Ultrafast Guanine Oxidation by Photoexcited Cationic Porphyrins Intercalated into DNA

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The photophysical and photochemical properties of a cationic porphyrin [bis(arginyl)porphyrin (BAP)] complexed to $[poly(dG\text{-}dC)]_2$ and $[poly(dA\text{-}dT)]_2$ have been investigated and compared to those of free BAP in aqueous solution. A drastic enhancement of the quantum yield of nonradiative deactivation of the lowest excited singlet state of BAP is observed upon intercalation between GC base pairs in $[poly(dG\text{-}dC)]_2$ but not upon complexation with $[poly(dA\text{-}dT)]_2$. Both picosecond time-resolved fluorescence and femtosecond transient absorption measurements give evidence for the occurrence of an ultrafast direct electron transfer ($k \geq 1.25 \times 10^{13}$ s^{-1}) from guanine to the lowest excited singlet state of BAP followed by an efficient back electron transfer ($k = 8.3 \times 10^{12}$ s^{-1}). A classical nonadiabatic Marcus model for this reverse electron transfer explains the experimental observations which allow one to estimate the electronic coupling energy (230 cm^{-1}) involved for BAP−$[poly(dG\text{-}dC)]_2$ complexes.

Introduction

The biological effects of UV,[1] ionizing radiations,[2,3] reactive oxygen species,[4,5] or drugs are greatly dependent on the level and the nature of the genetic damage and on the ability of the cells to react to lesions. Nucleic acids are the major targets of these mutagenic, carcinogen, and lethal effects. The reactions leading to DNA damage may involve different processes, a major one being oxidative damage which occurs preferentially at guanine sites, because guanines have the lowest oxidation potential of all bases.[6] Many reagents have been used to generate a radical cation (hole) in DNA including phenanthrenequinonediimine complexes of rhodium(III),[7] polypyridyl complexes of Ru(II),[8] naphthalimides,[9] anthraquinones,[10] benzophenones,[11] stilbenes,[12] Norrish reagents,[13] phenothiazinium dye thionine,[14] and meso-tetrakis(*N*-methyl-4-pyridiniumyl)porphyrin (H_2TMPyP-4).[15] The latter class of compounds, in addition to the fundamental interest of the understanding of the nucleic acid oxidation, is also important with regard to the study of photosensitizer reactivity. Although mechanisms involving singlet oxygen seem to occur with high yields, it has been suggested that the charge-transfer reaction leading to DNA oxidation could also be involved in the photoreactivity of cationic porphyrins capable of intercalative DNA binding.[15−17] Besides, even in the absence of oxygen, these photosensitizers may have negative side effects at the cellular level such as photosensitized molecular alteration at the DNA site. Therefore, the information available on the mechanisms responsible for DNA oxidation upon porphyrin photoexcitation is of fundamental interest.

A cationic porphyrin [bis(arginyl)porphyrin (BAP)] derived from H_2TMPyP-4 has been synthesized in the context of the research of chromophores able to target selectively the major groove of double strand DNA sequences encompassing the palindrome $d(GGCGCC)_2$.[18] BAP, contrary to H_2TMPyP-4, is a specific marker of DNA due to both porphyrin and arginine interactions with guanine.[18] On the other hand, BAP could also be an efficient hole donor toward guanine upon photoexcitation as previously suggested for H_2TMPyP-4.[15] To investigate such a possibility, we have compared the photophysical and photochemical properties of BAP complexed with $[poly(dG\text{-}dC)]_2$ with those of BAP bound to $[poly(dA\text{-}dT)]_2$ and of free BAP in aqueous solution. The intercalation of BAP between G−C base pairs was shown to activate a radiationless transition. This process was investigated by femtosecond transient absorption measurements. The evolution of the differential absorption spectra of free and complexed porphyrins was analyzed on a large spectral domain (420−600 nm). The effects identified in both kinetics and spectra provide evidence for an electron transfer from guanine to excited intercalated porphyrin and for a rapid back electron transfer.

Experimental Section

Pump−probe experiments were performed on the Ti-sapphire femtosecond transient absorption spectrometer described in detail in ref 19. Briefly, about 10% of the output of an amplified Ti-sapphire laser system (MIRA 900, Coherent), producing 110 fs pulses at a 1 kHz repetition rate with a pulse energy of 650 μJ at 808 nm, was split into two parts to generate the pump and probe pulse trains. For the pump, 90% of the incident 808 nm beam was doubled to excite the Soret band of the porphyrin (404 nm). Typically, the excitation spot size was 500 μm, and the pump energy was less than 1 μJ/pulse to eliminate saturation effects. In these conditions, less than 10% of the molecules are photoexcited. The remaining 10% of the fundamental beam (808 nm) was focused in a 0.5 cm path length quartz cell filled with water and was used as probe beam in the spectral region 400−650 nm. The polarization of the pump was turned at the "magic angle" (54.7°) with respect to that of the

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probe beam to remove any contribution from orientational relaxation. After traveling along a computer-controlled optical delay line, the probe beam was split again into two quasi-identical probing beams, one passing through the pumped sample, the other through an identical but unexcited sample. During the measurements, the sample was continuously moved in a plane perpendicular to the pump beam to avoid photodegradation effects. Moreover, the sample photodegradation was systematically checked by recording stationary absorption spectra.

The time dependence of the transient absorption spectra corrected for the group velocity dispersion was analyzed by fitting to a sum of exponential terms $A_0(\lambda,t)$. The measured absorbance $A(\lambda,t)$ is the convolution between the physical response $A_0(\lambda,t)$ and the instrumental response $F(t)$ represented by a Gaussian (fwhm of 180 fs).[19]

$$A(\lambda,t) = \int_{-\infty}^{+\infty} F(\tau - t)A_0(\lambda,t)\,\mathrm{d}t \qquad (1)$$

where

$$A_0(\lambda,t) = \sum a_i(\lambda)\exp(-t/\tau_i) \qquad (2)$$

$a_i(\lambda)$ is the wavelength-dependent amplitude, and τ_i is the wavelength-independent time constant. $A_0(\lambda,t)$ was reconstructed by a least-squares fit of the experimental transient absorption over a set of about 90 kinetics corresponding to a sampling of 2 nm of the probed spectral range. The reported uncertainties in the fit parameters are estimated to 10%, and the relative imprecision of the derived spectra is not worse than ±15%, confidence limits based on reproducibility from fitting a number of independent sets of data. Comparison scans of a cell containing pure buffer were also recorded to ensure that the measurements were free of significant buffer-induced transients.

Dynamic fluorescence measurements were performed using the time-correlated single-photon counting method by means of the experimental setup implemented at the Laboratoire de Photophysique et Photochimie Moléculaire et Supramoléculaire at the Ecole Normale Supérieure de Cachan, the detailed description of which has been given previously.[20] The excitation source was a Ti-sapphire laser (Tsunami, Spectra Physics) pumped by an argon laser and coupled with a BBO doubling crystal producing 2 ps pulses at 4 MHz repetition rate with a pulse energy of 10 nJ at 432 nm (excitation wavelength). The fluorescence decays were collected for at least 10 different wavelengths in the spectral range 610−730 nm and were analyzed by a global analysis method previously described.[20] The method allows the simultaneous determination of the kinetic parameters and the spectra associated with the different fluorescent species present in solution. The time resolution obtained after signal deconvolution by the instrumental response function (recorded by detecting the light scattered at a wavelength close to the excitation wavelength by a solution of Ludox) was about 30 ps.

Nanosecond transient absorption measurements were carried out using a Nd:YAG laser (Quantel, YG 441; pulse width 2 ns) at both 532 and 355 nm (energy/pulse 50 and 20 mJ) as an excitation source. The experimental setup was described in detail in ref 21. The triplet quantum yields of free BAP and BAP complexed to the polynucleotides were determined by a comparative method[22] using H_2TMPyP-4 as a reference ($\Phi_T = 0.92$ and $\epsilon_{470\ nm} = 28\,000\ M^{-1}\ cm^{-1}$)[23] upon excitation at 355 and 532 nm. Ground-state absorption and static fluorescence spectra were obtained by using, respectively, a double beam UV−visible spectrophotometer with 1 nm spectral bandwidth (Cary 300, Varian) and a spectrofluorimeter (Perkin-Elmer MPF-3L) using spectral bandwidth between 3 and 6 nm. The fluorescence spectra were corrected for the buffer Rayleigh and Raman scattered light. The fluorescence quantum yields of the various BAP/polynucleotide complexes have been determined as described in ref 24 by taking the H_2TMPyP-4 porphyrin as a standard ($\Phi_{F0} = 0.017$ [23]).

The BAP molecule has been synthesized in the Laboratoire de Chimie Bioorganique et Bioinorganique (Orsay, France).[25] The absence of metal atom contamination was controlled by mass spectroscopy. The porphyrins are supplied lyophilized and are then dissolved in a 25 mM potassium and sodium phosphate buffer (KH_2PO_4/Na_2HPO_4) (pH 7.0); in the presence of polynucleotides, 150 mM sodium chloride was added in to maintain the double helix structure. The polynucleotides $[poly(dG\text{-}dC)]_2$ and $[poly(dA\text{-}dT)]_2$ have been purchased from Amersham Pharmacia Biotech. The concentrations of the stock solutions were determined spectrophotometrically using the published extinction coefficients.[26] The final porphyrin concentrations in all of the experiments varied between 2 and 50 μM, and a molar ratio of base pairs to porphyrin (defined as $1/r_0$) of 25 was chosen to ensure total complexation of the porphyrin.[18,27] All experiments were carried out at ambient temperature (~23 °C).

Results

Photophysics of BAP−Polynucleotide Complexes. BAP in its ground state exhibits predominantly a planar conformation (95−97%), but we have demonstrated[28] that distorted geometries due to the formation of hydrogen bond complexes with the solvent can also occur (2−5%). Circular dichroism, IR spectroscopy, and UV−visible absorption spectroscopy measurements supported by molecular modeling calculations[18] have revealed that in the BAP planar conformation, the porphyrin ring intercalates into $[poly(dG\text{-}dC)]_2$ and the arginine arms bind to guanines in the major groove, while groove binding occurs with $[poly(dA\text{-}dT)]_2$. As concerns the minor BAP conformation, it was not possible to characterize its binding by such spectroscopic measurements due to its too weak contribution to the signal. However, time-resolved fluorescence measurements and fluorescence excitation spectra allowed us to conclude that it also interacts with the polynucleotides (see below).

The BAP absorption and fluorescence spectra, free and complexed with $[poly(dA\text{-}dT)]_2$ and $[poly(dG\text{-}dC)]_2$, are represented in Figure 1. Their patterns are highly dependent on the nucleic acid base environment. The weak hypochromicity ($\Delta H = 30\%$) and bathochromicity ($\Delta\lambda = 7$ nm) of the Soret band observed in the presence of $[poly(dA\text{-}dT)]_2$ are consistent with the formation of nonintercalated complexes. In contrast, interaction with $[poly(dG\text{-}dC)]_2$ leads to large hypochromicity ($\Delta H = 70\%$) and bathochromicity ($\Delta\lambda = 23$ nm), indicative of intercalation of the porphyrin core at $d(CpG)_2$ steps.[18,27,29] In the fluorescence spectrum of the BAP−$[poly(dA\text{-}dT)]_2$ complex, the $Q_x(0,0)$ band is slightly blue-shifted (6 nm), while a significant increase of both $Q_x(0,0)$ and $Q_x(0,1)$ bands is observed leading to a marked increase of the fluorescence quantum yield (36%, Table 1). The BAP fluorescence spectrum exhibits, like its absorption spectrum, more pronounced changes when BAP is complexed to $[poly(dG\text{-}dC)]_2$: a red-shift of both bands (9 nm) is observed, accompanied by a significant decrease of the $Q_x(0,1)$ band which leads to an important enhancement of the ratio of the $Q_x(0,0)$ and the $Q_x(0,1)$ peak intensities. Despite these modifications, the fluorescence quantum yield remains unchanged with respect to that of free BAP (Table 1).

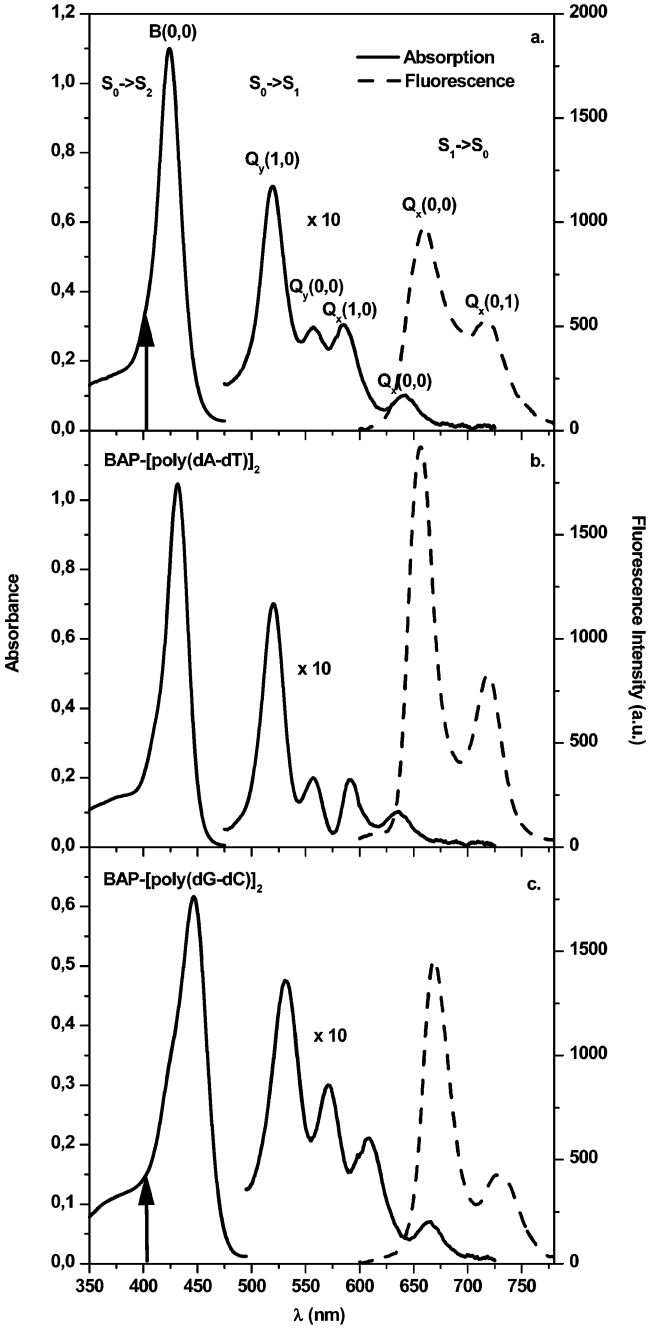

Figure 1. Absorption (solid line) and fluorescence emission (dashed line) spectra of free BAP (a), BAP−[poly(dA-dT)]$_2$ (b), and BAP−[poly(dG-dC)]$_2$ (c). [BAP] = 5 μM, r = 25, 25 mM phosphate buffer pH 7.0, 150 mM NaCl. (Arrows indicate the wavelength upon which the samples were excited in the femtosecond transient absorption experiments.)

TABLE 1: Summarized Photophysical Properties of Free BAP and BAP in the Presence of [Poly(dA-dT)]$_2$ and [Poly(dG-dC)]$_2$[a]

	τ_1 (ns) ±10%	A_1 (%) ±10%	τ_2 (ns) ±10%	A_2 (%) ±10%	Φ_f ±10%	Φ_T ±15%
free BAP	0.8	5.0	5.6	95	0.025	0.85
in the presence of [poly(dA-dT)]$_2$	3.8	8.0	11.4	92	0.034	0.95
in the presence of [poly(dG-dC)]$_2$	2.4	23	8.1	77	0.025	0.35

[a] τ_i, fluorescence lifetime; A_i, percentage of the fluorescent population; Φ_f, fluorescence quantum yield; Φ_T, triplet quantum yield.

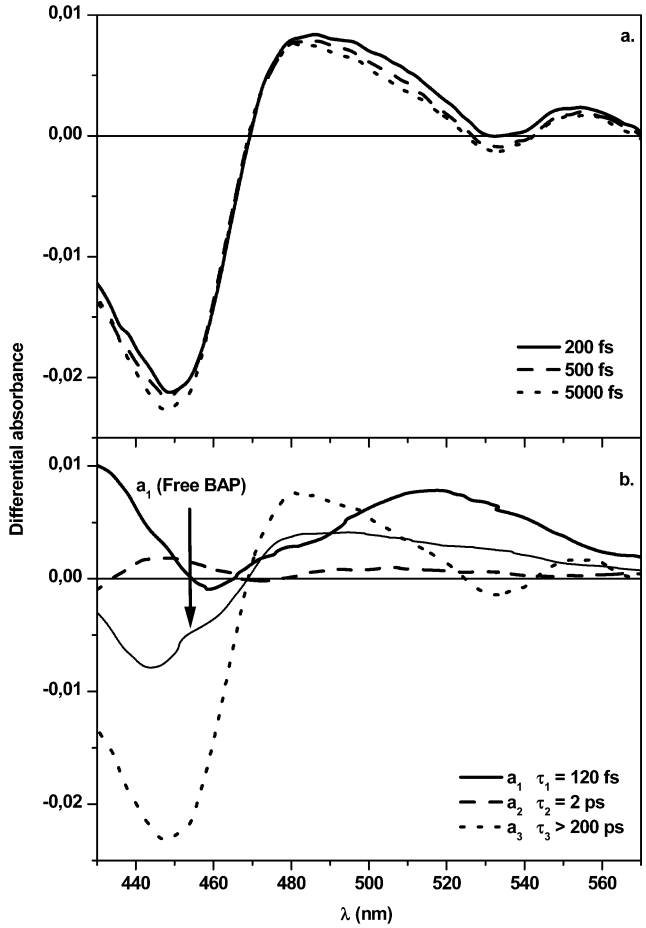

Figure 2. BAP 50 μM in the presence of [poly(dG-dC)]$_2$ ($1/r_0$ = 25) in 25 mM phosphate buffer pH 7.0, 150 mM NaCl. (a) Differential transient absorption spectra corrected for group velocity dispersion obtained for different delays between pump and probe, λ_{exc} = 404 nm. (b) Amplitudes in function of wavelength obtained after the analysis of the transient absorption signals according to the method described in the Material and Methods. The free BAP a_1 amplitude has been normalized to the same absorbance upon excitation at 404 nm.

The S_1 fluorescence decay times of BAP complexed to the polynucleotides are summarized in Table 1 and compared to those previously obtained for the free chromophore.[28] In the latter case, the longer lived fluorescence component was assigned to the planar BAP conformation (95%). In the presence of polynucleotides, the BAP fluorescence decays are always biexponential. This behavior can be due either to complexation of both BAP conformations (see above) or to the existence of two different geometries for the BAP−polynucleotides complexes as was already reported in the case of other DNA complexing molecules.[15,20,30] However, the measured lifetimes depend on the nucleic acid environment. In the presence of [poly(dA-dT)]$_2$, they are both significantly longer than those of the free chromophore, a result consistent with total complexation of both BAP conformations and a reduced nonradiative deactivation due to the shielding of the chromophore from the aqueous solvent. The data in Table 1 show that for free BAP as well as for the BAP−[poly(dA-dT)]$_2$ complex, the major part of the electronic energy relaxation from the S_1 state occurs via the porphyrin triplet state (Φ_T = 0.85 and 0.95 ± 15%, respectively) and the sum $\Phi_T + \Phi_f$ is close to unity. This implies that no direct $S_1 \rightarrow S_0$ radiationless transition is involved in the S_1 state deactivation of free BAP or BAP complexed to [poly(dA-dT)]$_2$, results similar to those previously obtained for H_2TMPyP-4.[15]

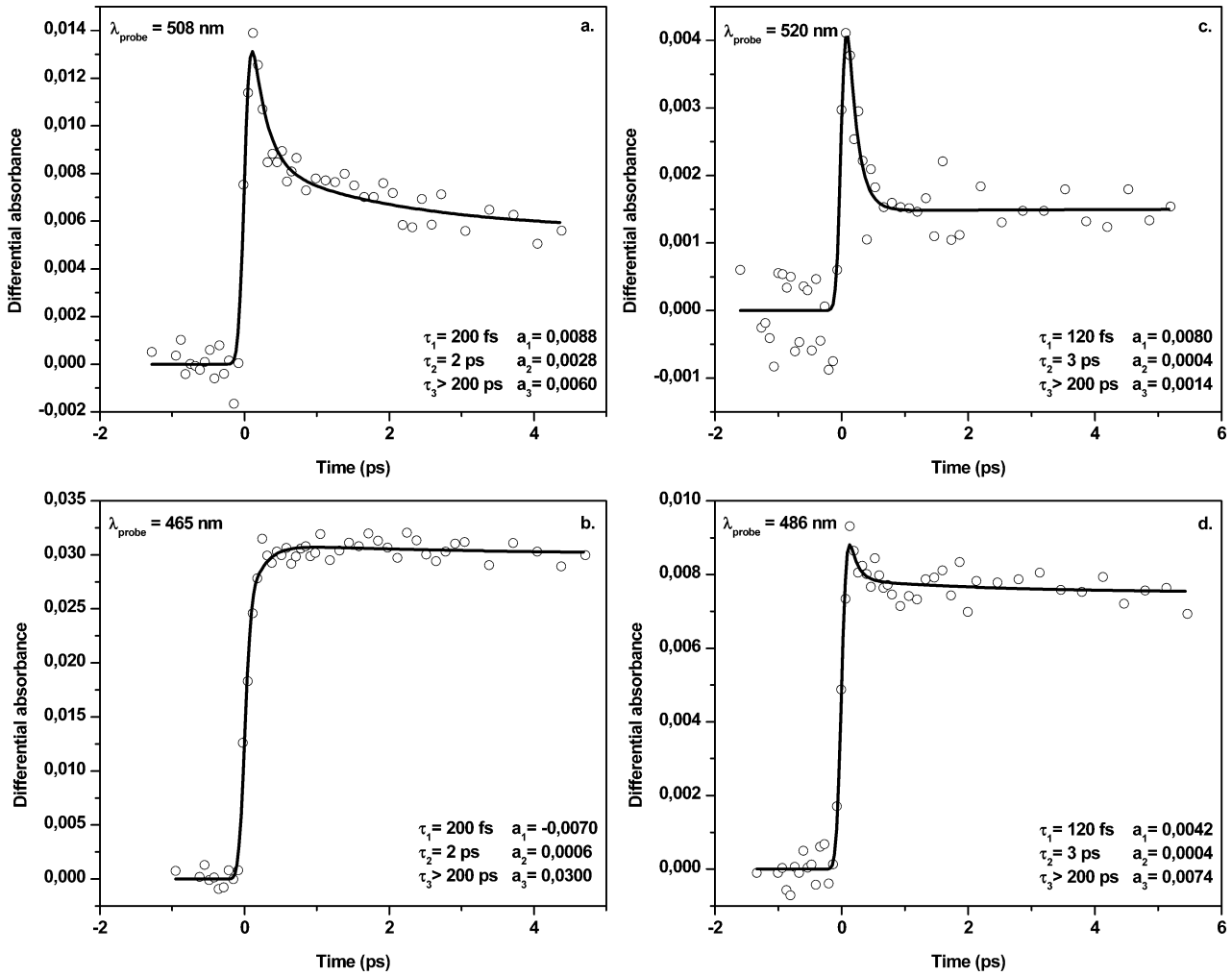

Figure 3. Representative kinetics of free BAP 50 μM at different probe wavelengths: 508 nm (a) and 465 nm (b). Kinetics of BAP 50 μM in the presence of [poly(dG-dC)]$_2$ ($1/r_0 = 25$) at corresponding probe wavelengths: 520 nm (c) and 486 nm (d). $\lambda_{pump} = 404$ nm, 25 mM phosphate buffer pH 7.0, 150 mM NaCl.

The photophysical behavior of BAP in the presence of [poly(dG-dC)]$_2$ is very different. In this case, the increase of both fluorescence lifetimes with respect to free BAP is not accompanied by an increase in the fluorescence quantum yield (Table 1), revealing the occurrence of a static quenching of the BAP fluorescence. One notes a significant change in the ratio of the amplitudes of both fluorescence decay components measured for complexed BAP with respect to free BAP (Table 1). In the case where the two conformations of BAP interact with [poly(dG-dC)]$_2$ without changing their characteristics, this observation suggests that it is the planar BAP conformation intercalated between GC base pairs (with a lifetime of 8.1 ns) which predominantly deactivates via this nonradiative pathway.

This static quenching of fluorescence does not result in an increase of the intersystem crossing efficiency because the quantum yield of triplet state formation of BAP−[poly(dG-dC)]$_2$ complex is 2−3-fold smaller than that of free BAP or BAP−[poly(dA-dT)]$_2$ complexes (Table 1). As solvent-induced internal conversion of intercalated BAP is inefficient (as observed in the presence of [poly(dA-dT)]$_2$), another nonradiative process, with a high quantum yield $\Phi_{nr} = 1 - (\Phi_T + \Phi_f) \approx 60\%$ and related to the porphyrin−polynucleotide interaction, should be responsible for both the static fluorescence quenching and the observed decrease of Φ_T. To identify this fast process, femtosecond transient absorption measurements were undertaken. It must be noted that only part of the intercalated molecules undergoes this radiationless process. Indeed, BAP fluorescence is not completely quenched and triplet absorption is still detected, which is in good agreement with the existence of different binding sites for the BAP−[poly(dG-dC)]$_2$ intercalated complexes.

Femtosecond Transient Absorption Spectroscopy. A global red-shift is observed in the time-resolved absorbance difference spectra of BAP upon its complexation with [poly(dG-dC)]$_2$ by comparison with that of the free chromophore[28] (Figure 2a). The strong negative absorbance band corresponding to the Soret band bleaching has its maximum at 450 nm versus 424 nm for the free chromophore,[28] and one can only distinguish the effects of the visible ground-state absorption bleaching at ∼530 nm (Q_y(1,0) band).

The corresponding transient absorbance kinetics has different profiles depending on the wavelength as illustrated in Figure 3, but in every case presents a plateau which remains flat to within a few percent of the total amplitude on the picosecond time scale (≫50 ps). Measurements of buffer alone showed negligible solvent contribution to the signal. A satisfactory fit of the transient absorption signals over the whole spectral domain is obtained with at least two exponential components (lifetimes of 120 ± 20 fs and 3 ± 1 ps) in addition to the long-lived component (≫50 ps). Thus, at least three different molecular states or species are involved in those kinetics. Given

the important differences between the three time constants, one can consider that each $a_i(\lambda)$ coefficient resulting from transient spectra analysis corresponds to the decay of one species.

The reconstructed wavelength-dependent amplitudes (Figure 2b) for BAP−[poly(dG-dC)]$_2$ complexes do not show any band susceptible to be attributed to stimulated emission from the S_2 state of BAP.[19] This result indicates that the lifetime of the excited electronic state associated to the Soret transition is too short to be resolved in our measurements (<80 fs), as was already observed for the free chromophore.[28] Thus, the lifetimes presently measured correspond either to a vibrational evolution into the S_1 state manifold and/or to another fast process possibly involving a low-lying intermediate charge-transfer state (see Discussion).

The effect of the intercalation of BAP between GC base pairs is predominantly visible in the amplitude $a_1(\lambda)$ of the femtosecond component. For free BAP, $a_1(\lambda)$ is significantly weaker and was attributed to a vibrational relaxation in the first excited singlet state.[28] In the presence of the polynucleotide, an important maximum around 520 nm and a growth below 450 nm toward shorter wavelengths were observed in the $a_1(\lambda)$ spectrum (Figure 2b). Furthermore, the exponential component associated to this a_1 amplitude spectrum is much shorter lived than that for free BAP (120 vs 200 fs).

In contrast, the amplitude spectra $a_2(\lambda)$ (picosecond process) and $a_3(\lambda)$ (long-lived component) measured for BAP−[poly(dG-dC)]$_2$ complexes are not significantly different from those measured for free BAP.[28] Thus, $a_2(\lambda)$ was similarly attributed to a cooling process or an excited-state conformational change, and $a_3(\lambda)$ was attributed to the differential absorbance of the thermally equilibrated S_1 state of the porphyrin having nanosecond lifetime (Table 1).

The effect of the BAP−polynucleotide interaction is also reflected in the transient absorbance kinetics (Figure 3) (one notes that the probe wavelengths for both samples were chosen to be equivalent, taking into account the red-shift of the absorption spectrum of the porphyrin upon its complexation). In the spectral domain of the red-edge tail of the Soret band (Figure 3b,d), the short time evolution of the transient absorption signal is very different in the two cases: the rise in absorbance detected for free BAP becomes an absorbance decay in the presence of G−C base pairs. Concerning the second set of kinetics (Figure 3a,c), one notes the much lower weight of the a_3 component in the case of BAP/[poly(dG-dC)]$_2$ as compared to free BAP. However, it may be noted that only partial ground-state recovery is observed in all kinetics of femtosecond transient absorption which include slower processes (described by the a_2 and a_3 spectral components) than the process associated to a_1, a result in good agreement with different excited BAP population upon its complexation with [poly(dG-dC)]$_2$.

Discussion

The present results reveal the occurrence of an efficient radiationless transition S_1-S_0 when BAP is intercalated between GC base pairs, a process not observed for the free chromophore or in an AT base pair environment. Photoinduced electron transfer from guanine (G) can be considered as a possible quenching process of the excited singlet state of BAP (BAP*), producing the electron-transfer intermediate [BAP$^{\bullet-}$, G$^{\bullet+}$]. Similar processes were already reported for other chromophores close to guanine sites.[14,31−33] To check this hypothesis, the Gibbs free energy was estimated using the Rehm−Weller equation.[34] Taking the reduction potential for BAP in its ground state as −0.26 eV,[35] its excited-state energy as 1.92 eV,[27] and the oxidation potential of guanine in [poly(dG-dC)]$_2$ as 1.16 eV (vs NHE),[36] the Gibbs free energy for the forward electron transfer (from guanine to the lowest excited singlet state of BAP) was calculated to be −0.50 eV.

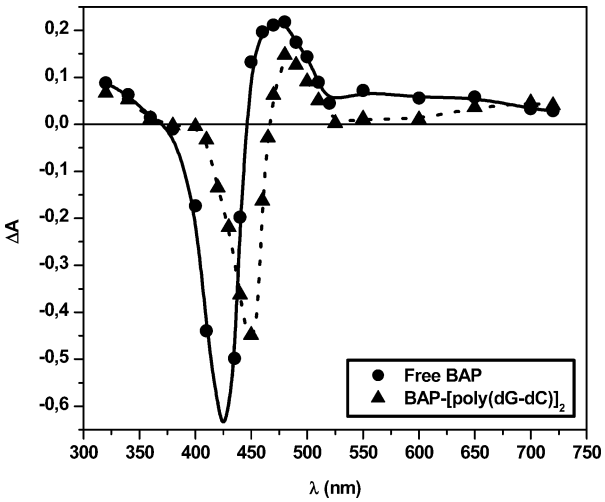

Figure 4. Transient absorption spectra of free BAP and BAP−[poly(dG-dC)]$_2$ complex in N_2O saturated buffered solution at the end of the laser pulse (10 ns). [BAP] = 10 μM, $1/r_0 = 25$, 25 mM phosphate buffer pH 7.0, 150 mM NaCl.

The femtosecond transient absorption spectroscopy experiments have allowed us to prove that BAP photoexcitation induces the oxidation of guanine. Indeed, the a_1 amplitude spectrum of the BAP−[poly(dG-dC)]$_2$ complex in Figure 2b shows a broad and intense maximum centered at 520 nm not observed for free BAP. This transient differential absorption is consistent with the reported absorption maxima of the guanine radical cation (525 nm)[37] and very likely also with the BAP radical anion. Although no data on the BAP radical anion absorption spectrum are yet available, several reports on different porphyrins including H_2TMPyP-4 have revealed a significant absorbance of their radical anions ($\epsilon > 10\,000$ M^{-1} cm^{-1} above 450 nm).[35,38−40] Due to the positive value of the a_1 amplitude, the earliest spectral evolution measured is an absorption decay, which means that the associated process corresponds to the reverse electron transfer from BAP radical anion to guanine radical cation and not to the forward electron transfer.

This back electron transfer is a very efficient process. Indeed, the radical ions formed in the forward electron transfer are expected to undergo thermalization to produce long-lived species. However, nanosecond transient absorption measurements did not show any evidence for any radical ion absorption and in particular for that of the guanine cation radical (maximum ∼525 nm). Indeed, the nanosecond transient absorption spectra are similar for free and intercalated BAP (the spectral shift being only due to the complexation) and correspond in both cases to the differential absorption between the ground and triplet states of BAP (Figure 4). Besides, no significant kinetic evolution occurs over 50 ps (our upper limit of time observation), which could be attributed to electron recombination (Figure 3).

Taking into account this result, the forward electron transfer from guanine to excited BAP is still faster and occurs within the exciting laser pulse duration ($k \geq 10^{13}$ s^{-1}). It probably proceeds through the formation of a charge-transfer state (CT) lying below the first locally excited singlet state S_1 and above the lowest triplet state T_1 of BAP. Indeed, S_1 is quenched but not T_1 because the T_1 lifetime is not shortened upon complexation with [poly(dG-dC)]$_2$. Direct electron transfer from the

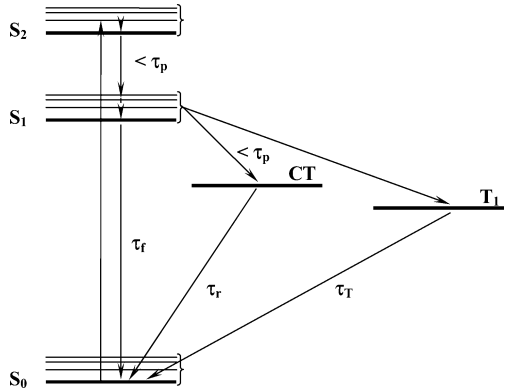

Figure 5. Schematic diagram showing the assumed relaxation channels of the BAP−[poly(dG-dC)]$_2$ complex: S_2 to S_1 decay within the pulse duration τ_p, electron transfer from the S_1 state within the pulse duration τ_p, back electron transfer ($\tau_r = 120 \pm 20$ fs), S_1 to T_1 intersystem crossing, fluorescence decay ($\tau_f = 8.2 \pm 0.8$ ns). The lifetime of the triplet state τ_T is in the order of 20 μs.

locally excited S_2 state might also be considered a priori in view of its very fast rate, in competition with the S_2-S_1 internal conversion. However, this possibility can be ruled out because the triplet formation quantum yield was found to be quite similar upon excitation either into the S_1 state (532 nm) or into the S_2 state (355 nm). A schematic illustration of the states and processes involved is shown in Figure 5.

Such ultrafast electron-transfer rates have been already reported for aromatic molecules intercalated into polynucleotides, with the electron acceptor chromophore and the donor nucleobase located adjacent to each other.[31,33] Although no statement can be made a priori about the adiabatic or nonadiabatic character of the forward electron transfer, the fast back electron transfer, a CT $\rightarrow$ S_0 transition, may be expected to be nonadiabatic and thus considered within the frame of the Marcus classical treatment. According to this model, the rate constant for the reverse electron transfer is given by

$$k = \frac{4\pi^2}{h\sqrt{4\pi\lambda k_B T}}|V|^2 \exp\left(-\frac{(\Delta G + \lambda)^2}{4\lambda k_B T}\right)$$

where λ is the reorganization energy (of reactants and solvent), $|V|$ is the electronic coupling matrix, k_B and h are the Boltzmann and Planck constants, respectively, and T is the absolute temperature.[41] Using the calculated free energy for the reverse electron transfer $\Delta G_r = -1.42$ eV, the measured rate constant $k = 8.3 \times 10^{12}$ s^{-1}, and $\lambda = 1$ eV (a typical value of the reorganization energy in a polar solvent like water), one finds $|V| = 230$ cm^{-1}, which is of the order of magnitude of k_BT and also close to the values reported in recent papers[31,33] for similar face-to-face acceptor−guanine intercalation complexes undergoing very fast electron transfer. This $|V|$ value supports the assumption of the validity of the Marcus treatment for this back electron transfer.

We have shown in the present study that photoexcited BAP is able to oxidize the guanine base when it is intercalated at a d(CpG)$_2$ step and that the reverse electron transfer in [poly(dG-dC)]$_2$ is very efficient, thus a priori preventing DNA from permanent damage. Similar examples of very efficient recombination following guanine oxidation by intercalated molecules have been reported recently in the literature.[14,42,43] This result is not in contradiction with the assumption that the electron-transfer reaction may participate in the enhancement of the photodynamic activity of cationic porphyrins by comparison to anionic or metallic porphyrins especially in anaerobic conditions.[15−17] Indeed, irreversible DNA damages have been observed in the case of long-lived charge-separated states;[43] the charge separation lifetime closely depends on DNA sequences which play a key role in both initial hole localization and probability of hole migration.[14,44,45] This relationship is now currently under investigation with BAP complexed to various oligonucleotides.

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Supporting Information Available: Structure of the BAP molecule at pH 7.0. This material is available free of charge via the Internet at http://pubs.acs.org.

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