA.[1] Recent studies[2-4] carried out with *N,N'*-diazapyrenium and *N,N'*-diazaperopyrenium dyes intercalated into synthetic and natural polynucleotides have provided clear spectroscopic evidence[4] indicating that fluorescence quenching is due to rapid electron abstraction from an adjacent nucleic acid base by the excited singlet state of the dye. Related studies have reported[5,6] that the excited singlet state of intercalated methylene blue abstracts an electron from one of the bases in poly[dGdC], on a time scale of a few picoseconds,[6] but not from poly[dAdT]. This high specificity is surprising in view of the fact that the one-electron oxidation potentials[7] of guanine and adenine differ by only 100 mV. Furthermore, the dramatic reduction in excited singlet state lifetime that occurs upon binding to poly[dGdC] is matched by only modest reductions in the quantum yields for fluorescence and for formation of the triplet excited state.[8] Other workers,[9] however, have reported that both quantum yields are lowered upon binding to poly[dGdC] and that, due to steric and polarity effects, methylene blue binds more effectively to poly[dGdC] than to poly [dAdT].[9-11]

Because so little is known about the dynamics of electron-transfer processes involving polynucleotide matrices,[4,12] we have

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studied the photochemistry of intercalated methylene blue using transient spectroscopic techniques. We conclude that intercalated methylene blue undergoes photoinduced hydrogen atom (or electron) abstraction from both guanine (in poly[dGdC]) and adenine (in poly[dAdT]). At least in the case of poly[dGdC], the reaction is highly reversible and the ground state is restored within a few tens of picoseconds. The very different binding affinities and different distances over which the hydrogen atom must migrate make it difficult to compare the dynamics of quenching in the two polynucleotides.

Experimental Section

Methylene blue, chloride salt, (Aldrich Chemicals) was purified by repeated chromatography on a sephadex LH20 column using methanol as eluent. The final material gave satisfactory ^{1}H-NMR, FAB-MS, and elemental analyses and appeared as a single component on TLC (silica gel with methanol:acetic acid 9:1 as solvent). Samples of calf-thymus deoxyribonucleic acid (CT-DNA), double-stranded poly[deoxyguanylic-deoxycytidylic acid] (poly[dGdC]), and double-stranded poly-[deoxyadenylic-deoxythymidylic acid] (poly[dAdT]) were obtained from Sigma Chemicals and used as received. Solutions were prepared from freshly distilled deionized water containing 5 mM sodium sulfate and 1 mM phosphate buffer (pH 7). Concentrations of dye and polynucleotide were determined by absorption spectroscopy using the following molar extinction coefficients: methylene blue, 82 000 M^{-1} cm^{-1} at 665 nm;[13] CT-DNA, 6600 M^{-1} cm^{-1} at 260 nm;[14] poly[dGdC], 8400 M^{-1} cm^{-1} at 254 nm;[8,15] poly[dAdT], 6600 M^{-1} cm^{-1} at 262 nm.[8,15] Solutions for spectroscopic measurements were adjusted to give appropriate absorbances at the excitation wavelength and, unless stated otherwise, retained a fixed phosphate:dye molar concentration ratio (P/D) of 60:1 and were air-equilibrated. Concentrations of polynucleotide are expressed throughout the text in terms of phosphate. Wherever appropriate, the molar concentration of methylene blue ([MB^+]) and the fraction of dye bound to the polynucleotide (F) are given.

Absorption spectra were recorded with a Hitachi U3210 spectrophotometer, and fluorescence spectra were recorded with a fully corrected Perkin-Elmer LS5 spectrofluorimeter. Solutions for steady-state fluorescence spectroscopy were adjusted to possess an absorbance of 0.05 at the excitation wavelength (630 nm), and relative fluorescence quantum yields were determined by integration of the emission between 650 and 800 nm. Fluorescence quantum yields were calculated relative to aluminum trisulfonatophthalocyanine as standard,[16] and lifetimes were measured by time-correlated, single-photon counting using a mode-locked, synchronously-pumped, cavity-dumped Rhodamine 6G dye laser. The excitation wavelength was 610 nm, and emission was detected at 695 nm with a high radiance monochromator (used in conjunction with a 650-nm glass cutoff filter) and a Hamamatsu Model R2809U microchannel plate phototube. Data analysis was made after deconvolution[17] of the instrument response function (FWHM 60 ps). Solutions for time-resolved fluorescence studies were adjusted to possess an absorbance of ca. 0.03 at 610 nm and were air-equilibrated.

Transient absorption spectroscopy was performed using a frequency-doubled Antares 76S mode-locked (76 MHz) Nd:YAG-pumped Rhodamine 6G dye laser.[18] A Quantel Model RGA67-10 regenerative amplifier, operated at 10 Hz, and a Quantel Model PTA-60 dye laser were used to generate 3-mJ pulses at 593 nm having a fwhm of about 0.8 ps. The output beam was split; part was used as the excitation pulse and the remainder was optically delayed and focused into 1:1 D_2O:D_3PO_4 in order to generate a continuum for use as the probing pulse. The continuum was split into sample and reference beams and monitored with an intensified dual diode array spectrograph (Princeton Instruments) interfaced to a microcomputer. Transient absorption changes were determined by computer substraction of sample and reference beams averaged over 600 individual shots. Decay kinetics were established by overlaying about 50 spectra recorded at different delay times which were collected in random order. Analysis was made using nonlinear least-squares iterative procedures. Solutions were adjusted to possess an absorbance of 0.12 at 593 nm and were air-equilibrated.

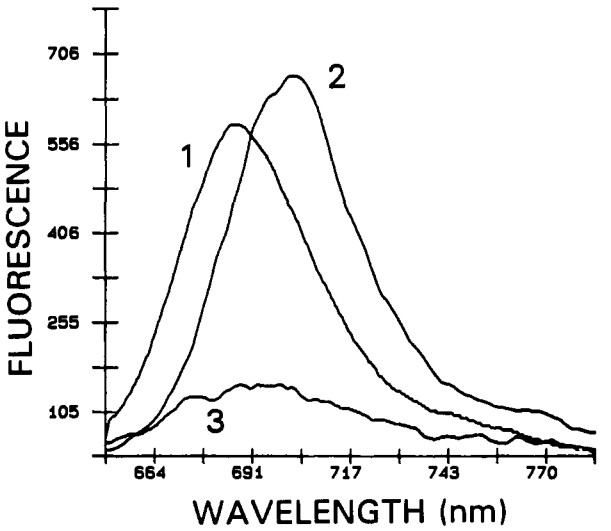

Figure 1. Fluorescence spectra recorded for methylene blue ([MB^+] = 1.3 μM) in (1) aqueous solution, (2) poly[dAdT] (P/D = 60; F = 0.72), and (3) poly[dGdC] (P/D = 60; F = 0.99). Spectrum 3 is shown at 10× amplification for clarity of presentation.

The triplet excited state of methylene blue[19,20] was monitored by conventional nanosecond laser flash photolysis using a frequency-doubled, Q-switched Quantel YG481 Nd:YAG laser (pulse width 10 ns). Output (80 mJ) from the laser was Raman shifted to 640 nm with hydrogen gas, giving 7-mJ pulses, and attenuated with crossed-polarizers, as required. The triplet was monitored using a pulsed xenon arc lamp as probing beam. Data analysis was made using nonlinear least-squares iterative procedures after averaging 50 individual records. Solutions were adjusted to possess an absorbance of 0.20 at 640 nm and were either air-equilibrated or deoxygenated by purging with N_2.

Cyclic voltammetry was performed with a Pine Instruments potentiostat using a glassy carbon working electrode, a Pt disc counter electrode, and an SCE reference. Solutions contained methylene blue (2×10^{-4} M), phosphate buffer (2 mM), and KCl (0.1 M) and were deoxygenated by purging with N_2. The pH was varied between 4.2 and 12 by changing the composition of the buffer. Related studies were made with methylene blue (5×10^{-5} M) bound to CT-DNA (3 mM) in the presence of KCl (0.1 M) and phosphate buffer (pH 7, 2 mM).

Results

Photophysics of Methylene Blue in Water. Previous work has established pK_a values for the ground state,[21] triplet excited state,[13] and one-electron reduced form[22] of methylene blue, respectively, to be 0.0, 7.2, and 9.0. The ground state self-associates, with an association constant of $(4 \pm 1) \times 10^3$ M^{-1} under our experimental conditions,[23] to form a nonfluorescent dimer. The monomer[24] is weakly fluorescent ($\Phi_f = 0.020 \pm 0.005$) (Figure 1) and the first excited singlet state is short-lived ($\tau_f = 345 \pm 10$ ps) (Figure 2a). Intersystem crossing to the triplet manifold occurs with high efficiency ($\Phi_t = 0.58$),[25] and the first excited triplet state is relatively long-lived. In mildly alkaline (pH 9.0) solution, the triplet lifetime was 45 ± 4 μs in the absence of oxygen and 3 ± 1 μs following aeration. In deoxygenated mildly acidic (pH 4.2)

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solution, the triplet lifetime was reduced to 5 ± 1 μs and was further reduced to 3 ± 1 μs upon aeration. Consequently, in deoxygenated solution, the triplet lifetime depends upon the degree of protonation[13,19,20] and, under our standard conditions, was 8 ± 1 μs.

Binding to Polynucleotides. Previous studies have shown[9-11,26] that methylene blue binds to CT-DNA, where it preferentially intercalates next to a G-C base pair. At a P/D of 60 ([MB$^+$] = 25 μM), the absorption maximum was red-shifted by 10 and 7 nm, respectively, for poly[dGdC] ($F \approx 1.0$) and poly[dAdT] ($F = 0.98$) relative to aqueous solution and there were decreases in the maximum molar extinction coefficient of 25–35%. Fluorescence spectra exhibited appreciable red shifts upon binding to poly[dAdT] but not to poly[dGdC] (Figure 1), and there were significant changes in fluorescence quantum yield relative to aqueous solution (see later).

Scatchard plots[27] for binding of methylene blue to poly[dGdC] were linear, giving an association constant (K) of (1.7 ± 0.2) × 10^6 M^{-1} and a saturation number (n) of 0.17 ± 0.03 per phosphate group under our conditions. Previous work, relying on linear dichroism, anisotropy, and hydrodynamic measurements, has established[9] that methylene blue intercalates tightly between base pairs in poly[dGdC]. We consider, therefore, that in the presence of poly[dGdC], methylene blue exists free in solution or as an intercalated species. Scatchard plots recorded for binding of methylene blue to poly[dAdT] were slightly curved, indicating the presence of at least two binding sites with similar association constants.[28] Approximating the titration data to a single binding site model gave a crude association constant of (3.2 ± 0.8) × 10^4 M^{-1}; the accompanying value of n is 0.15 ± 0.05 per phosphate group. Earlier studies[9,11] have also shown that binding of methylene blue to poly[dAdT] is unfavorable relative to poly[dGdC] but that the dye does intercalate between base pairs.[9] Thus, methylene blue may be considered to bind to poly[dAdT] both via intercalation between base pairs and through surface (electrostatic) association.

Photophysics of Methylene Blue Bound to Poly[dGdC]. Upon binding to poly[dGdC] methylene blue showed a significant reduction in fluorescence relative to dye dissolved in neutral aqueous solution. At high P/D the fluorescence quantum yield reached a lower limit, corresponding to 98% quenching, and, under the same conditions, the triplet excited state was formed in extremely low yield. For P/D = 60 ([MB$^+$] = 1.3 μM; $F = 0.99$), the level of fluorescence quenching was 94% (Figure 1), and time-resolved fluorescence decay profiles ([MB$^+$] = 1.1 μM; $F \approx 0.99$) required analysis in terms of two exponential components. The most

$$I_f(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) \quad (1)$$

satisfactory fit was obtained for lifetimes of ca. 20 (A = 98%) and 345 ± 15 ps (A = 2%). The same two lifetimes were preserved throughout the entire titration (Figure 2b), but the fractional contribution of the shorter-lived component increased systematically from 0 to almost 100% as the P/D was raised from 0 to 100. [The magnitude of the shorter lifetime is limited by the instrument response, and, in fact, the "true" lifetime is ≪20 ps.] The longer lifetime (τ_2) is identical to that measured for methylene blue in aqueous solution, and, consequently, this component is assigned to dye molecules free in solution. The shorter lifetime, therefore, is assigned to dye molecules bound to poly[dGdC]. By "fixing" the two lifetimes as τ_1 = 20 ps and τ_2 = 345 ps, the fractional amplitudes derived from the time-resolved fluorescence studies gave an association constant (K = (1.4 ± 0.3) × 10^6 M^{-1}) in excellent agreement with that derived from steady-state fluorescence measurements (K = (1.7 ± 0.2) × 10^6 M^{-1}). Moreover, the extremely effective quenching observed for bound material suggests to us that it refers to dye molecules intercalated between base pairs in the poly[dGdC] duplex.[12] Intercalation would position the dye within van der Waals contact with a guanine molecule and, therefore, favor rapid electron or hydrogen atom transfer.

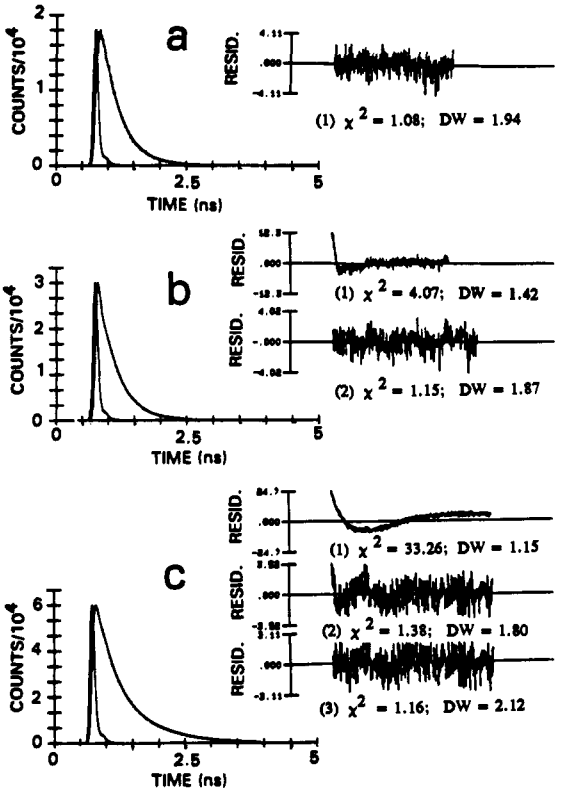

Figure 2. Fluorescence decay profiles recorded over a time scale from 0 to 5 ns for methylene blue ([MB$^+$] = 1.1 μM) in (a) aqueous solution, (b) poly[dGdC] (P/D = 5; $F = 0.88$), and (c) poly[dAdT] (P/D = 60; $F = 0.69$). The upper trace represents the experimental data together with the best fit to (a) one, (b) two, and (c) three exponentials whilst the lower trace is the instrument response function. The chi-square (χ^2) and Durbin–Watson (DW) parameters, together with the weighted residuals, derived from fitting the decay data to the sum of (1) one, (2) two, or (3) three exponentials are given adjacent to each decay profile.

Excitation of methylene blue in the presence of poly[dGdC] at a P/D = 60 ([MB$^+$] = 7.1 μM; $F \approx 1.0$) with a 10-ns laser pulse at 640 nm generated the triplet excited state of the dye,[19,20] albeit in very low yield. Deactivation of the triplet occurred by first-order kinetics in both air-equilibrated and deoxygenated solutions, giving lifetimes of 7 ± 2 and 5 ± 2 μs, respectively. The observed triplet, therefore, is assigned to methylene blue molecules that are free in solution. Rapid quenching of the excited singlet state of intercalated dye precludes significant population of the triplet, such that only nonbound triplet is observed.

Excitation of methylene blue in the presence of poly[dGdC] at a P/D = 60 ([MB$^+$] = 9.2 μM; $F \approx 1.0$) with a 0.8-ps laser pulse at 593 nm generated the excited singlet state of the dye (Figure 3). This species decayed rapidly, with a lifetime of 4.0 ± 0.5 ps, to leave a residual transient which decayed with a lifetime of 30 ± 3 ps (Figure 3). The longer-lived transient did not exhibit the absorption spectral features characteristic of the dye triplet state,[19,20] and, instead, it was assigned to a reduced form of methylene blue.[29] Reduction must occur by way of abstraction of an electron or a hydrogen atom from an adjacent nucleic acid base by the excited singlet state of the dye, and, based on redox potentials measured for one-electron oxidation of the various bases,[7] the most likely donor is guanine. The fast decay of this transient is attributed to reestablishment of the ground-state equilibrium since there was no destruction of the dye, even upon prolonged exposure to the laser beam.

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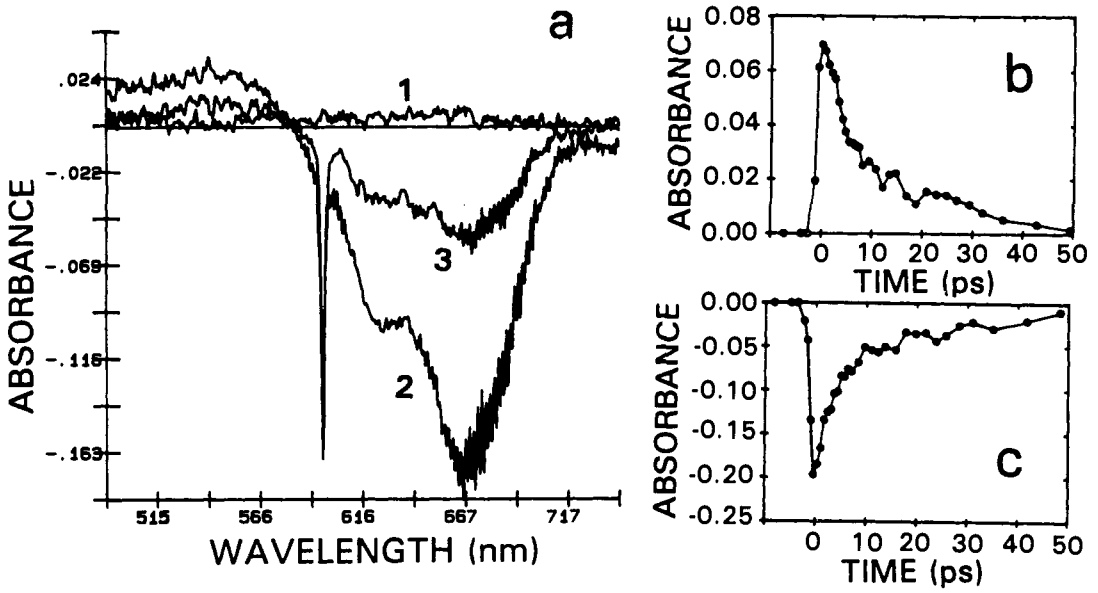

Figure 3. (a) Transient differential absorption spectra recorded (1) 2 ps before the excitation pulse, (2) 1 ps after the pulse, and (3) 20 ps after the pulse for methylene blue in poly[dGdC] (P/D = 60; $[MB^+]$ = 9.2 μM; $F \approx 1.0$). The excitation pulse (FWHM = 0.8 ps) was at 593 nm. Also shown are decay profiles recorded at (b) 545 nm and (c) 650 nm.

Photophysics of Methylene Blue Bound to poly[dAdT]. Relative to neutral aqueous solution, the fluorescence quantum yield for methylene blue ($[MB^+]$ = 1.3 μM) increased upon binding to poly[dAdT] (Figure 1). The magnitude of this enhancement remained constant at P/D > 100, where the increase was ca. 25%. Under similar conditions ($[MB^+]$ = 4.2 μM), the quantum yield for formation of the triplet excited state of the dye decreased by ca. 10%. For P/D = 60 ($[MB^+]$ = 1.3 μM; F = 0.72), there was a 15% increase in Φ_f while the corresponding decrease in τ_1 was 5%. Time-resolved fluorescence studies showed that, for dye bound to poly[dAdT] at a P/D = 60 ($[MB^+]$ = 1.1 μM; F = 0.69), the decay profile was best analyzed as the sum of three exponential components (Figure 2c):

$$I_f(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) + A_3 \exp(-t/\tau_3) \quad (2)$$

The most satisfactory analysis of the decay profiles gave lifetimes of τ_1 = 60 ± 5 ps (A_1 = 25%), τ_2 = 350 ± 15 ps (A_2 = 39%), and τ_3 = 730 ± 30 ps (A_3 = 36%).

The same three lifetimes were preserved throughout the entire titration, but the fractional contribution of the intermediate lifetime (A_2) decreased systematically with increasing P/D. This component is assigned, therefore, to methylene blue molecules that remain free in solution. The ratio A_3:A_1 remained fixed at 1.5 ± 0.2 throughout the titration, with the combined contribution $A_1 + A_3$ increasing systematically from 0 to almost 100% as P/D increased from 0 to 100. These findings, together with the nonlinear Scatchard plots, suggest to us that both τ_1 and τ_3 refer to dye molecules bound to poly[dAdT]. From the time-resolved fluorescence studies, the total association constant was derived to be (3.9 ± 0.6) × 10^4 M^{-1}, which is in very good agreement with that determined from the (nonlinear) Scatchard plots (K = (3.2 ± 0.8) × 10^4 M^{-1}). Following from the results obtained with poly[dGdC], we assign the shorter lifetime (τ_1 = 60 ± 20 ps) to methylene blue molecules intercalated into the polynucleotide duplex and the longer lifetime (τ_3 = 730 ± 50 ps) to dye bound to the phosphate layer. This assignment demands that methylene blue preferentially binds (K = 2.3 × 10^4 M^{-1}) to the outside of the strand rather than intercalates between base pairs (K = 1.6 × 10^4 M^{-1}). Accepting this premise, the specificity for intercalation into poly[dGdC] compared to poly[dAdT] is about 100-fold.

It was found that triplet decay profiles for methylene blue in the presence of poly[dAdT] required analysis in terms of two exponential components. In air-equilibrated solution at a P/D = 60 ($[MB^+]$ = 9.5 μM; F = 0.94), a good fit to the decay data was obtained with lifetimes of 8 ± 2 μs (14%) and 17 ± 4 μs (86%). Upon deoxygenation of the solution, the most satisfactory fits involved lifetimes of 8 ± 2 μs (10%) and 32 ± 5 μs (90%). In each case, the longer lifetime is attributed to dye molecules bound to the phosphate layer while the shorter-lived component is assumed to refer to dye molecules free in solution; as mentioned for poly[dGdC], the very short lifetime of the excited singlet state of intercalated dye precludes significant population of the triplet manifold for this species. The increased triplet lifetime for methylene blue bound to the phosphate layer relative to that free in solution arises because the polynucleotide protects the dye against both oxygen and protonation.

Excitation of methylene blue bound to poly[dAdT] at a P/D = 60 ($[MB^+]$ = 11 μM; F = 0.95) with a 0.8-ps laser pulse at 593 nm generated the excited singlet state of the dye (Figure 4). This species decayed via complex kinetics over some hundreds of picoseconds to form the corresponding triplet excited state (Figure 4). The transient spectroscopic records gave no indication of the presence of a species other than the singlet or triplet excited states of the dye. The decay profiles could be fit satisfactorily to a three-exponential component model using the lifetimes and fractional amplitudes derived by time-resolved fluorescence studies. It should be noted, however, that the precision of decay data generated by ultrafast transient absorption spectroscopy does not warrant such intricate analysis.

Energetics. The excited singlet-state energy levels of methylene blue in water and intercalated into a polynucleotide, respectively, are 180 and 177 kJ mol^{-1}, as calculated from absorption spectra. The triplet energy[30] in benzene/ethanol = 9/1 is 144 kJ mol^{-1}, and we assume a similar value for intercalated dye. In water, the triplet energy depends on the extent of protonation, and values of 96 and 139 kJ mol^{-1}, respectively, have been reported for the monoprotonated and unprotonated triplets.[31]

Attempts to measure the redox potential for one-electron reduction of methylene blue in water (4.2 < pH < 12) were unsuccessful because the reduced form rapidly disproportionated.[32] The redox potential for one-electron reduction at pH 1.7 has been determined previously[33] to be 0.19 V vs NHE. Using this value together with pK values of 0.0 for the ground state[21] and 9.0 for the semiquinone form,[22] the redox potential at pH 7 was calculated to be −0.12 V vs NHE. The corresponding value for the un-

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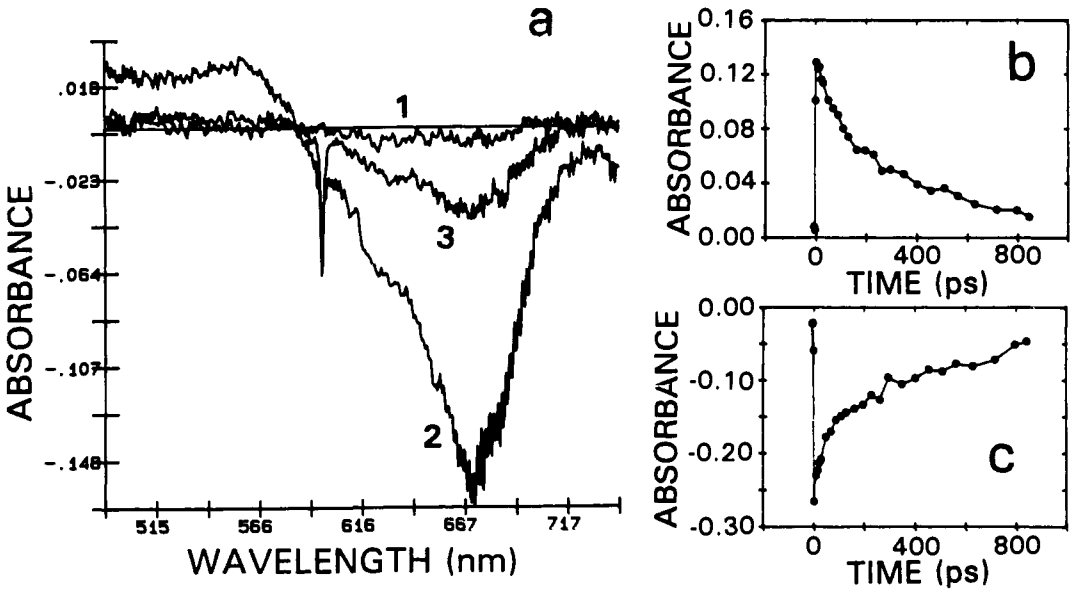

Figure 4. (a) Transient differential absorption spectra recorded (1) 2 ps before the excitation pulse, (2) 3 ps after the pulse, and (3) 800 ps after the pulse for methylene blue in poly[dAdT] (P/D = 60; $[MB^+]$ = 11 μM; F = 0.95). The excitation pulse (fwhm = 0.8 ps) was at 593 nm. Also shown are decay profiles recorded at (b) 545 nm and (c) 650 nm.

protonated radical (i.e., at pH 11) was calculated to be −0.24 V vs NHE.[34,35] Unlike in water, the cyclic voltammograms recorded for methylene blue intercalated into CT-DNA at pH 7 were quasireversible, and the one-electron reduction potential was found to be −0.28 V vs NHE. The polynucleotide, because of its low diffusion coefficient, inhibits disproportionation of the radical. Using the measured redox potential together with the above determinations of singlet and triplet state energy levels, one-electron reduction potentials for intercalated excited singlet and triplet state species,[36] respectively, were derived as 1.55 and 1.21 V vs NHE.

Discussion

This work has confirmed earlier findings[9-11] and provided the first quantitative determination of the preference with which methylene blue intercalates into poly[dGdC] rather than poly[dAdT]. According to the derived intercalation constants, the specificity is about 100-fold. Previous workers have explained this preference in terms of polarity and steric differences between the polynucleotides.[10] Related studies have provided association constants for methylene blue binding to CT-DNA which appear to be midway between our extreme values for the two synthetic polynucleotides.[9,26] We have shown also that methylene blue binds to the outer surface of poly[dAdT], presumably due to electrostatic attraction. It is reasonable to assume that the dye binds to the phosphate layer of poly[dGdC] with a comparable association constant. However, since intercalation into poly[dGdC] is so effective, surface binding will be significant only at very low P/D, and we have avoided such conditions. Using space-filling molecular models, we have concluded that methylene blue is likely to intercalate into polynucleotides via the major groove with the *N,N*-dimethylamino groups remaining outside the strand. This orientation positions the aza-N atom near the center of the duplex. The primary binding forces arise from π-stacking and charge-transfer interactions.

The excited singlet state lifetime of intercalated methylene blue is reduced drastically relative to dye free in solution. From the measured lifetimes, the quenching rate constants[37] are 25 × 10^{10} and 1.4 × 10^{10} s^{-1}, respectively, for poly[dGdC] and poly[dAdT]. An identical rate constant was reported by Beddard et al.[6] for methylene blue intercalated into poly[dGdC], but these authors found no evidence for quenching of dye intercalated into poly[dAdT]. However, the low affinity with which methylene blue intercalates into poly[dAdT] means that intercalated dye will always be a minority speices, even at high P/D, and difficult to resolve, especially by transient absorption spectroscopy. Our contention that there is extensive quenching of the excited singlet state of methylene blue which is intercalated into poly[dAdT] is based exclusively on the analytical fitting of the time-resolved fluorescence data. When collected at low levels of precision, these decay profiles could be adequately described in terms of two exponential components, having lifetimes of 230 ± 40 and 680 ± 60 ps, which might be assigned to free and surface-bound dye, respectively. When collected at much higher levels of precision, however, the decay profiles demanded analysis in terms of three exponential components, as described in the Results section.

Transient absorption studies carried out with poly[dGdC] showed the presence of a short-lived intermediate species (τ = 30 ± 3 ps) which we believe to be a reduced form of methylene blue (Figure 3). Unfortunately, the absorption spectral changes are dominated by bleaching of ground state dye and are not diagnostic of the state of reduction. The intermediate could not be detected for poly[dAdT], presumably because it decays faster than it is formed or because the complexity of this system complicates kinetic analysis (Figure 4). In fact, an intermediate could be formed with a lifetime longer than that of the intercalated excited singlet state, but the precision of the decay data (Figure 4b,c) does not permit its resolution. Beddard et al.[6] observed similar behavior in poly[dGdC] and reported the lifetime of the intermediate to be 130 ps. This value differs significantly from our observed lifetime of 30 ps, but their longer lifetime may be a consequence of having excess dye free in solution.

It is well known[7,38-40] that the pyrimidines are much easier to oxidize than the complementary purines and that guanine is more

(34) A previous calculation of the one-electron redox potential for methylene blue gave a value of −0.23 V vs NHE (ref 31).

(35) Kelly et al.[8] report that the redox potential for one-electron reduction of methylene blue in water is 0.05 V vs NHE, but they provide no experimental details. Kittler et al.[7a] give the value as −0.02 V vs NHE.

(36) The redox potential for one-electron reduction of the excited singlet state ($E°_S$) was calculated according to ($E°_S = E° + E_S$), where $E°$ is the redox potential for the ground state and E_S is the energy of the first excited singlet state. The value for the first excited triplet state was calculated accordingly.

(37) The rate constant for fluorescence quenching (k_f) was calculated from $k_f = [(1/\tau) - (1/\tau_0)]$, where τ is the lifetime of the excited singlet state of the intercalated dye, as measured by transient absorption studies, and τ_0 is the inherent lifetime of the excited singlet state. Since the latter value cannot be measured experimentally, we used the value found for methylene blue in aqueous solution (τ_0 = 345 ps).

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readily oxidized that adenine. The redox potentials for one-electron oxidation of the bases within a polynucleotide duplex, however, are unknown, although data exist for the individual bases and nucleotides in aqueous solution.[7] Using the one-electron redox potentials measured[7b] by pulse radiolytic oxidation of 2′-deoxyguanosine (E° = 0.71 V vs NHE) and 2′-deoxyadenosine (E° = 0.81 V vs NHE) in water at pH 13, together with the measured pK_a values for ground state and oxidized species,[41,42] we have calculated[43] redox potentials for formation of the corresponding π-radical cations. The derived values are 1.38 and >1.65 V vs NHE, respectively, for 2′-deoxyguanosine (GUAH) and 2′-deoxyadenosine (ADEH). These values indicate that electron abstraction by the first excited singlet state of intercalated methylene blue ($^*MB^+$) should be slightly favorable for poly[dGdC] (ΔG° = −16 kJ mol^{-1}) and unfavorable for poly[dAdT] (ΔG° >10 kJ mol^{-1}), assuming identical redox potentials for the polynucleotides. Beddard et al.[6] have used such reasoning to explain why fluorescence quenching occurs for poly[dGdC] but not for poly[dAdT]. The corresponding triplet-state reactions would be unfavorable for both polynucleotides; ΔG° = 16 and >42 kJ mol^{-1}, respectively, for poly[dGdC] and poly[dAdT].

$$^*MB^+ + GUAH \rightarrow MB^\bullet + GUAH^{\bullet+} \quad (3)$$

$$^*MB^+ + ADEH \rightarrow MB^\bullet + ADEH^{\bullet+} \quad (4)$$

Even allowing for the close proximity between reactants and the considerable uncertainty in reaction exergonicity, the quenching rate constants appear to us to be too fast for a nonadiabatic electron-transfer reaction. Thus, there is little, if any, thermodynamic driving force for photoinduced electron transfer, and the semirigid nature of the highly-ordered duplex seems certain to impose a high reorganization energy for oxidation of one of the bases, especially if oxidation results in loss of one or more of the Watson–Crick hydrogen bonds between the bases.[42] The nonpolar nature of the interior of the strand does not favor solvation of intermediate species, and the dynamics for reorientation of adjacent bases are expected to be slow. The thermodynamic situation is greatly improved, however, if electron transfer is accompanied by proton transfer.

Thus, the one-electron reduced form of methylene blue is more basic than the corresponding ground state. The ΔpK_a is 9.0, and this corresponds to a differential free energy change for protonation of the radical of −51 kJ mol^{-1}.[44] The reverse situation holds for

$$MB^\bullet + H^+ \rightleftarrows MBH^{\bullet+} \quad (5)$$

2′-deoxyguanosine, since the π-radical cation is more acidic than the ground state,[41,42] the differential free energy change for deprotonation of the π-radical cation being −31 kJ mol^{-1}.[45]

$$GUAH^{\bullet+} \rightleftarrows GUA^\bullet + H^+ \quad (6)$$

Therefore, photoinduced electron transfer between intercalated methylene blue and guanine would become thermodynamically more favored by ca. 82 kJ mol^{-1} if accompanied by proton transfer. This overall process corresponds to photoinduced hydrogen atom transfer from the N(1) position of guanine[42] to the aza-N atom of methylene blue. Our understanding of the intercalation site

$$^*MB^+ + GUAH \rightarrow MBH^{\bullet+} + GUA^\bullet \quad (7)$$

has the methylene blue aza-N atom in very close proximity to the N(1) atom of a guanine residue, such that only short-range transfer (i.e., 3 Å) is needed. Loss of the proton from the N(1) guanine position, however, destroys the central hydrogen bond to the complementary cytosine residue. This would lower the overall reaction exergonicity, possibly by as much as 20 kJ mol^{-1}. Even so, photoinduced hydrogen atom transfer would be more favorable, by at least 60 kJ mol^{-1}, than electron transfer and remains consistent with the observed transient absorption spectral changes.[29]

Photoinduced hydrogen atom transfer can also be invoked to explain the quenching reaction observed for methylene blue intercalated into poly[dAdT]. Here, the differential free energy change[45] for deprotonation of the adenine π-radical cation is <−68 kJ mol^{-1}. Proton transfer from the adenine π-radical cation to the neutral methylene blue radical, as formed by one-electron transfer, would be thermodynamically favorable by >120 kJ mol^{-1}. Thus, despite the unfavorable thermodynamics for photoinduced electron transfer (ΔG° > 10 kJ mol^{-1}), photoinduced hydrogen atom transfer would be strongly exergonic (ΔG° <−110 kJ mol^{-1}).[46] Pulse radiolytic studies carried out with 2′-deoxyadenosine have shown that deprotonation of the π-radical cation occurs via loss of a proton from the primary amino function. According to our intercalation model, this would require relatively long-range hydrogen atom transfer since the amino group donor and the N-aza acceptor are separated by ca. 7 Å. This increased distance may be responsible for the slower rate of quenching observed for poly[dAdT] relative to poly[dGdC].

$$^*MB^+ + ADEH \rightarrow MBH^{\bullet+} + ADE^\bullet \quad (8)$$

Because of the extremely efficient excited singlet state quenching observed for intercalated methylene blue, we have not been able to detect the corresponding triplet excited state. As an approximation, assuming the inherent (i.e., unquenched) fluorescence lifetime for intercalated dye is identical to that measured in water, we would expect to observed triplet quantum yields for intercalated dye of 0.007 and 0.10, respectively, for poly[dGdC] and poly[dAdT]. Since there are residual dye molecules free in solution and/or bound to the phosphate layer that have very much higher triplet quantum yields, it is difficult to clearly identify the intercalated triplet. We cannot comment, therefore, on the ability of the triplet to abstract a hydrogen atom (or an electron) from the polynucleotide.

A final issue concerns our observation that methylene blue bound to the outer surface of poly[dAdT] is more fluorescent than dye dissolved in water. Time-resolved fluorescence studies indicate that the lifetime of surface-bound dye is 730 ± 50 ps, compared to 345 ± 10 ps for dye free in aqueous solution. Preliminary studies reported by Beddard et al.[6] have also attributed a long-lived component ($\tau \approx$ 500 ps) in the time-resolved fluorescence decay profiles to surface-bound dye. Because of its short lifetime, the excited singlet state will not be quenched by either oxygen or protonation, such that the polynucleotide does not protect the singlet state against these processes. Instead, the enhanced fluorescence observed upon binding to the surface is attributed to a change in polarity; since methylene blue is readily polarized, its photophysics should be dependent on the polarity of the surrounding medium. The observed red shifts in both absorption and fluorescence that accompany surface binding are consistent with this assessment.

Concluding Remarks

The primary finding to emerge from this study is that intercalated methylene blue undergoes rapid photoinduced hydrogen atom transfer with guanine and adenine residues. In poly[dGdC], hydrogen atom transfer must cause considerable disruption of the local structure, since at least one of the Watson–Crick hydrogen bonds will be broken and subsequently reformed.[42,47] Thus, the reorganization energy accompanying the reaction might be high,

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(44) Calculated as $\Delta G^\circ = -2.303RT[\Delta pK_a]$, where ΔpK_a is the difference in pK_a values for protonation of the one-electron reduced species (pK_a = 9.0) and of the methylene blue ground state (pK_a = 0.0).

(45) Calculated as for ref 44 with ΔpK_a being the difference in pK_a values for deprotonation of the 2′-deoxyguanosine ground state (pK_a = 9.4) and of the corresponding π-radical cation (pK_a = 3.9). For 2′-deoxyadenosine, the pK_a values for the ground state and π-radical cation, respectively, are >13 and <1.

(46) The overall free energy change was calculated according to $\Delta G^\circ = [-nF(E^\circ_S - E^\circ_{ad})] - [2.303RT(\Delta pK_a^{dye} + \Delta pK_a^{ad})]$, where E°_{ad} refers to the redox potential for one-electron oxidation of adenine (E°_{ad} > 1.65 V vs NHE) while ΔpK_a^{dye} (9.0) and ΔpK_a^{ad} (>12), respectively, refer to the differential pK_a values for methylene blue and 2′-deoxyadenosine.

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although our work does not allow us to make an estimate of the magnitude of this parameter. For poly[dGdC], the rate of reverse transfer ($k = 3.3 \times 10^{10}$ s^{-1}; $\Delta G° = -146$ kJ mol^{-1}) is significantly slower than that of the forward reaction ($k = 2.5 \times 10^{11}$ s^{-1}; $\Delta G° = -76$ kJ mol^{-1}) despite its higher thermodynamic driving force. This cannot be taken to indicate that the reverse reaction falls within the Marcus "inverted region", however, since the forward and reverse steps may display quite distinct rate vs driving force profiles.[48] Further studies are needed before we can estimate the reorganization energy for a polynucleotide matrix, and we are attempting to evaluate this important parameter by using a series of intercalators of similar structure but differing reduction potential.

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Crystal Structure and Magnetic Properties of $[Ln_2Cu_4]$ Hexanuclear Clusters (where Ln = trivalent lanthanide). Mechanism of the Gd(III)-Cu(II) Magnetic Interaction

Marius Andruh,[†] Isabelle Ramade,[†] Epiphane Codjovi,[†] Olivier Guillou,[†] Olivier Kahn,*[,†] and Jean Christian Trombe[‡]

Contribution from the Laboratoire de Chimie Inorganique, URA 420, Université de Paris Sud, 91405 Orsay, France, and CEMES-LOE, UPR 8011, PO Box 4337, 31055 Toulouse, France.
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Abstract: The hexanuclear clusters $[Ln_2Cu_4(fsaaep)_4(NO_3)_6]\cdot 0.5(CH_3OH\cdot H_2O)$ (abbreviated as $[Ln_2Cu_4]$) have been synthesized; Ln is a trivalent lanthanide and $(fsaaep)^{2-}$ is the ligand deriving from 3-(*N*-2-(pyridylethyl)formimidoyl)salicylic acid. The crystal structure of the compound with Ln = Pr has been solved. This compound crystallizes in the orthorhombic system, space group $P2_12_12_1$. The lattice parameters are $a = 18.746(4)$ Å, $b = 24.196(4)$ Å, $c = 17.053(4)$ Å, and $Z = 4$. The structure consists of $[Pr_2Cu_4]$ entities in which the metal ions form a chair-shaped hexagon. The two praseodymium atoms are located on both sides of a double layer containing the four copper atoms. The copper environments are elongated distorted octahedra, and the praseodymium environments are bicapped square antiprisms. The magnetic susceptibility and the magnetization of $[La_2Cu_4]$ and $[Gd_2Cu_4]$ have been investigated. As far as the magnetic properties are concerned, the hexanuclear clusters may be viewed as two independent Cu(II)Ln(III)Cu(II) triads. The magnetic susceptibility data for $[La_2Cu_4]$ have revealed a weak Cu(II)-Cu(II) interaction through the closed-shell rare earth ion characterized by the interaction parameter $J_{CuCu} = -3.13$ cm^{-1} (the interaction Hamiltonian being of the form $H = -JS_A\cdot S_B$). The magnetic susceptibility data for $[Gd_2Cu_4]$ have shown that the ground-state spin for the Cu(II)Gd(III)Cu(II) triad is $S = {}^9/_2$; the Gd(III)-Cu(II) interaction is ferromagnetic with an interaction parameter $J_{GdCu} = 6.0$ cm^{-1}. The field dependence of the magnetization measured at both 2 and 30 K confirms the nature of the ground state and of the Gd(III)-Cu(II) interaction. The heart of the paper is devoted to the mechanism of the Gd(III)-Cu(II) interaction. The ferromagnetic nature of this interaction is attributed to the coupling between the Gd(III)Cu(II) ground configuration and the Gd(II)Cu(III) charge-transfer excited configuration in which an electron is transferred from the singly-occupied 3d-type copper orbital toward an empty 5d-type gadolinium orbital. A semiquantitative estimate of J_{GdCu} is performed which agrees fairly well with the value deduced from the magnetic data. It is emphasized that the Gd(III)-Cu(II) interaction is quite peculiar in the sense that owing to the contraction of the 4f orbitals the usual mechanisms involving 4f-3d overlap densities are inoperative.

Introduction

In the past two decades or so, a large number of heteropolymetallic compounds have been described. The studies of these compounds have often been performed either in relation to the modeling of some metalloenzymes containing several kinds of metal ions or with the perspective to design novel molecular materials, in particular molecular-based magnets. A particular emphasis has been brought to the magnetic properties.[1] The main idea emerging from those studies is that the interaction between two nonequivalent magnetic centers may lead to situations which cannot be encountered with species containing a unique kind of spin carrier. In fact, the recent investigation of the magnetic properties of heteropolymetallic compounds has represented quite an important contribution to the development of molecular ferromagnetism as a whole[2] and has allowed the introduction of several important new concepts: (i) the importance of the relative symmetries of the interacting magnetic orbitals;[3,4] (ii) the strict orthogonality of the magnetic orbitals favoring the stabilization of the state of highest spin;[3,5-7] (iii) the irregular spin-state structure leading to molecular systems with a high spin in the ground state despite antiferromagnetic interactions between nearest

[†] Université de Paris Sud.
[‡] CEMES-LOE.

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