# Exciplex Formation Dynamics of Photoexcited Copper(II) Tetrakis(4-*N*-methylpyridyl)porphyrin with Synthetic Polynucleotides Probed by Transient Absorption and Raman Spectroscopic Techniques

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To elucidate the interaction mechanism of water-soluble copper(II) tetrakis(4-*N*-methylpyridyl)porphyrin ($Cu^{II}$-(TMpy-P4)) with synthetic polynucleotides such as poly(dA-dT)$_2$ and poly(dG-dC)$_2$, the exciplex formation dynamics of photoexcited $Cu^{II}$(TMpy-P4) with added polynucleotides have been investigated by using femtosecond transient absorption as well as transient Raman spectroscopic methods. The nanosecond transient Raman spectra of $Cu^{II}$(TMpy-P4) mixed with poly(dA-dT)$_2$ clearly demonstrate the exciplex formation between photoexcited $Cu^{II}$(TMpy-P4) and poly(dA-dT)$_2$. On the other hand, the exciplex formation of photoexcited $Cu^{II}$(TMpy-P4) with poly(dG-dC)$_2$ is not so manifest as compared with that mixed with poly(dA-dT)$_2$. The transient absorption of $Cu^{II}$(TMpy-P4) mixed with poly(dG-dC)$_2$ exhibits a rise component of 1.3 ps in addition to the very slow decay component ($\tau \sim 22$ ns). This observation is quite different from that of $Cu^{II}$(TMpy-P4) in aqueous solution or $Cu^{II}$(TMpy-P4) mixed with poly(dA-dT)$_2$, because the exciplex formed in poly(dG-dC)$_2$, if any, is so short-lived that it returns quickly back to four-coordinate $Cu^{II}$(TMpy-P4) intercalated at poly(dG-dC)$_2$, which blocks the axial coordination by water molecules and consequently gives rise to a long lifetime ($\tau \sim 22$ ns).

## Introduction

Since Fiel et al.[1] have shown that cationic porphyrins are capable of interacting with DNA double helix, the interaction of water-soluble porphyrins with biopolymers has attracted considerable interest for the possible biomedical application.[2] Indeed, the two basic modes in porphyrin binding to biopolymers can be utilized as models for the binding of anticancer drugs. The previous studies[3] revealed that planar porphyrins (four-coordinate) are exclusively intercalated at GC/CG sites while porphyrins having axial ligand ($H_2O$) form groove-bound complexes at ATAT sites of DNA. Furthermore, porphyrins such as hematoporphyrin derivatives are known to act as photosensitizers in the photodynamic therapy.[2] In this case the attachment of porphyrins to oligonucleotides exhibits a sequence-specific photocleavage. Finally, it was recently reported that porphyrins can inhibit HIV-1 function, the virus responsible for AIDS.[4]

The photodynamics of copper(II) porphyrins have been extensively investigated by a variety of spectroscopic methods: picosecond transient absorption,[5−8] picosecond and nanosecond transient resonance Raman (RR),[7−11] and CARS (coherent anti-Stokes Raman spectroscopy)[8] techniques. These studies have demonstrated that the binding of nitrogen- or oxygen-containing solvent molecules to the central copper(II) metal of photoexcited copper(II) porphyrins results in a drastic decrease in the lifetimes of photoexcited copper(II) porphyrins by lowering the energy of a quenching state below the normally emissive $^2T/^4T(\pi,\pi^*)$ states. As for the complexes of water-soluble copper(II) porphyrins with DNA or various synthetic polynucleotides, several investigations[3a,8,12−15] have revealed the exciplex formation between photoexcited copper(II) porphyrins and poly(dA-dT)$_2$. On the contrary, no evidence on the exciplex formation was found in poly(dG-dC)$_2$ from nanosecond and picosecond transient RR spectra in our experiments. Kruglik et al.[8] explained the polynucleotide dependence of the exciplex formation in terms of the secondary structural difference in the two synthetic polynucleotides.

We investigated previously the photodynamics of copper(II) porphyrins in various solvents to examine the electronic nature of the excited states involved in the energy relaxation process.[10,11] In those works, it was found that the overall decay time of photoexcited copper(II) porphyrins has a correlation with the appearance of the transient Raman bands corresponding to the exciplex formation between photoexcited copper(II) porphyrins and nucleophillic solvents. In other words, the transient Raman bands become weaker with a decrease in the lifetime of the exciplex under the same experimental condition such as the laser pulse width and the excitation power density. Furthermore, the lifetimes of the exciplexes are strongly influenced by changing the solvents from oxygen- to nitrogen-containing solvents. To investigate the dependence of the exciplex formation dynamics of $Cu^{II}$(TMpy-P4) on the nature of added synthetic polynucleotides, we have carried out a comparative study on $Cu^{II}$(TMpy-P4) mixed with poly(dA-dT)$_2$ and poly(dG-dC)$_2$ by femtosecond transient absorption as well as transient RR measurements.

## Experimental Section

The alternating polynucleotides, poly(dG-dC)$_2$ and poly(dA-dT)$_2$, were purchased from Pharmarcia Biochemicals (Picataway, NJ). $Cu^{II}$(TMpy-P4) was purchased from Porphyrin Products

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(Logan, UT) and used without further purification. Samples were prepared by mixing metalloporphyrin in phosphate buffer solution (pH 7) with polynucleotide, keeping the porphyrin concentration constant. The final porphyrin concentration was approximately $0.5 \times 10^{-5}$ M. All the transient Raman experiments were performed by flowing the sample solution through a glass capillary (0.8-mm i.d.) at a rate sufficient enough to ensure that each laser pulse encounters a fresh volume of the sample.

The transient Raman spectra were obtained by using the 416 and 436 nm pulses, generated by the hydrogen Raman shifting of the third (355 nm) and second (532 nm) harmonics, respectively, from a nanosecond Q-switched Nd:YAG laser and a combination system of a mode-locked Nd:YAG laser (Coherent Antares 76S) and a Nd:YAG regenerative amplifier (Continuum RGA-20).[10,11] The Raman spectra were collected with a HR 640 spectrograph (Jobin-Yvon), a gated intensified photodiode array detector (Princeton Instruments IRY700), and a pulse generator (Princeton Instruments FG100).

The dual-beam femtosecond time-resolved transient absorption spectrometer consisted of a self-mode-locked femtosecond Ti:sapphire laser (Spectra Physics, Tsunami), Ti:sapphire regenerative amplifier (Quantronix) pumped by a Q-switched Nd:YLF laser, a pulse stretcher/compressor, and an optical detection system. A femtosecond Ti:sapphire oscillator pumped by a continuous-wave Ar ion laser produces a train of 60 fs mode-locked pulses with an average power of 600 mW at 800 nm. To generate high-energy pulses, the output pulses from the oscillator were stretched and sent to a Ti:sapphire regenerative amplifier pumped by a Q-switched Nd:YLF laser having ca. 150 ns pulse duration and 1 kHz repetition rate. The femtosecond seed pulses and Nd:YLF laser pulses were synchronized by adjusting an electronic delay between Ti:sapphire oscillator and Nd:YLF laser. Then, the amplified pulse train inside the Ti:sapphire regenerative amplifier was cavity dumped by using the Q-switching technique so that 10 000-fold amplification at 1 kHz was achieved. These pulses were compressed again to reduce the pulse width. The resulting amplified laser pulse had a pulse width of ~120 fs and an average power of 300 mW at 1 kHz repetition rate in the range 790−840 nm. The pump pulses at the desired wavelength were generated by frequency doubling in a $\beta$-BBO crystal. These pulses were separated by using a dichroic mirror and then focused to 1 mm diameter spot at the 2 mm path length quartz cell containing the sample. The reflected fundamental beam was focused onto a quartz window to generate a white light continuum, which was again split into two parts: one for probing the transient and the other for the reference. The time delay between pump and probe beams was controlled by making pump beam travel along a variable optical delay line. The white light continuum pulses after the sample were dispersed by an 18 cm focal length spectrograph (Scientific Instruments) and then hit onto the dual 512 channel photodiode arrays (Princeton Instruments).

The intensity of the white light of each 512 channel photodiode array was processed to calculate the absorption difference spectrum at the desired time delay between pump and probe pulses. For precise measurements of decay profiles of transient absorption signals, the monitoring wavelength was selected by putting an appropriate interference filter (fwhm = 10 nm) and then split into the two parts (probe and reference). With chopping the pump pulses at 40 Hz, the modulated probe pulses as well as the reference ones were detected by photodiodes. The output current was amplified with a homemade fast preamplifier, and then the resultant voltage of the probe pulses was normalized by a boxcar average with pulse-to-pulse configuration. The resultant signal modulated by a chopper was measured by a lock-in-amplifier and then fed into a personal computer for further signal processing.

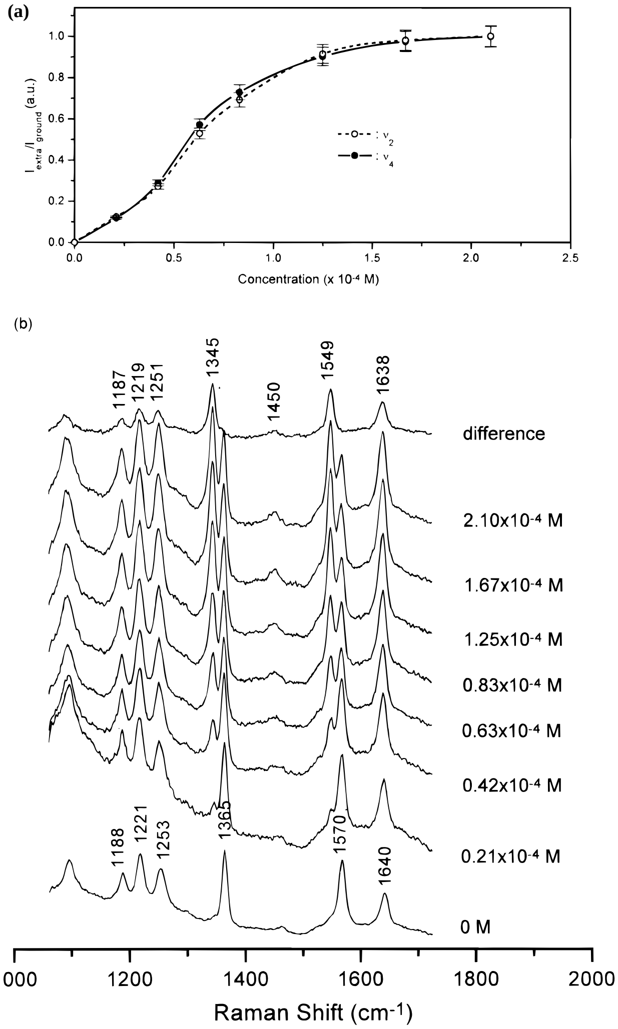

**Figure 1.** Transient RR spectra of $Cu^{II}$(TMpy-P4) mixed with poly(dA-dT)$_2$. The porphyrin concentration and the laser power were kept constant lower than $10^{-5}$ M and 1 mJ, respectively. (a) Measured intensity ratios of extra Raman bands to the corresponding ground-state ones as a function of added poly(dA-dT)$_2$. (b) The series of transient Raman spectra of $Cu^{II}$(TMpy-P4) aqueous solution with increasing the relative portion of the synthetic polynucleotide upon photoexcitation at 416 nm. The top spectrum denotes the difference between the RR spectra (see text).

## Results

We recorded the transient RR spectra of $Cu^{II}$(TMpy-P4) in aqueous solution mixed with the two different synthetic polynucleotides, poly(dA-dT)$_2$ and poly(dG-dC)$_2$. Figure 1 displays a series of nanosecond transient Raman spectra of $Cu^{II}$(TMpy-P4) in aqueous solution with a gradual increase of the concentration of poly(dA-dT)$_2$ keeping the laser power density and the porphyrin concentration constant. Photoexcitation with 3.5 ns laser pulses at 416 or 436 nm produced the reversible transient Raman spectra without an indication of permanent photodegradation. The intensity ratios between the transient Raman bands at 1549 and 1345 cm$^{-1}$ ($\nu_2$ and $\nu_4$ modes) and their corresponding ground state ones at 1570 and 1365 cm$^{-1}$, respectively, have gradually increased with the addition of poly(dA-dT)$_2$ (Figure 1a). The pure Raman spectrum of the exciplex was obtained by subtracting the Raman spectrum obtained by photoexcitation with low laser power density (not shown) from

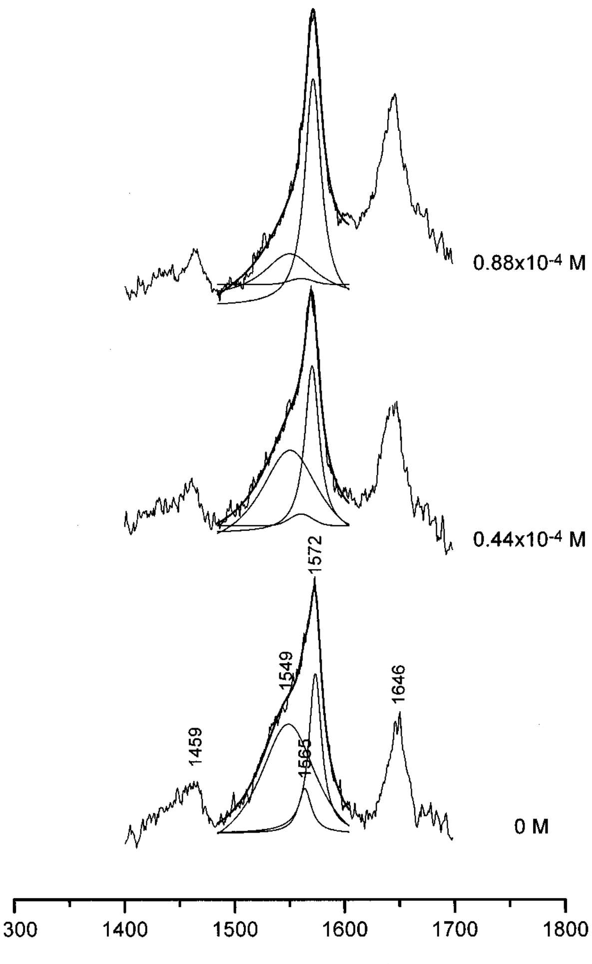

**Figure 2.** Series of picosecond transient RR spectra of Cu$^{II}$(TMpy-P4) mixed with poly(dG-dC)$_2$ with increasing the concentration of the polynucleotide with an excitation at 436 nm. The porphyrin concentration was ca. 0.5 × 10$^{-5}$ M.

that measured by photoexcitation with high laser power density in solution with the polynucleotide concentration of 2.1 × 10$^{-4}$ M ([DNA pair]/[Cu$^{II}$(TMpy-P4)] ≈ 40). The difference spectrum gives the transient Raman bands at 1638, 1549, 1450, 1345, 1251, 1219, and 1187 cm$^{-1}$, respectively. The peak positions of the transient Raman bands are in good accordance with those assigned to five-coordinate exciplex formed between photoexcited Cu$^{II}$(TMpy-P4) and dioxane or tetrahydrofuran (THF) solvent.[10,11] These observations strongly suggest that the structural nature of the exciplex of Cu$^{II}$(TMpy-P4) in poly(dA-dT)$_2$ is similar to that observed in other simple oxygen-containing solvents.[10,11]

It has been reported[3a,8,12−14] that the transient Raman bands corresponding to the exciplex are not observed for Cu$^{II}$(TMpy-P4) in aqueous solution and for that mixed with poly(dG-dC)$_2$ by using nanosecond laser pulses. We were able to observe, however, the transient Raman bands of Cu$^{II}$(TMpy-P4) in aqueous solution with an illumination of picosecond laser pulses (~70 ps pulse width); (Figure 2). To determine the precise Raman frequency shifts of weak bands to appear as a shoulder of the intense ground-state Raman ones, the Raman bands around the $\nu_2$ mode are reconstructed by using Lorentzian line-shape functions. From this simulation, we can see that the two types of transient states (TS$_1$ (1565 cm$^{-1}$) and TS$_2$ (1549 cm$^{-1}$) states) are involved in the decay process of photoexcited Cu$^{II}$(TMpy-P4) in aqueous solution. Moreover, the slight increase of poly(dG-dC)$_2$ concentration induces an appreciable decrease in the intensities of the transient Raman bands without a change in the spectral feature.

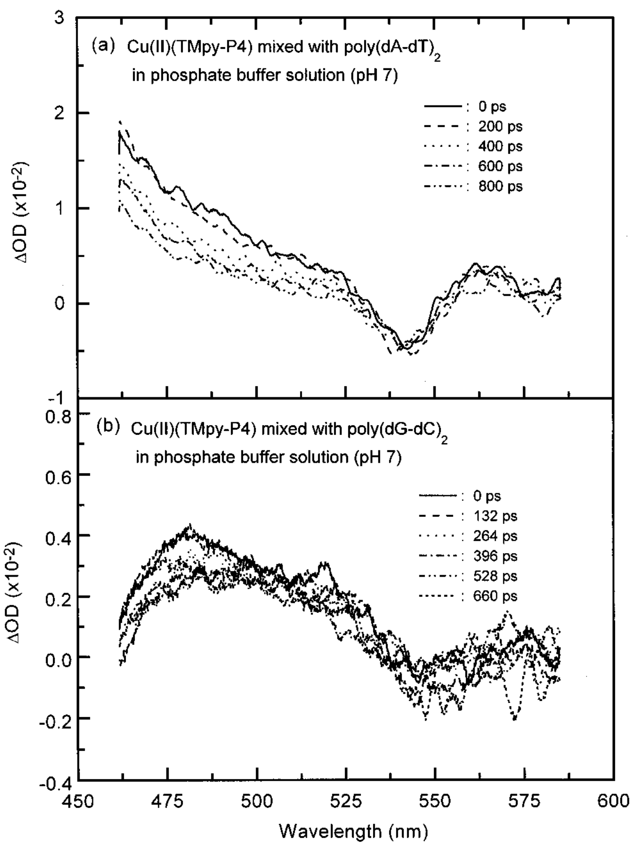

**Figure 3.** Transient absorption spectra at various time delays for Cu$^{II}$(TMpy-P4) solution mixed with poly(dA-dT)$_2$ (a) and poly(dG-dC)$_2$ (b). The concentrations of the porphyrin and polynucleotides are ca. 0.5 × 10$^{-5}$ and 2.1 × 10$^{-4}$ M, respectively.

To obtain further information on this complicated photodynamics, we recorded the transient absorption spectra of Cu$^{II}$(TMpy-P4) in aqueous solution mixed with poly(dA-dT)$_2$ and poly(dG-dC)$_2$ as shown in Figure 3. The featureless broad absorption spectra of Cu$^{II}$(TMpy-P4) in both polynucleotides, however, are almost similar to each other except a weak broad transient absorption maximum near 490 nm in poly(dG-dC)$_2$ and the spectral shift as expected from the red-shift in the ground-state absorption spectrum of Cu$^{II}$(TMpy-P4) mixed with poly(dG-dC)$_2$ as compared with that mixed with poly(dA-dT)$_2$ (439 nm vs 428 nm of the Soret band maxima for [DNA-pair]/[Cu$^{II}$(TMpy-P4)] ≈ 50).[15] Although there is no significant difference in the transient absorption spectra of Cu$^{II}$(TMpy-P4) mixed with the two different polynucleotides, the decay profiles of the transient absorption signals exhibit interesting behaviors. Figure 4 shows the transient absorption decay profiles of photoexcited Cu$^{II}$(TMpy-P4) in various solvent environments. In aqueous buffer solution (pH 7), the temporal profile of the transient absorption exhibits a biphasic decay (closed circles in Figure 4a): the lifetime of a fast component is 3.2 ps and that of a slow one is 11.9 ps. However, the two decay time constants are decreased to 1.4 and 10.3 ps, respectively, with a shift in the probe wavelength from 460 to 480 nm (closed circles in Figure 5a).[16] The one-color pump−probe transient RR spectra by using the picosecond laser with 70 ps pulse width suggest that the photoexcited relaxation dynamics of Cu$^{II}$(TMpy-P4) in aqueous solution reflect the exciplex formation through accepting water molecules as an axial ligand.[11]

The addition of small quantity of poly(dA-dT)$_2$ (0.5 × 10$^{-4}$ M) to the aqueous solution of Cu$^{II}$(TMpy-P4) significantly

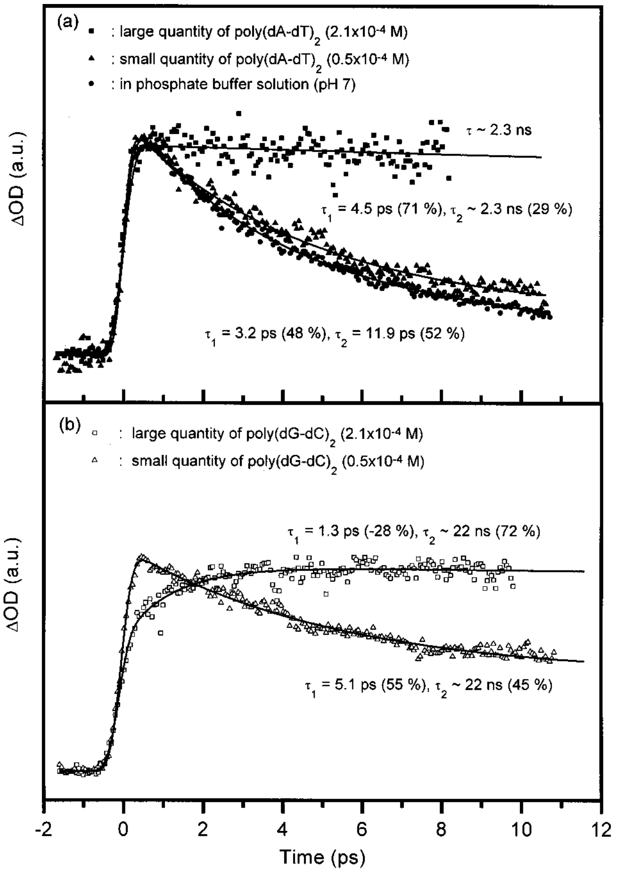

**Figure 4.** Decay profiles of photoinduced absorbance changes of Cu$^{II}$(TMpy-P4) in aqueous solution and mixed with poly(dA-dT)$_2$ (a) and poly(dG-dC)$_2$ (b) in phosphate buffer (pH = 7) monitored at 460 nm. The average power of the pump pulse at 400 nm is about 8 mW at 1 kHz repetition rate. The lifetime of the long-lived component shown in (a) ($\tau \sim 2.3$ ns) was measured by the moving the optical delay line in the long range. The lifetime of the long-lived component ($\tau \sim 22$ ns) was taken from ref 15 based on the time-resolved emission measurements.

increased the amplitude of a slow component as shown in Figure 4a. The curve fitting of transient absorption decay profile exhibits a relatively fast component with a lifetime of 4.5 ps in addition to a very slow component ($\tau \sim 2.3$ ns). The more poly(dA-dT)$_2$ is added to the porphyrin solution, the larger becomes the amplitude of the slow component. Moreover, no fast decay component was observed in the transient absorption decay profiles measured with the addition of large quantity of poly(dA-dT)$_2$ ($2.1 \times 10^{-4}$ M). Since the concentration of poly(dA-dT)$_2$ employed in the above experiment is high enough to result in the formation of the exciplex with photoexcited Cu$^{II}$(TMpy-P4) in aqueous solution (see Figure 1), the contribution of water solvent to the relaxation process of photoexcited copper(II) porphyrins is probably negligible as compared with that of poly(dA-dT)$_2$. The large increase in the lifetime of the transient species upon addition of poly(dA-dT)$_2$ to Cu$^{II}$(TMpy-P4) in aqueous solution was similar to that observed as THF or dioxane solvent was added to the aqueous solution of Cu$^{II}$(TMpy-P4).[10,11] This similarity provides evidence to account for the appearance of the transient Raman bands corresponding to the exciplex formation of photoexcited Cu$^{II}$(TMpy-P4) with poly(dA-dT)$_2$. On the other hand, the decay profile of photoexcited Cu$^{II}$(TMpy-P4) mixed with poly(dG-dC)$_2$ is different from that obtained in the presence of poly(dA-dT)$_2$ (Figure 4). The decay profiles measured at the two different poly(dG-dC)$_2$ concentrations exhibit an appearance of a very slow component ($\tau \sim 22$ ns)[15] as compared with that of Cu$^{II}$(TMpy-P4) in aqueous solution as in the case of poly(dA-dT)$_2$ solution. However, there is an apparent rise component with a time constant of 1.3 ps in addition to the very slow decay component at high concentration of poly(dG-dC)$_2$ ($2.1 \times 10^{-4}$ M). The similar rise component for Cu$^{II}$(TMpy-P4) in aqueous solution mixed with poly(dG-dC)$_2$ was again observed at 480 nm while Cu$^{II}$(TMpy-P4) solution mixed with poly(dA-dT)$_2$ does not show rise component (Figure 5).

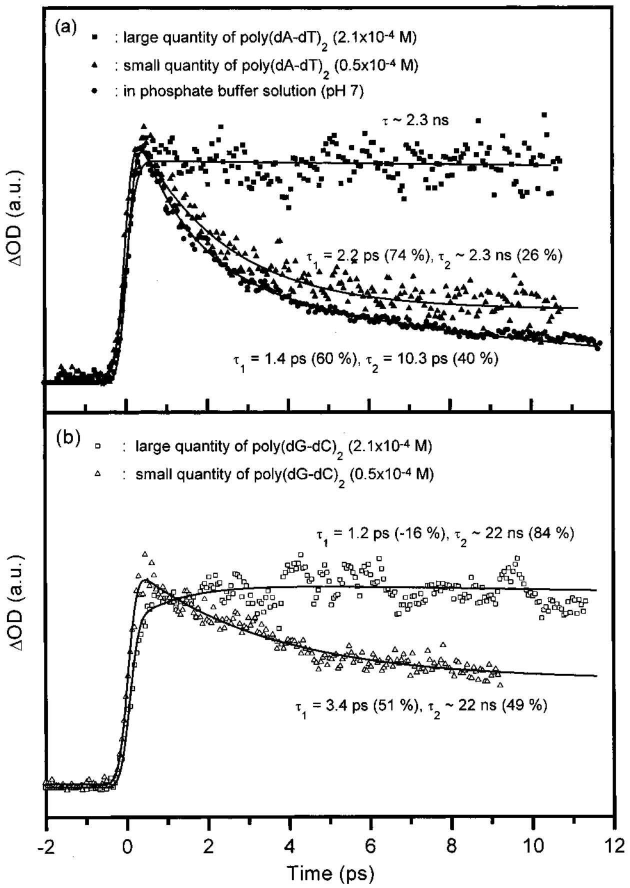

**Figure 5.** Decay profiles of photoinduced absorbance changes of Cu$^{II}$(TMpy-P4) mixed with poly(dA-dT)$_2$ (a) and poly(dG-dC)$_2$ (b) monitored at 480 nm. The long-lived components with the time constants of 2.3 and 22 ns in (a) and (b), respectively, are the same as shown in Figure 4.

## Discussion

Figure 1 shows the intensity ratio of the transient Raman bands ($\nu_2$ and $\nu_4$ modes) relative to their corresponding ground-state Raman ones in Cu$^{II}$(TMpy-P4) mixed with poly(dA-dT)$_2$. At lower concentrations of poly(dA-dT)$_2$, the transient Raman bands gain rapidly their intensities with an increase in the polynucleotide concentration. But the ratio becomes almost constant at higher concentrations of poly(dA-dT)$_2$. These results suggest that poly(dA-dT)$_2$ is the major exciplex-forming site with photoexcited Cu$^{II}$(TMpy-P4). On the other hand, water molecules probably participate in the decay processes of photoexcited copper(II) porphyrins at lower concentrations of poly(dA-dT)$_2$. If this is the case, the transient absorption decay profiles measured at high concentrations of poly(dA-dT)$_2$ shown in Figures 4a and 5a are attributable to the decay of the exciplex formed between photoexcited Cu$^{II}$(TMpy-P4) and poly(dA-dT)$_2$. However, the decay profiles measured at lower concentrations of polynucleotides are probably competitive processes in the exciplex formation of photoexcited copper(II) porphyrin with water and polynucleotide. In other words, the fast and slow components observed at lower concentrations of polynucleotides are attributable to the exciplex formation with water and poly(dA-dT)$_2$, respectively. These considerations demonstrate that the photodynamics of the exciplex play an important role in the appearance of transient Raman bands with nanosecond laser pulses as well as the overall photodynamics of copper(II) porphyrins mixed with synthetic polynucleotides.

Recently, Kruglik et al.[8] investigated the current system by transient absorption spectroscopy with a time resolution of 10 ps, reporting that the exciplex is formed by the binding of one of CO groups of thymine residue of poly(dA-dT)$_2$ in the lowest excited triplet state of copper(II) porphyrins. The lifetimes of the triplet state and the exciplex were reported to be 35 ps and 3.2 ns, respectively, from the decay of the bleaching of the Soret band at 417 nm. In our experiments, however, the transient absorption decay profiles at 460 and 480 nm at the concentration of $2.1 \times 10^{-4}$ M poly(dA-dT)$_2$ do not exhibit any rise components with our time resolution (150 fs). No evidence was found for several tens of picoseconds decay which was assigned to the time constant of the triplet excited state.[8] Since the wavelengths of 460 and 480 nm are far from the ground-state absorption region, the transient absorption signal is supposed to be originated from the excited state. Thus we can suggest that the transient absorption signals at 460 and 480 nm result from the formation of the exciplex. These experimental observations indicate that the exciplex of Cu$^{II}$(TMpy-P4) with poly(dA-dT)$_2$ is formed within our laser pulse width (150 fs). Thus these results strongly suggest that the exciplex is formed not in the lowest excited triplet state which is rapidly populated from the initially excited singlet state through the binding with solvents or polynucleotides employed.

The intensity changes of the transient Raman bands of Cu$^{II}$(TMpy-P4) and the appearance of the rise component depending on the types of polynucleotides suggest that the excited-state dynamics of Cu$^{II}$(TMpy-P4) mixed with poly(dA-dT)$_2$ and poly(dG-dC)$_2$ are different each other. The transient Raman bands induced by the exciplex formation should have been detected in the transient Raman spectra of Cu$^{II}$(TMpy-P4) mixed with poly(dG-dC)$_2$, because the decay time of the transient absorption for Cu$^{II}$(TMpy-P4) solution mixed with poly(dG-dC)$_2$ is long enough to give rise to the transient Raman bands by the picosecond laser excitation. These experimental observations indicate that the transient absorption spectra shown in Figure 3b observed in Cu$^{II}$(TMpy-P4) aqueous solution mixed with poly(dG-dC)$_2$ is attributable not to the exciplex formation but to the $^2$T/$^4$T($\pi$,$\pi$*) manifold, because the intercalation of Cu$^{II}$(TMpy-P4) at GC/CG sites blocks water molecules to ligate to photoexcited Cu$^{II}$(TMpy-P4) as an axial ligand.

The previous spectroscopic investigations revealed that the lifetimes of the exciplexes of copper(II) porphyrins formed with nitrogen-containing solvents are much shorter than those with oxygen-containing ones.[6] In the case of poly(dA-dT)$_2$, the lifetime of the resultant five-coordinate exciplex through the ligation with the CO group in thymine residue is long enough to exhibit the transient Raman bands by nanosecond laser pulses. However, the five-coordinate exciplex, which might be formed in poly(dG-dC)$_2$, should decay much faster due to the ligation with the nitrogen atom in cytosine residue in its duplex because this polynucleotide does not contain oxygen atom capable of forming the exciplex with photoexcited Cu$^{II}$(TMpy-P4). However, the exciplex of photoexcited Cu$^{II}$(TMpy-P4) with poly(dG-dC)$_2$ is believed to be short-lived, if it is really formed, probably due to the instability of the exciplex induced by the difference in the secondary structure between poly(dA-dT)$_2$ and poly(dG-dC)$_2$. This argument is consistent with the observation that the increase of poly(dG-dC)$_2$ concentration reduces the intensities of transient Raman bands significantly as shown in Figure 2, because the intercalation of Cu$^{II}$(TMpy-P4) into poly(dG-dC)$_2$ hinders the exciplex formation of photoexcited Cu$^{II}$(TMpy-P4) through the axial coordination by water molecules.

This behavior probably is responsible for the rise component observed in the transient absorption decay profiles of Cu$^{II}$(TMpy-P4) in aqueous solution mixed with poly(dG-dC)$_2$. More specifically, the exciplex formation of photoexcited Cu$^{II}$(TMpy-P4) with poly(dG-dC)$_2$ through the binding of nitrogen atom of cytosine residue is so unstable probably due to the unfavorable conformation of poly(dG-dC)$_2$. Thus the rise component we observed is responsible for the fast dissociation process of exciplex between photoexcited Cu$^{II}$(TMpy-P4) and nitrogen atom of cytosine residue to generate relatively long-lived $^2$T/$^4$T ($\pi$,$\pi$*) manifold of Cu$^{II}$(TMpy-P4). An alternative explanation can be made that the rise component we observed in Cu$^{II}$(TMpy-P4) in aqueous solution mixed with poly(dG-dC)$_2$ is mainly induced by the fast deactivation process from the initially excited singlet state to the $^2$T/$^4$T($\pi$,$\pi$*) manifold of Cu$^{II}$(TMpy-P4). The previous investigation on Cu$^{II}$TPP in noncoordinating solvents, however, demonstrates that the unperturbed B- or Q-excited singlet states have lifetimes shorter than 350 fs. Thus, the rise component in Cu$^{II}$(TMpy-P4) mixed with poly(dG-dC)$_2$ is probably not caused by the relaxation from the initially excited singlet state.

Meanwhile, the previous nanosecond transient Raman studies[14] revealed that the extra transient Raman bands induced by the exciplex formation are observed in Cu$^{II}$(TMpy-P4) solution mixed with a 32-mer containing 26 possible GC/CG sites and only one ATAT site with 8 ns laser pulses, even though most copper(II) porphyrins are expected to be intercalated at GC/CG sites under this condition. Strahan et al.[14] interpreted the above abnormal experimental results by introducing a translocation of photoexcited Cu$^{II}$(TMpy-P4) from the intercalation sites at GC/CG pairs to the groove binding at ATAT site, because Cu$^{II}$(TMpy-P4) solution mixed with only poly(dG-dC)$_2$ does not exhibit any extra transient Raman bands under the same experimental condition. Thus the observed rise component ($\tau \sim 1.3$ ps) is probably responsible for the translocation process, although the time constant of $\sim$1.3 ps seems to be too short for this process to occur. But it is interesting to seek the reason the translocation process occurs so fast in Cu$^{II}$(TMpy-P4) aqueous solution mixed with poly(dG-dC)$_2$. The numerous investigations on the binding mode of metalloporphyrins with biopolymers unequivocally demonstrated that planar porphyrins (four-coordinate) are exclusively intercalated into GC/CG sites, while axially ligated porphyrins (five-coordinate) form groove-bound complexes at ATAT sites.[3] Thus the five-coordinate exciplex formed through the binding of nitrogen atom in cytosine residue of poly(dG-dC)$_2$ to photoexcited Cu$^{II}$(TMpy-P4) probably has a strong tendency to change its binding mode from the intercalation site to the groove binding one. On the other hand, upon photoexcitation Cu$^{II}$(TMpy-p4) groove bound to poly(dA-dT)$_2$ forms five-coordinate exciplex easily with the CO group of thymine residue. This dramatic alternation between the two binding modes of copper(II) porphyrins with a change in the nature of polynucleotides plays an important role in the exciplex formation process.

## Conclusion

To elucidate the interaction mechanism of Cu$^{II}$(TMpy-P4) with polynucleotides such as poly(dA-dT)$_2$ and poly(dG-dC)$_2$, the exciplex formation dynamics of photoexcited Cu$^{II}$(TMpy-P4) with added polynucleotides has been investigated by using femtosecond transient absorption and transient Raman spectroscopic methods. The photoexcitation decay dynamics of $Cu^{II}$(TMpy-P4) mixed with poly(dA-dT)$_2$ is much different from that mixed with poly(dG-dC)$_2$, because the exciplex formed in poly(dA-dT)$_2$ was clearly demonstrated by nanosecond transient Raman and femtosecond transient absorption spectra. The transient absorption decay profiles measured in $Cu^{II}$(TMpy-P4) mixed with poly(dA-dT)$_2$ give only the slow component ($\tau \sim$ 2.3 ns) without a rise component. The nanosecond transient Raman spectra and transient absorption decay profiles of $Cu^{II}$(TMpy-P4) mixed with poly(dA-dT)$_2$ clearly indicate that polynucleotides and water molecules compete each other in the exciplex formation with photoexcited $Cu^{II}$(TMpy-P4), because $Cu^{II}$(TMpy-P4) is believed to be groove-bound at poly(dA-dT)$_2$ sites. On the other hand, upon increasing the relative concentration of poly(dG-dC)$_2$ in $Cu^{II}$(TMpy-P4) aqueous solution, the trace of the exciplex formation between photoexcited $Cu^{II}$(TMpy-P4) and water molecules disappears in the picosecond transient Raman spectra. In addition, the transient absorption of $Cu^{II}$(TMpy-P4) mixed with poly(dG-dC)$_2$ exhibits a rise component of 1.3 ps in addition to the very slow decay component ($\tau \sim$ 22 ns). The short rise component might be attributable to the short-lived unstable exciplex between photoexcited $Cu^{II}$(TMpy-P4) and nitrogen atom of cytosine residue in the intercalation site of ploy(dG-dC)$_2$. The long-lived component ($\tau \sim$ 22 ns) which was measured by time-resolved photoluminescence for $Cu^{II}$(TMpy-P4) in poly(dG-dC)$_2$ indicates the decay from the $^2T/^4T(\pi,\pi^*)$ manifold of $Cu^{II}$(TMpy-P4).

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