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Dynamics and Mechanism of the Exciplex Formation between Cu(TMpy-P4) and DNA Model Compounds Revealed by Time-Resolved Transient Absorption and Resonance Raman Spectroscopies

Sergei G. Kruglik,[†] Victor A. Galievsky,[‡] Vladimir S. Chirvony,[‡] Pavel A. Apanasevich,[†] Vladimir V. Ermolenkov,[†] Valentine A. Orlovich,[†] Laurent Chinsky,[§] and Pierre-Yves Turpin^{*,§}

B. I. Stepanov Institute of Physics, Academy of Sciences of Belarus, 70 F. Skaryna Ave., Minsk 220072, Belarus; Institute of Molecular and Atomic Physics, Academy of Sciences of Belarus, 70 F. Skaryna Ave., Minsk 220072, Belarus; and Institut Curie and Université Pierre et Marie Curie, 11 rue Pierre et Marie Curie, F-75231 Paris Cedex 05, France

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The dynamics and mechanism of the photoinduced reversible process of formation and decay of an exciplex species created between the water-soluble cationic metalloporphyrin copper 5,10,15,20-tetrakis[4-(*N*-methylpyridyl)]porphyrin (Cu(TMpy-P4)) and the DNA model compound poly(dA-dT) have been studied in detail. Such a photoinduced process had been previously observed in transient resonance Raman (RR) spectra under high-power laser irradiation of complexes of Cu(TMpy-P4) with calf thymus DNA and some oligo- and polynucleotides containing thymine (T) or uracile (U) residues. It was found that the interaction of excited Cu(TMpy-P4) with carbonyl groups of T or U involved in polymers having an appropriate secondary structure was responsible for the new transient species detected in high-power Raman spectra. In the present work, direct kinetic measurements of the exciplex formation between Cu(TMpy-P4) and poly(dA-dT) were carried out by using both picosecond transient absorption pump–probe technique (10-ps time resolution) and two-color time-resolved RR technique (100-ps time resolution). A comparative nanosecond Raman study of this exciplex and of the excited (d,d) state of copper *meso*-tetraphenylporphyrin (CuTPP) model compound dissolved in a number of oxygen-containing solvents has also been performed, to clarify the excited electronic state which is at the origin of this process. It has been found that the binding of one of the CO-groups of T or U to Cu(TMpy-P4) in its lowest excited triplet state results in a shortening of the triplet-state lifetime to 35 ± 7 ps. In addition, a population of an excited $^2[d_x^2, d_{x^2-y^2}]$ state, i.e., the most low-lying and long-lived excited state for the five-coordinated Cu(TMpy-P4) (exciplex state), occurs in the process of excitation relaxation. Large wavenumber shifts of structure-sensitive vibrational marker lines from the porphyrin skeleton reveal the promotion of one of the copper d electrons into the half-filled $d_{x^2-y^2}$ orbital and the expansion of the porphyrin core to accommodate the occupation of this d orbital. The exciplex deactivation process (excited (d,d) state decay) has a time constant of 3.2 ± 0.5 ns and is accompanied by the CO-group deattachment with a disruption of the exciplex into initial components.

Introduction

In the recent past, an ability for water-soluble cationic porphyrins and metalloporphyrins to interact with DNA has been revealed and studied.^{1–4} At least partially, the motivation for these studies derives from the fact that porphyrins are photosensitizers and on exposure to light are antitumor active.^{1,5–7} On the other hand, a variety of metal ions can be introduced into porphyrins that in turn influences the mode of their interaction with DNA. Among cationic porphyrins, H₂-5,10,15,20-tetrakis[4-(*N*-methylpyridyl)]porphyrin (H₂(TMpy-P4)) and its metal (Me) derivatives have been specially well studied, because they yield only low self-association in water solutions.^{8,9} Fiel et al.⁵ were the first to demonstrate, by using optical absorption, circular dichroism (CD) and viscosimetry techniques, an ability for H₂(TMpy-P4) to be intercalated into calf thymus DNA. Then a variety of metal derivatives were later found to interact with nucleic acids.^{10,11} Various methods

were used to study their mode of interaction: luminescence,^{12,13} EPR,¹⁴ NMR,^{15–17} linear dichroism,^{18,19} viscosimetry,^{20,21} and resonance Raman (RR) spectroscopies.^{22–27}

There are at least three modes of binding of Me(TMpy-P4) to DNA:^{1–3}

(i) Outside or groove binding preferentially at A-T sites, and possibly at G-C sites of DNA but with a much lower affinity. This is characteristic for methylporphyrins having axial ligands.

(ii) Planar (four-coordinate) methylporphyrins preferentially intercalated at G-C sites.

(iii) External mode of binding, in which neighbor porphyrin molecules can interact with each other, with a possible stacking along the outside surface of DNA.^{28,29}

Two of these modes of DNA interaction are characteristic for Cu(TMpy-P4) investigated in the present work: a major part of the molecules is intercalated between G-C base pairs, while a minor part can also be groove-bound at ATAT sites.²⁶

Recently, an additional light-driven process of interaction with DNA was found exclusively for Cu(TMpy-P4): an interaction of electronically excited Cu(TMpy-P4) with DNA and some DNA model compounds which provides an intermolecular excited complex (so-called exciplex).^{24–27} The exciplex was

[†] B. I. Stepanov Institute of Physics.

[‡] Institute of Molecular and Atomic Physics.

[§] Institut Curie and Université Pierre et Marie Curie.

* To whom correspondence should be addressed.

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featured by the appearance of new prominent porphyrin marker lines in RR spectra recorded at high laser excitation power in the Soret or the Q electronic absorption bands of the porphyrin molecule. The probable photophysical origin of the exciplex building process was assumed at first to be a charge separation between Cu(TMpy-P4) and DNA with a formation of a porphyrin π -cation radical $[\text{Cu}(\text{TMpy-P4})]^+$ and a nucleic residue anion radical, $[\text{dA-dT}]^-$ or $[\text{dT}]^-$.^{24,25} Further RR study of Strahan et al.²⁶ revealed that this process involves only the porphyrin species groove bound at AT sites of DNA: a photoinduced translocation was proposed for Cu(TMpy-P4) from GC/CG to ATAT sites, resulting in the exciplex formation under specific requirements of a flexibility of Pu-Py alternating double-helical structure. At last, very recent RR studies²⁷ have shown that thymine or uracil (but not cytosine) residues are needed to build up the exciplex, provided that they occur within a sequence of a minimum length and of a favorable secondary structure. Direct participation of thymine in the exciplex formation was confirmed in two-color UV-vis RR experiments, where dramatic changes in some thymine line intensities were observed due to exciplex formation.³⁰ A photoinduced axial ligation of the central porphyrin copper ion by thymine or uracil carbonyl groups with a participation of the excited (d,d) state of Cu(TMpy-P4) was proposed as a mechanism for the exciplex formation.²⁷ However, the results presented in ref 27 were not sufficient to definitely determine the whole picture of photophysical processes that occur during exciplex formation.

Until now, the specific interactions described above of water-soluble Cu(TMpy-P4) with DNA and DNA model compounds were investigated mainly by RR techniques, in using nanosecond laser pulses.²⁴⁻²⁷ Meanwhile, pump-probe RR experiments have proven that the exciplex lifetime is shorter than a 20 ns time resolution.^{24,26} In the present work, we focus on the dynamics and mechanism of the processes photoinduced in a mixture of Cu(TMpy-P4) with poly(dA-dT), in which Cu(TMpy-P4) molecules are known to be groove bound to AT sites. Kinetics measurements were performed by using a picosecond transient absorption technique with a 10-ps time resolution. On the other hand, two-color time-resolved RR measurements were carried out to directly determine the decay time of the exciplex transient Raman bands and to set up a correspondence between excited-state species registered in both transient absorption and RR studies. A comparative study of the Cu(TMpy-P4)-poly(dA-dT) complex and of a copper *meso*-tetraphenylporphyrin model compound (CuTPP) in a number of oxygen-containing solvents has also been made to define more precisely the excited electronic state which is at the origin of the exciplex under investigation.

We found the following dynamical picture of the exciplex formation and decay for Cu(TMpy-P4) mixed with poly(dA-dT) in a phosphate buffer: after excitation, the ligation of Cu(TMpy-P4) by a CO group of thymine occurs in the excited triplet state $^2\text{^4T}_1$, with a porphyrin lifetime shortening down to 35 ± 7 ps as a consequence. This ligation process is followed by the population of an energetically downshifted "intrametal" $^2[\text{d}_{x^2-y^2}, \text{d}_{xy}]$ excited state of the $[\text{Cu}(\text{TMpy-P4})\text{-L}]$ five-coordinated complex (L = CO group of thymine), this (d,d) state being the most long-lived key "bottleneck" transient state detectable in RR spectra at high-power laser irradiation: this constitutes the exciplex. The exciplex deactivation corresponds to the excited (d,d) state decay and actually occurs with a time constant of 3.2 ± 0.5 ns.

For comparison, the results of a preliminary transient absorption study of free Cu(TMpy-P4) in a phosphate buffer solution are also presented. In this case, the lifetime of the triplet excited

state $^2\text{^4T}_1$ was found to be 21 ± 3 ps, this likely indicating the quenching role of water molecules.

Experimental Section

The Cu(II) derivative of 5,10,15,20-tetrakis(4-*N*-methylpyridyl) porphyrin is a generous gift from Prof. K. Nakamoto (Marquette University, Milwaukee WI. Poly(dA-dT) was purchased from Pharmacia Biochemicals. These compounds have been used as received.

The sample solutions have been prepared by mixing an aqueous solution of Cu(TMpy-P4) with a phosphate buffer solution of the nucleic acids. The final solution thus obtained (pH = 6.8 and ionic strength $\mu = 0.2$) contained Cu(TMpy-P4) and nucleic acids (concentration in base pairs for double-stranded polynucleotides) at ca. 3.0×10^{-5} and 1.0×10^{-3} M, respectively, as determined spectrophotometrically.²⁷ The molar ratio of base pairs/Cu(TMpy-P4) was ca. 30.

Transient absorption spectra and kinetics of absorption changes were measured on a homemade tunable pump-probe absorption spectrometer having a 10-ps time resolution. Pumping was provided by the second harmonic of a YAlO₃: Nd solid-state master laser ($\lambda_{\text{pump}} = 540$ nm), while the output beam of an optical parametric oscillator was used as a probing beam tunable over the entire visible region (360–1000 nm). An intracavity negative-feedback electrooptical system in combination with the intracavity pulse selection provided a high laser intensity stability (intensity dispersion < 2%) and resulted in sufficiently high sensitivity and accuracy of the spectrometer (3×10^{-4} OD units).

Raman spectrometer has been built on the basis of a homemade pulsed Nd:YAG laser with repetition rate 12.5 Hz and pulse duration of either 10 ns (Q-switch regime^{31,32}) or 100 ps (mode locker plus cavity dumper regime³³). A frequency conversion of YAG harmonics in a H₂ Raman shifter was implemented to obtain radiations at 416 nm (the first Stokes component of the Nd:YAG third harmonic^{34,35}) or 436 nm (the first anti-Stokes component of the Nd:YAG second harmonic), for near-Soret excitation of the samples. For measurements of Raman excitation profiles (REP), radiations from the homemade narrow-band dye laser pumped by the second and the third harmonics of the master Nd:YAG laser were used for Raman probing, as well as the radiations obtained from the frequency summations of Nd:YAG fundamental and dye laser outputs. To create a photoinduced population of excited states of the molecules under investigation, we used the intense pumping radiation at 532 nm (Nd:YAG second harmonic) falling in resonance with the Q₀₋₁ band of metalloporphyrin absorption. Pumping and probing beams were brought into coincidence on the sample by a dichroic mirror. All Raman spectra were excited under a backscattering geometry and were dispersed by a double grating monochromator. For low-intensity measurements, the laser radiation was focused near the front surface of a sample cell by a cylindrical lens in a band of ca. 1×5 mm. In contrast, high excitation power was provided by a sharp focusing, by using a spherical lens giving a circular spot of 0.1–1 mm in diameter. The Raman analog signal obtained from the photomultiplier was processed and digitized by a rapid stroboscopic voltmeter. Monochromator scanning, control of the laser beams intensities, data acquisition, and processing were provided by a PC computer and CAMAC standard equipment.

All spectra were recorded at room temperature. Samples were contained in a rotating quartz cell with 1 mm thickness to avoid heating effects and photodecomposition by successive laser pulses. The absence of sample degradation has been checked by measuring stationary absorption spectra before and after each

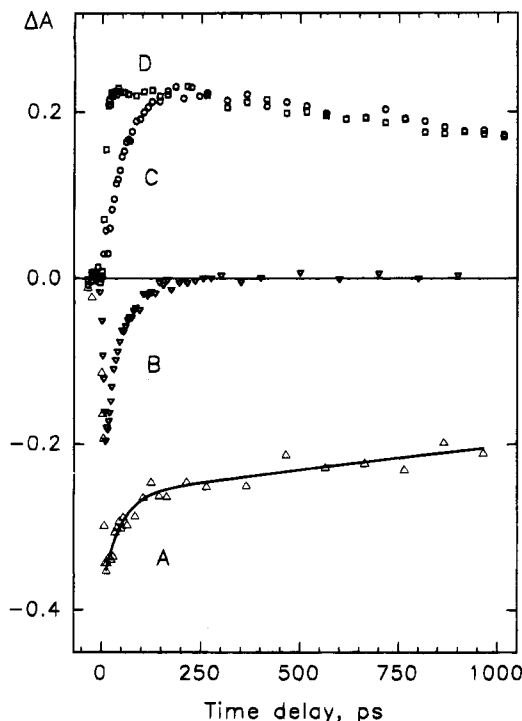


Figure 1. Time evolution of absorption difference of Cu(TMpy-P4) mixed with poly(dA-dT) probed at different wavelengths: 417 (A), 437 (B), 441 (C), and 453 nm (D). The solid line curve A represents a least-squares fit to a double-exponential function having time constants of 35 ± 7 ps and 3.2 ± 0.5 ns. Pumping was provided by a radiation flux of 100 mJ/cm^2 at 540 nm. The maximum standard deviation in ΔA was 0.005.

RR experiment and immediately during measurements in the case of picosecond absorption experiments.

Results

By exciting the Cu(TMpy-P4)-poly(dA-dT) solution with 10-ps pulses at 540 nm, fully reversible optical density changes (OD) are induced in the spectral region corresponding to the porphyrin Soret band (390–470 nm). Typical kinetics of OD changes exhibits a two-phase character and can be well fitted with a two-exponential function, with time constants of 35 ± 7 ps and 3.2 ± 0.5 ns. An example of such a typical kinetics is shown in Figure 1, curve A, recorded at $\lambda_{\text{probe}} = 417$ nm. Difference absorption spectra corresponding to both kinetical phases were recorded at time delays of 5 and 150 ps after excitation. These two difference spectra (Figure 2) differ from each other by the position of their isosbestic points (i.e., wavelengths where $\Delta OD = 0$) and, despite of their shape resemblance, they characterize the two different transient species observed in the kinetical measurements as short-lived (35 ps) and long-lived (3.2 ns) components.

To estimate the absolute absorption spectra of these two transient species from their experimentally measured difference spectra, it is necessary to know the percentages of probed molecules which are pumped from the ground to the corresponding transient excited state. This has been done by measuring the dependence of the observed OD changes as a function of the pumping pulse energy. A saturation curve has been obtained for ΔOD measured at 427 nm (Figure 3), which shows a plateau for pumping energy densities $E_{\text{pump}} \times 3 \geq 60 \text{ mJ/cm}^2$: about 100% of the molecules are thus pumped into their first (short-lived) transient state for pumping densities greater than this threshold. Therefore, since we routinely used $E_{\text{pump}} = 100 \text{ mJ/cm}^2$, we can simply add the measured $\Delta OD(\lambda)$

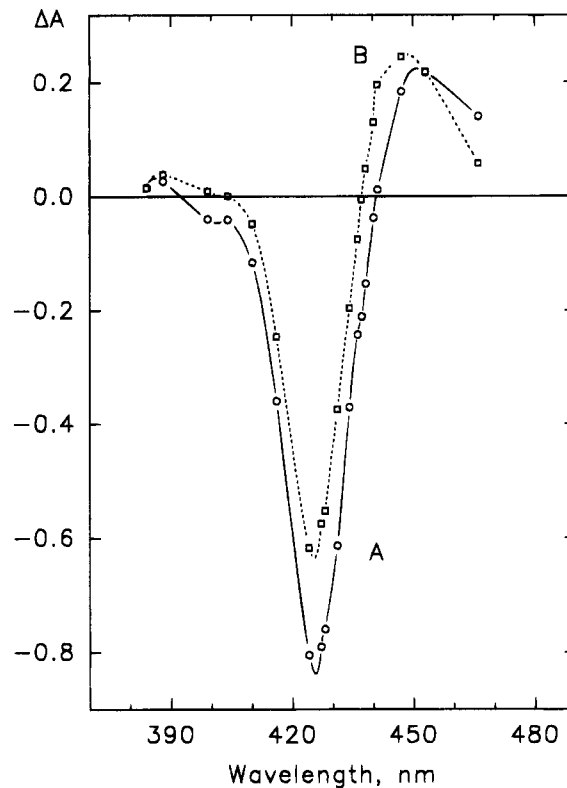


Figure 2. Difference absorption spectra within the Soret-band region for Cu(TMpy-P4)-poly(dA-dT) at time delays 5 (A) and 150 ps (B) following excitation with 540 nm.

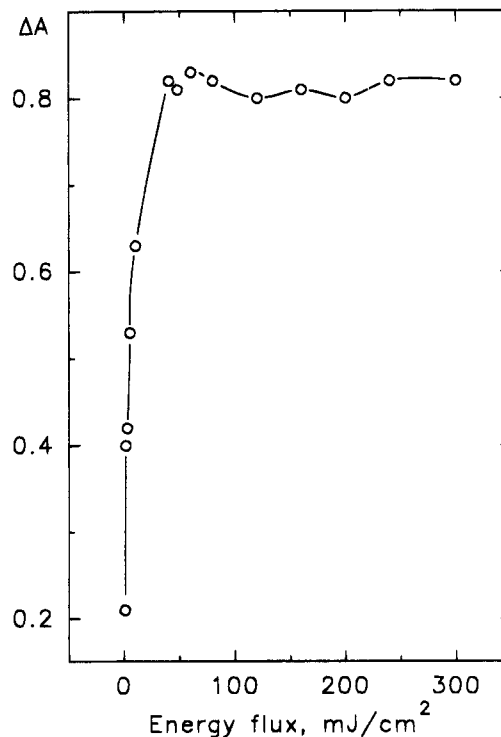


Figure 3. Energy density dependence of absorbance changes at 427 nm for Cu(TMpy-P4)-poly(dA-dT). The measurements were carried out at 5 ps delay time after 540 nm excitation.

values to the ground state absorption spectrum $A(\lambda)$ to obtain the absolute absorption spectrum of the first transient species. The reconstructed absorption spectra of both the first and second transient species are shown in Figure 4, along with the ground state absorption in the Soret-band region (curve C). Note that a 100% conversion from the first transient species to the second

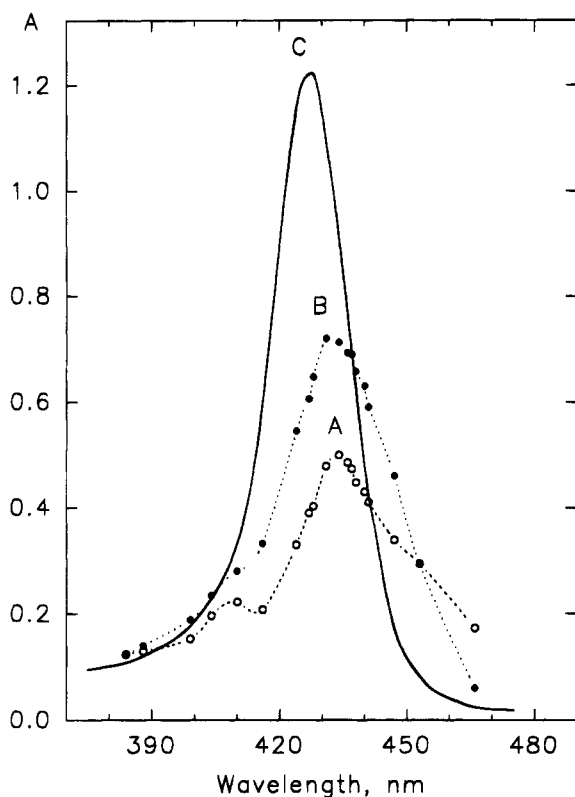


Figure 4. Ground-state absorption spectrum of Cu(TMpy-P4)-poly(dA-dT) (C) and its reconstructed transient absorption spectra at time delays of 5 (A) and 150 ps (B) after excitation pulses. The A and B absorption spectra correspond to transient species having lifetimes of 35 ± 7 ps and 3.2 ± 0.5 ns, respectively.

one has been assumed to reconstruct the absorption spectrum of the second (long-lived) transient species. It is worthy to be noted that half-widths of the Cu(TMpy-P4) Soret band for Cu(TMpy-P4) bound to poly(dA-dT) (Figure 4, curve C) and of free Cu(TMpy-P4) in a phosphate buffer solution (Figure 6, curve A) are quite different: $\Delta\nu_{0.5} = 990 \text{ cm}^{-1}$ for Cu(TMpy-P4)-poly(dA-dT) complex, while $\Delta\nu_{0.5} = 1210 \text{ cm}^{-1}$ for free Cu(TMpy-P4). This observation likely reflects a more ordered arrangement of the porphyrin molecules inside the polynucleotide, resulting in a partial elimination of an inhomogeneous broadening, rather than a porphyrin-porphyrin excitonic interaction process.¹³

Besides the observation of the two-exponential decay kinetics (Figure 1A) at $\lambda_{\text{probe}} = 417 \text{ nm}$, there are three other wavelengths which yield single-exponential decays of OD changes: at 453 nm (Figure 1D), 441 nm (Figure 1C), and 437 nm (Figure 1B). It is seen from the spectra of Figure 4 that these wavelengths also correspond to isosbestic points: between the first (1) and the second (2) transient species ($\lambda_{\text{probe}} = 453 \text{ nm}$), between the ground state (0) and the first transient species (1) ($\lambda_{\text{probe}} = 441 \text{ nm}$), and between the ground state (0) and the second transient species (2) ($\lambda_{\text{probe}} = 437 \text{ nm}$), respectively. One of the three kinetics in a series of transitions $0 \rightarrow 1$, $1 \rightarrow 2$, and $2 \rightarrow 0$ becomes invisible in these isosbestic points. Indeed, the kinetics at 453 nm (Figure 1D) exhibits a fast rise time, reflecting the population of the first transient species and then a slow decay with the lifetime characteristic for the $2 \rightarrow 0$ transition, because the $1 \rightarrow 2$ transition is not seen at this wavelength (the corresponding extinction coefficients are equal: $\epsilon_1 = \epsilon_2$). The kinetics at 441 nm (curve 1C) shows a slow rising rate corresponding to the $1 \rightarrow 2$ transition, followed by a decay corresponding to species 2 because the $0 \rightarrow 1$ transition is not seen at this wavelength. At last, the 437 nm kinetics (curve

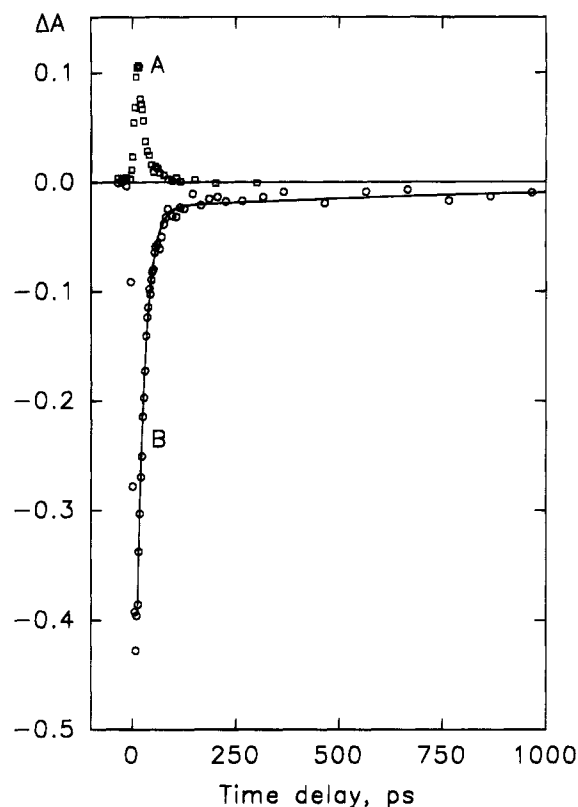


Figure 5. Time evolution of absorption difference for free Cu(TMpy-P4) in a phosphate buffer observed at probing wavelengths 453 (A) and 426 nm (B). The solid line represents a least-squares fit to a single-exponential function with 21 ± 3 ps time constant.

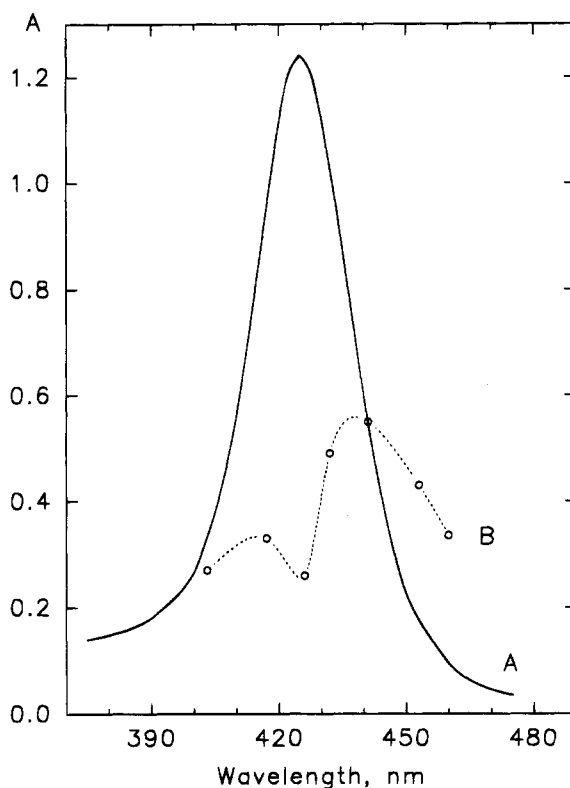


Figure 6. Ground-state absorption spectrum of free Cu(TMpy-P4) in a phosphate buffer (A) and its reconstructed transient absorption spectrum at 5 ps delay after excitation (B).

1B) reflects the population of species 1, followed by "pure" $1 \rightarrow 2$ transition kinetics because the ΔOD at this wavelength is not sensitive to the subsequent $2 \rightarrow 0$ relaxation.

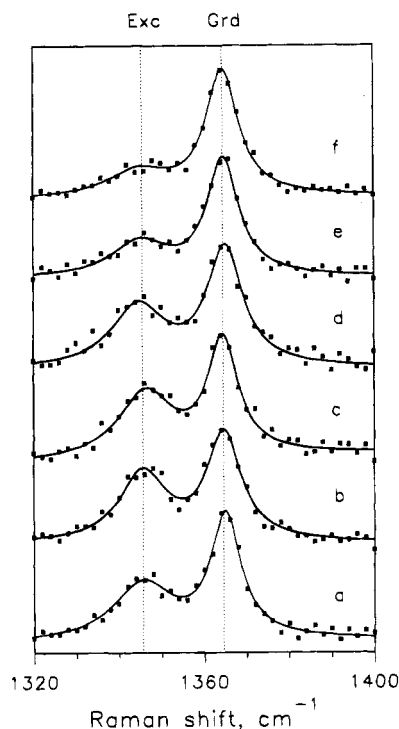


Figure 7. Two-color time-resolved RR spectra of Cu(TMPy-P4) bound to poly(dA-dT), recorded with pumping at 532 nm ($t_{\text{pump}} = 100$ ps, $E_{\text{pump}} \approx 1$ mJ) and probing at 416 nm ($t_{\text{probe}} \leq 80$ ps, $E_{\text{probe}} \approx 20$ μ J). Pump-probe delay times are (a) 0 ps, (b) 100 ps, (c) 200 ps, (d) 1 ns, and (e) 3 ns. Spectrum f is a probe-only spectrum.

For sake of comparison, picosecond transient absorption measurements were also carried out for free Cu(TMPy-P4) in a phosphate buffer solution within the 400–465 nm spectral region. Almost all observed Δ OD changes (Figure 5A) were found to decay in following a single-exponential kinetics with a 21 ± 3 ps time constant. However, a weak contribution from a longer transient species having nanosecond decay time has been observed at probing wavelengths where a total value of Δ OD signal was sufficiently high (Figure 5B). The difference absorption spectrum of a short-lived transient species recorded at a 5 ps time delay after pumping has been made (not shown), and the corresponding reconstructed absorption spectrum is given in Figure 6B, while Figure 6A represents the 400–460 nm ground-state Soret band of free Cu(TMPy-P4) in phosphate buffer solution. Again, for making this reconstruction the dependence of the Δ OD values versus the pumping energy density was evaluated (saturation curve, not shown) to find the fraction of excited molecules in the probed volume. This fraction has been found to be equal to 55% for the experimental conditions ($E_{\text{pump}} = 100$ mJ/cm²). Note that the intensity of the long-lived component was too weak to allow its absorption spectrum to be reconstructed.

To establish a direct correspondence between the transient species detected in picosecond absorption and nanosecond RR saturation measurements, time-resolved two-color RR spectra of the Cu(TMPy-P4)-poly(dA-dT) complex were recorded by using a picosecond Raman spectrometer specially designed for this purpose. Figure 7 gives RR spectra in the 1320–1400 cm⁻¹ spectral region where the most prominent symmetric ν_4 vibrational mode of the porphyrin shows up both in the ground ($\nu_{\text{grd}} = 1364$ cm⁻¹) and excited exciplex state ($\nu_{\text{exc}} = 1346$ cm⁻¹). These RR spectra were recorded with pump-probe delay times of (a) 0 ps, (b) 100 ps, (c) 200 ps, (d) 1 ns, and (e) 3 ns. Spectrum f is a probe-only spectrum. Pumping was provided by the intense green Nd:YAG laser radiation ($\lambda_{\text{pump}} = 532$ nm,

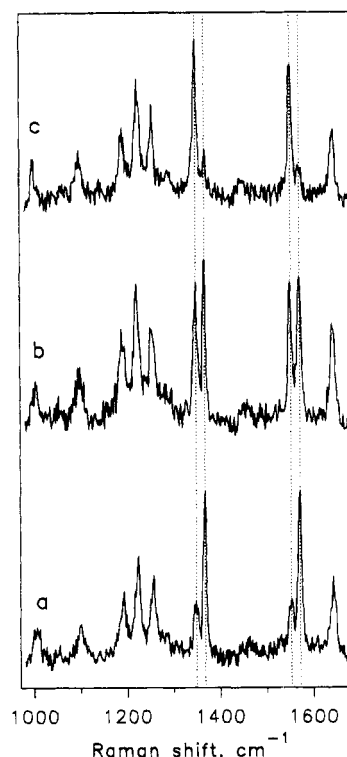


Figure 8. One-color saturation RR spectra of Cu(TMPy-P4) mixed with poly(dA-dT) obtained with excitation by 10 ns pulses at 436 nm. Incident power densities are (a): 1×10^5 , (b) 8×10^5 , and (c) 7×10^6 W/cm².

$t_{\text{pump}} = 100$ ps, $E_{\text{pump}} \approx 1$ mJ), while for probing a weak blue radiation was implemented ($\lambda_{\text{probe}} = 416$ nm, $t_{\text{probe}} \leq 80$ ps, $E_{\text{probe}} \approx 20$ μ J). Experimentally measured intensities are presented in Figure 7 by markers, and solid line profiles are the best fit obtained with two Lorentzian contours centered at 1364 and 1346 cm⁻¹. It should be noted that the appearance of a small shoulder at 1346 cm⁻¹ in spectrum f is due to the perturbation caused by the proper probing beam. Transient Raman spectra given in Figure 7 unambiguously reveal that the exciplex species has a decay time of several nanoseconds, since the intensity of the 1346 cm⁻¹ Raman band remains practically unaltered during 200 ps after excitation (Figure 7a–c), and appreciably decreases only for nanosecond delay times (Figure 7e). Consequently, this estimation is in good agreement with the 3.2 ns value for the exciplex lifetime estimated from transient absorption experiments.

We have also performed detailed comparative studies between photoinduced transients of Cu(TMPy-P4) bound to poly(dA-dT) and those of model CuTPP in various O-containing solvents, by using the saturation Raman technique with 10-ns laser pulses.

Figure 8 presents 436 nm RR spectra of Cu(TMPy-P4)-poly(dA-dT) recorded at three different laser excitation powers: from these spectra it is possible to estimate RR contributions belonging to pure electronic ground state (Figure 9a) and pure excited state (Figure 9b) of the complex. These spectra were obtained by using the following subtracting procedure:

$$a \text{ (Figure 9)} = a \text{ (Figure 8)} - k_1 c \text{ (Figure 8)}$$

$$b \text{ (Figure 9)} = c \text{ (Figure 8)} - k_2 a \text{ (Figure 8)}$$

where k_1 and k_2 factors were empirically evaluated to eliminate negative and/or positive peak contributions for the ν_2 and ν_4 modes in Figure 9.

Furthermore, transient RR spectra of CuTPP (unsoluble in water) in O-containing organic bases such as THF and dioxane/

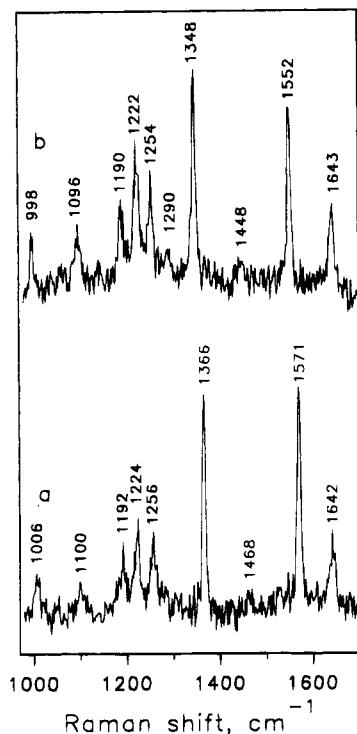


Figure 9. RR spectra of Cu(TMpy-P4)-poly(dA-dT) complex in the electronic ground (a) and excited exciplex (b) states. These spectra were obtained from the spectra depicted in Figure 10 by using the subtracting procedure described in the text.

toluene (1:1) mixture, recorded under the same experimental conditions as for the spectra presented in Figure 8, have been reported in one of our previous papers.⁵⁴

Raman excitation profiles (REP) of both the excited and ground states of Cu(TMpy-P4) bound to poly(dA-dT) were measured earlier by Chinsky et al.²⁵ To compare the resonance enhancement characteristics of the photoinduced transient excited state of this complex with that of CuTPP in O-containing solvents, we have performed pump-probe REP measurements for the ν_4 mode of CuTPP in THF within the Soret-band region. Pump pulse energy at 532 nm was maintained at a fixed energy value of 1 mJ in all experiments, while probing beam, tunable within the 401–448 nm region, was set at the energy level of $\approx 50 \mu\text{J}$ so that no transient features originating from the excited state would appear during probe-only measurements. Raman band of THF solvent at 914 cm^{-1} has been used as an internal standard of intensity. Figure 10 presents plots of normalized Raman intensity of the ν_4 skeletal marker band in the ground (A) and in the transient excited (B) states versus the excitation wavelength. Markers represent experimental points, while curves represent their best least square fits by Gaussian functions. Profile A was corrected from differential reabsorption by using the steady-state absorption spectrum. It was not possible to perform an analogous procedure for the profile B, since the fraction of molecules pumped to the excited state and consequently the values of optical density of this state are not known exactly. Therefore, only the long-wavelength part of the REP corresponding to the excited state is presented in Figure 10, where the effect of differential reabsorption has been considered as negligible. Nevertheless, the remaining points were perfectly well fitted by a Gaussian function centered at ca. 422 nm. The ground-state REP for the ν_4 mode, containing points on the short-wavelength limb of the profile as well, can be sufficiently well fitted by a double-Gaussian function, with the first contour being centered at ca. 414 nm. This agrees with previous theoretical estimations³⁷ of the contributions of both

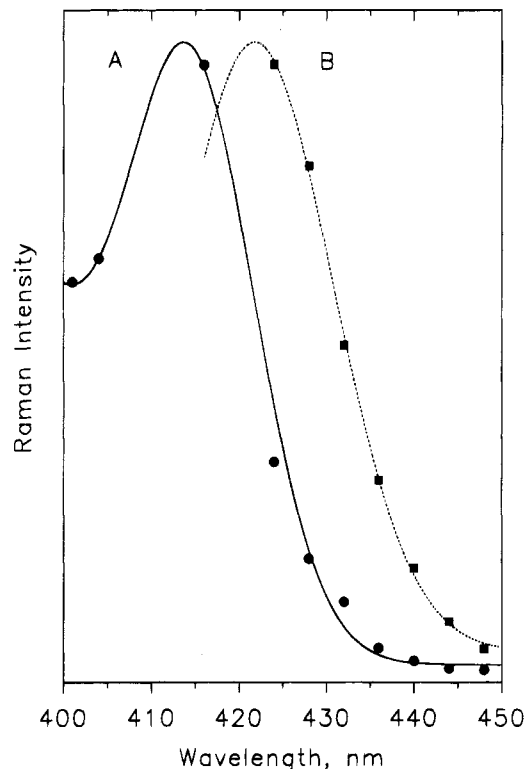


Figure 10. RR excitation profiles of the ν_4 mode in the ground (A) and excited (B) states for CuTPP in THF. Markers represent experimental points, lines represent best fits by Gaussian functions.

the B_{0-0} and B_{0-1} states to the REP measured within the Soret band. It should be noted that the profiles of the ν_4 mode of CuTPP in THF presented in Figure 10, both in the ground and excited states, show a strong resemblance with the corresponding REPs measured for Cu(TMpy-P4)-poly(dA-dT),²⁵ with the exception of an overall red shift of the latter.

Discussion

Before discussing the preceding results, it is worthwhile to present a brief outline of Cu(II) porphyrin photophysics. Excited states of "standard" Cu(II) porphyrins (CuP) soluble in organic solvents like copper(II) octaethylporphyrin (CuOEP), copper(II) *meso*-tetraphenylporphyrin (CuTPP), etc., have been extensively studied during the past 10–15 years.^{38–54} Due to the d^9 configuration of the central Cu(II) ion and π - d interaction, doublet and quartet electronic state manifolds can be realized for Cu(II)P. However, because of the weakness of this π - d interaction, all observed spectral (but not photophysical) properties of CuPs remain qualitatively the same as for other regular metalloporphyrins.³⁸ To emphasize this, the ground and lowest excited doublet states of CuP are usually called as "singdoublets" 2S_0 and 2S_1 , respectively, while the designation "tripdoublet-quartet" state $^{2,4}T_1$ is used for the former lowest (π, π^*) triplet state T_1 ³⁸ (for sake of simplicity, we shall use here the names "singlet states 2S_0 and 2S_1 " and "triplet state $^{2,4}T_1$ "). In the framework of this three-level scheme, the main feature of the CuP photophysics is a very fast (tens of femtosecond⁴⁶) and very effective formally spin-allowed radiationless transition $^2S_1 \rightarrow ^{2,4}T_1$ which is followed by a slower establishment of the thermal equilibrium $^{2,4}T_1 \leftrightarrow ^4T_1$.³⁹

Besides 2S_1 and $^{2,4}T_1$ (π, π^*) excited states, an intramolecular charge-transfer (CT) state of (π, d) origin can take part in the processes of excitation energy relaxation of CuP, although the CT state most likely lies (in noncoordinating solvents like toluene, benzene) slightly above the lowest triplet state (Figure

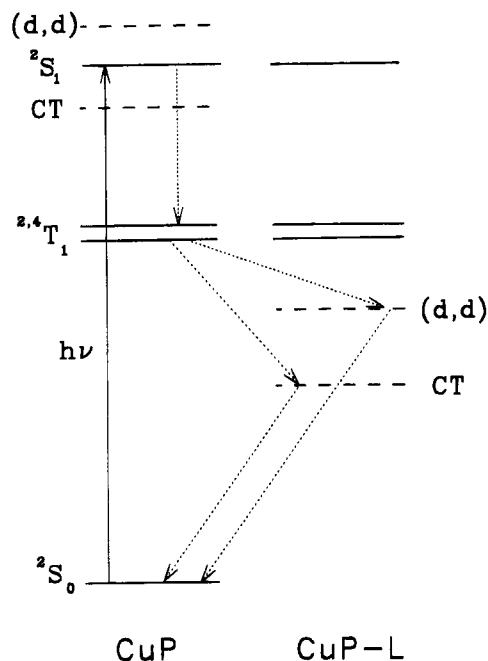
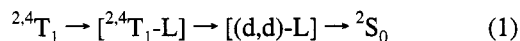


Figure 11. Schematic diagram of electronic energy levels and excitation/deactivation pathways for Cu(TMPy-P4) in noncoordinating solvents (Cu(TMPy-P4)) and for Cu(TMPy-P4), axially ligated by O-containing molecules (Cu(TMPy-P4)-L).

11). Owing to this fact, temperature activated quenching of the $^{2,4}T_1$ manifold is observed in noncoordinating solvents at room temperature, which results in a shortening of the decay time of the $^{2,4}T_1 \rightarrow ^2S_0$ transition, in the range of tens to hundreds of nanoseconds.^{47–49}

In nitrogen containing organic solvents such as pyridine, piperidine, etc., axial liganding of CuP by one of these molecules causes, both in the ground 2S_0 and in the triplet $^{2,4}T_1$ excited states, a downshift of the CT state below the $^{2,4}T_1$ state, with as a result a shortening of the triplet state lifetime down to tens of picoseconds.^{40–44}

At last recently, the dynamics and mechanism of quenching of CuP $^{2,4}T_1$ state by oxygen-containing molecules such as tetrahydrofuran (THF), 1,4-dioxane, etc., were observed and comprehensively studied.^{36,50–54} It was found that the binding of an O-containing molecule L as a fifth (axial) ligand to CuP in the $^{2,4}T_1$ excited state results in a lowering not only of the CT but also of the (d,d) excited state of $^2[d_{x^2-y^2}, d_{z^2-y^2}]$ origin (a schematic picture of this process is presented in Figure 11). As a consequence, two competing paths of excitation energy relaxation are realized after attachment of the molecule L:



It is noteworthy that the [(d,d)-L] excited state is spectroscopically observable in both transient absorption and RR measurements and has a lifetime of ca. 360 ps for CuOEP in THF,^{52,54} while the [CT-L] state was not spectroscopically detected (in transient absorption or RR spectra and kinetics), this being most likely due to a very short [CT-L] state lifetime.⁴²

In the present case dealing with Cu(TMPy-P4), it has earlier been proved²⁷ that the exciplex species formed between Cu(TMPy-P4) in its excited state and DNA or DNA model compounds is created via an interaction of the porphyrin central copper atom with thymine or uracile carbonyl groups, provided the existence of an appropriate fixation mode and of a proper

secondary structure of the polymer. In general, this process has some similarity to that of "standard" CuP liganding (in its $^{2,4}T_1$ state) by O-containing solvent molecules. Indeed, our experimental results enable the excited electronic state origin of the exciplex to be established and to clarify the mechanism of its formation/decay.

Let us first compare the transient absorption data obtained for Cu(TMPy-P4)-poly(dA-dT) with already known photophysical properties of other "standard" CuP, in assuming that Cu(TMPy-P4) exhibits the same photophysical behavior. This suggestion is quite plausible since the photophysical behaviour of CuP is mainly determined by the Cu(II) ion rather than by the porphyrin macrocycle structure. Therefore, after excitation of Cu(TMPy-P4) in the $^2S_0 \rightarrow ^2S_1$ pathway, extremely fast (<100 fs) intersystem crossing to the triplet-state manifold has to occur. This means that an excited $^{2,4}T_1$ state has to be observed in our picosecond absorption measurements, as the first transient state obtained after excitation by a 10-ps pulse: this was earlier shown for CuOEP and CuTPP molecules in both noncoordinating and coordinating organic solvents.^{39–44} Indeed, spectral properties of the first transient species (Figure 5A) well agree with the well-known characteristic properties of the triplet-triplet absorption spectra of metalloporphyrins and include a two-band absorption profile in the Soret region, with a main maximum localized in the region of the red side of the Soret band and structureless absorption in the blue-green region smoothly vanishing in intensity in going from the Soret to Q bands.

The very short 35-ps lifetime of the excited triplet state of Cu(TMPy-P4) bound to poly(dA-dT) strongly suggests the existence of a quenching process due to the porphyrin environment. The present transient absorption data (Figure 1) prove that the quenching of the Cu(TMPy-P4) triplet state results in a simultaneous formation of a second transient species having a 3.2 ns lifetime. Two questions are now arising: (i) from what transient species detected in picosecond absorption measurements does the exciplex Raman bands originate, and (ii) what is the origin of the exciplex in terms of Cu(TMPy-P4) electronic states?

As for the first question, time-resolved RR study reveals that the exciplex species has a nanosecond decay time. This observation is in perfect agreement with the 3.2 ns value for the lifetime of the second transient species measured in picosecond absorption experiments. Therefore, it is reasonable to set up a correspondence between the transient exciplex bands arising in RR spectra at high laser excitation power (Figure 7, $\nu_{exc} = 1346 \text{ cm}^{-1}$) and the second, more long-lived transient species detected in difference absorption measurements (Figure 4B).

Let us now consider the possible electronic-state origin of the exciplex. First of all, "extra" exciplex Raman lines, which appeared in transient RR spectra of Cu(TMPy-P4)-poly(dA-dT) at high excitation power, cannot be assigned to RR scattering from the triplet state of ligated Cu(TMPy-P4). Numerous recent investigations^{55–60} have revealed that transient Raman spectra originating from excited triplet (π, π^*) state of porphyrins and metalloporphyrins greatly differ from the ground-state ones, breaking down the one-to-one analogy of the Raman-active vibrations of the ground- and excited-states species. Indeed, transient triplet Raman spectrum of CuTPP in benzene⁵⁹ is characterized by a considerable broadening of the lines and the weakness (or even absence) of the most prominent Raman bands from the porphyrin skeleton (ν_2 and ν_4 modes).

Therefore, we assume that the [(Cu(TMPy-P4)*-poly(dA-dT))] exciplex has to be assigned to a transient electronic state

populated in the process of triplet-state relaxation. Having in mind the mechanism of "standard" CuP triplet-state quenching in O-containing organic solvents, three transient electronic states of Cu(TMpy-P4) can be proposed as probable candidates for the exciplex: (1) A thermodynamically unstable ground state of a five-coordinated Cu(TMpy-P4) species before deattachment of the axial ligand L. (2) A [(d,d)-L] excited electronic state of five-coordinated Cu(TMpy-P4). (3) A [CT-L] excited electronic state of five-coordinated Cu(TMpy-P4).

The first possibility was earlier studied in details during our investigations of stable five-coordinated CuP complexes.^{53,54} We have recorded Raman spectra in the ground electronic state of CuOEP in THF at liquid nitrogen temperature (77 K, ca. 50% of CuP molecules are complexed with THF), as well as of CuOEP and CuTPP in piperidine (>50% and >90% of CuP molecules are then complexed with piperidine, respectively) at ambient temperature. The results of these studies as well as those of Shelnutt et al.⁶¹ prove that the binding of O- or N-containing molecules as axial ligands in the ground electronic state of CuP does not significantly perturb the porphyrin structure, and CuP Raman marker bands only slightly change their wavenumbers ($\Delta\nu \leq 7 \text{ cm}^{-1}$). The reason is that, in contrast to the case of Ni-porphyrins,^{62,63} axial binding of a σ -donor molecule to CuP in the ground electronic state does not cause the promotion of one of the inner d-electrons of the copper ion into the upper half-filled $d_{x^2-y^2}$ orbital. On the contrary, for Cu(TMpy-P4)-poly(dA-dT) the values of frequency shifts of structure sensitive marker lines due to the exciplex population are quite large ($\Delta\nu \leq 19 \text{ cm}^{-1}$), this indicating the occupation of the copper $d_{x^2-y^2}$ orbital and the expansion of the porphyrin core to accommodate this occupation. Therefore, these data reveal the excited electronic state origin of the transient species observed in Raman spectra of Cu(TMpy-P4)-poly(dA-dT) at high laser excitation power.

Moreover, all of the obtained experimental results unambiguously prove the similarity of the electronic excited-state origin for the [(Cu{TMpy-P4})*-poly(dA-dT)] complex and for model CuP in O-containing solvents which have been thoroughly investigated elsewhere.^{36,50-54} Following the scheme of CuP excitation relaxation in the presence of oxygen-containing molecules (Figure 11), only the excited (d,d) or CT states of Cu(TMpy-P4) ligated by O-containing CO group of thymine might correspond to the long-lived species observed by transient absorption and RR methods. The most probable candidate for the transient bottleneck state of [(Cu(TMpy-P4))*-poly(dA-dT)] exciplex is the excited (d,d) state, as it was proposed in ref 27. The CT origin of the exciplex, either an intramolecular or intermolecular (π ,d) state with an electron transfer from the porphyrin π -macrocycle to DNA,²⁴⁻²⁶ is less probable. Let us consider in more details the reasons for this statement.

(1) Values of the Frequency Shifts of Raman Marker Bands. RR spectra originating from the above mentioned CT state of CuP can be modeled, in a first approximation, by the spectra of CuP π -cation radicals.^{64,65} The wavenumbers of ν_2 and ν_4 modes in the ground state and their transient or cation radical counterparts (ν_2' , ν_4') and shifts ($\Delta\nu_2$, $\Delta\nu_4$) are presented in Table 1. There is a clear evidence that the values of wavenumber shifts of Raman bands characteristic for Cu(TMpy-P4)-poly(dA-dT) and for CuTPP in oxygen-containing solvents are about equal, while frequency shifts for CuTPP^{•+} cation radicals are quite different. Since it was reliably proved⁵²⁻⁵⁴ that the appearance of transient peaks in Raman spectra of CuP in O-containing solvents is assigned to the excited $^2[d_{x^2}, d_{x^2-y^2}]$ state, it is reasonable to assume the same origin for [(Cu{TMpy-P4})*-poly(dA-dT)] transient species.

TABLE 1: Frequencies (cm^{-1}) of Marker Raman Bands in the Ground Electronic State (ν_2 and ν_4 Modes), Their Transient Excited or Cation Radical Counterparts (ν_2' , ν_4'), and Frequency Shifts ($\Delta\nu_2$, $\Delta\nu_4$) for Water-Soluble Cu(TMpy-P4) Bound to poly(dA-dT) and for Model CuTPP in Organic Solvents

| sample | ref | ν_2 | ν_2' | $\Delta\nu_2$ | ν_4 | ν_4' | $\Delta\nu_4$ |
|--------------------------------|-----------|---------|----------|---------------|---------|----------|---------------|
| Cu(TMpy-P4)-poly(dA-dT) | this work | 1571 | 1552 | 19 | 1366 | 1348 | 18 |
| CuTPP in THF | 36, 54 | 1565 | 1543 | 22 | 1366 | 1342 | 24 |
| CuTPP in dioxane/toluene (1:1) | 54 | 1564 | 1544 | 20 | 1366 | 1344 | 22 |
| CuTPP ^{•+} | 64 | 1562 | 1530 | 32 | 1365 | 1355 | 10 |
| CuTPP ^{•+} | 65 | 1561 | 1539 | 22 | 1361 | 1361 | 0 |

(2) Raman Excitation Profiles and Transient Absorption Spectra. The investigations of resonance enhancement of Raman scattering originating from the ground and excited states of Cu(TMpy-P4)-poly(dA-dT) obtained by Chinsky et al.²⁵ and those of CuTPP in THF obtained in this work have revealed the following peculiarities. It was found that, first of all, for both of the molecules, the REP of the ν_4 mode both in the ground and in the excited states have similar shapes, and, second, the exciplex profiles for the ν_4' excited-state mode exhibit small red shifts ($\leq 8 \text{ nm}$) with respect to the ground-state profiles. Moreover, Raman bands of the Cu(TMpy-P4) exciplex species are comparable in intensity with their ground state counterparts. Picosecond transient absorption investigations of this molecule (Figure 4) showed that the most long-lived transient species has an absorption spectrum similar to that of the ground state but slightly red-shifted (less than 10 nm), in good agreement with REP measurements performed for the exciplex ν_4' line. All these observations are compatible with the metalloporphyrin excited (d-d) state origin,^{44,52-54,66-69} but not with the CT state origin^{42,44,69} of the exciplex.

It is interesting to note that RR saturation study also reveals a clear difference between relaxation times of the processes of excited states depopulation for Cu(TMpy-P4)-poly(dA-dT) and for CuTPP in THF or dioxane. Transient Raman lines originating from the excited state appear in spectra of Cu(TMpy-P4)-poly(dA-dT) even at low power density ($I_{\text{ex}} \sim 10^5 \text{ W/cm}^2$, Figure 8a). Moreover there is a noticeable ground state depletion at moderate excitation power ($I_{\text{ex}} \sim 10^6 \text{ W/cm}^2$, Figure 8b). In contrast, RR spectrum of CuTPP in dioxane/toluene mixture recorded at the same excitation power only displays weak shoulders due to the scattering from the excited (d-d) state.⁵⁴ The distinctive feature of RR spectra of CuTPP in O-containing solvents is the absence of pronounced ground state depletion even at highest possible excitation powers ($I_{\text{ex}} \geq 10^8 \text{ W/cm}^2$,^{52,54}). In contrast, for Cu(TMpy-P4)-poly(dA-dT) complex almost a complete depopulation of the ground electronic state can be achieved (Figure 8c). These tendencies are in good agreement with the direct kinetical measurements of the excited (d-d) state lifetimes ($t_{\text{d,d}} = 190 \text{ ps}$ for CuTPP in THF, $t_{\text{d,d}} = 3.2 \text{ ns}$ for Cu(TMpy-P4)-poly(dA-dT)). It should be also noted that in saturation Raman studies of Cu(TMpy-P4)-poly(dA-dT) we did not observe clear manifestations of a parallel fast relaxation pathway from the triplet manifold via the CT state, effect which was reliably established in the relaxation process of CuTPP in O-containing solvents.^{52,54} However, we left open in Figure 11 this possibility of relaxation pathway via the CT state, as it has been extensively discussed in our previous paper.⁵⁴

In our picosecond absorption studies, the transient species for free Cu(TMpy-P4) in a phosphate buffer solution is surprisingly short ($21 \pm 3 \text{ ps}$). We believe that this transient state is likely the $^2\text{T}_1$ triplet state: first, by its typical absorption

spectrum (Figure 6) similar to the spectrum A of Figure 3, and second, on the basis of a general statement that the $^2\text{A}_1$ excited state is first to be observed in the process of electronic relaxation after excitation by picosecond pulses. Therefore, the triplet state lifetime for free Cu(TMpy-P4) in water is found to be as short as 21 ps. On the other hand, Cu(TMpy-P4) in water is known as a planar four-coordinated molecule, and in the framework of our assumption of photophysical similarity of Cu(TMpy-P4) with CuTPP, the former has to have a triplet-state lifetime within a nanosecond time scale. There are two ways to solve this contradiction: (i) to assume that the $^2\text{A}_1$ excited state is not the lowest one of the proper four-coordinated Cu(TMpy-P4) and that it can be eventually "quenched" by lower-lying excited states, and (ii) to assume that the $^2\text{A}_1$ state of Cu(TMpy-P4) in water is dynamically quenched due to axial liganding of triplet molecule by H_2O molecules, with a resulting downshift of the (d,d) and/or CT states and subsequent quenching of the $^2\text{A}_1$ state. It is now impossible to prefer one of these two hypotheses, and additional experiments will be carried out to clarify this problem.

The role of water as a quencher of the triplet state for Cu(TMpy-P4) bound to polynucleotides and DNA has been suggested by Hudson et al.¹³ In fact, these authors proposed that exciplex formation involves an increase in coordination number at copper ion, with water as a fifth ligand, and conversion to a d-d state. However, our data presented in this work as well as direct measurements of frequency changes of thymine Raman lines³⁰ unambiguously proved that namely the CO group of the nucleic residue interacts with the porphyrin central copper ion in the process of exciplex formation. Nevertheless, in our opinion, the idea about the possible quenching influence of water may be valid in the case of free Cu(TMpy-P4) in a buffer solution. The second (more long-lived) transient species observed for free Cu(TMpy-P4) as a less intense tail on some kinetics curves may also correspond to the excited (d,d) state because the CT state is known to be unobservable for Cu(TMpy-P4). Additional experiments are necessary for better understanding the origin of this long-lived transient species.

Conclusion

On the basis of our investigations of the Cu(TMpy-P4)-poly(dA-dT) intermolecular complex by using picosecond transient absorption and resonance Raman spectroscopies, the following sequence of relaxation processes has been revealed, which takes place after Cu(TMpy-P4) photoexcitation in the $^2\text{S}_0 \rightarrow ^2\text{S}_1$ transition. First of all, an unobserved very fast (<100 fs) intersystem crossing $^2\text{S}_1 \rightarrow ^2\text{A}_1$ likely occurs, and, therefore, the excited $^2\text{A}_1$ triplet-state manifold is the first transient state spectroscopically observed after photoexcitation. Then, the process of binding of a carbonyl group of thymine residue as a fifth ligand to porphyrin central Cu(II) ion of the excited Cu(TMpy-P4) occurs, since the appropriate geometric conditions are realized in poly(dA-dT). An electron density donation from the p_z orbital of the carbonyl group oxygen disturbs the metalloporphyrin excited-states structure and causes a downshift of the excited $^2[\text{d}_{x^2-y^2}, \text{d}_{xz-yz}]$ state below the $^2\text{A}_1$ state, which results in a population of the former and a quenching of the latter. A time constant of 35 ± 7 ps was found for the $^2\text{A}_1$ state decay and for the $^2[\text{d}_{x^2-y^2}, \text{d}_{xz-yz}]$ state population. A decay of the (d,d) (exciplex) state then occurs with a time constant of 3.2 ± 0.5 ns and is simultaneously accompanied by a carbonyl group deattachment with a return of all the system to the initial state. Comparative studies revealed same processes of exciplex formation for water-soluble Cu(TMpy-P4) bound to poly(dA-

dT) and for model CuTPP dissolved in O-containing solvents. The (d,d) nature of the exciplex is proven (i) by the character of the resonance enhancement of transient Raman bands as well as by transient absorption spectra originating from exciplex species, and (ii) by the large-frequency downshifts of structure-sensitive marker lines of the porphyrin skeleton, which reveal the promotion of one of the copper d-electrons to the half-filled $\text{d}_{x^2-y^2}$ orbital and the expansion of the porphyrin core to accommodate this occupation.

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