Invited Paper

AFM-Correlated CSM-Coupled Raman/Fluorescence Studies on Selective Interactions of Water Soluble Porphyrins with DNA[1]

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The Raman and fluorescence spectroscopic properties of water-soluble oxo-titanium(IV) meso-tetrakis (1-methyl pyridium-4-yl) porphyrin ($O=Ti(TMPyP)^{4+}$) bound with calf thymus DNA and artificial DNAs such as double stranded poly[d(A-T)$_2$] and poly[d(G-C)$_2$] have been investigated on the single DNA molecule basis by AFM-correlated confocal scanning microscope (CSM)-coupled Raman and fluorescence spectroscopic techniques as well as the ensemble-averaged spectroscopy. The ensemble-averaged spectroscopic studies imply that the porphyrin interacts with DNA in different groove binding patterns depending on the base pairs. AFM-images of the different DNAs bound with $O=Ti(TMPyP)^{4+}$ were measured, and their morphologies are found to depend on kind of base pairs interacting with $O=Ti(TMPyP)^{4+}$. Being correlated with the AFM images, the CSM-coupled Raman and fluorescence spectral properties of the three different single $O=Ti(TMPyP)^{4+}$-DNA complexes were observed to be highly resolved and sensitive to base pair-dependent axial ligation of Ti-O bond as compared to the corresponding ensemble-averaged spectral properties, which affect the groove binding and its strength of the $O=Ti(TMPyP)^{4+}$ with DNA. The axial ligation was found to be accompanied by vibration structural change of the porphyrin ring, leading to keep the shape of double stranded poly[d(A-T)$_2$] rigid while poly-[d(G-C)$_2$] and calf thymus DNA flexible after binding with the oxo-titanyl porphyrin. The base pair dependence of the fluorescence decay times of the DNA-bound porphyrins was also observed, implying that an excited-state charge transfer takes place in the G-C rich major groove in calf thymus DNA. These results suggest that binding of $O=Ti(TMPyP)^{4+}$ is more preferential with the G-C rich major groove than with the A-T rich minor groove in calf thymus DNA so that the morphology of DNA is changed.

Keywords: Atomic force microscopy (AFM); Confocal scanning microscopy (CSM); Raman/fluorescence spectroscopy; DNA-porphyrin interaction; Single DNA; Morphology; Excited state charge transfer; Base pair dependence.

INTRODUCTION

Since the discovery of Fiel et al.[2] that water soluble porphyrins can be intercalated into B-form DNA, numerous studies on porphyrin complexes with DNA, RNA, and their model compounds have been performed, with the aim to exploit the great potential of medical, biological, and photophysical applications of porphyrins, on the basis of a good knowledge of their physicochemical properties. Particularly, water-soluble cationic (metallo)porphyrins are proved to be of interest in many areas, for example, as probes of the local nucleic acid structure and dynamics, as artificial nucleases, and as possible DNA photosensitizers to be used in photodynamic therapy. Therefore, the importance of an accumulation of fundamental knowledge on interaction of water-soluble cationic (metallo)porphyrins with DNA has been increasingly recognized and various analytical tools have been required.[3]

In this regard, Raman and fluorescence spectroscopic techniques have been useful for studying the binding patterns of water-soluble cationic (metallo)porphyrins with DNA. However, the conventional spectroscopic techniques require large volume of sample solution and their strong laser power can damage the sample. In order to solve these problems, we have developed the AFM (Atomic Force Microscope)-correlated CSM (Confocal Scanning Microscope)-coupled Raman technique.

In this lecture, the binding patterns of water soluble cationic oxotitanium(IV) meso-tetrakis(1-methylpyridium-4-yl) porphyrin ($O=Ti^{IV}(TMPyP)^{4+}$) with double stranded poly[d(A-T)$_2$], poly[d(G-C)$_2$] polynucleotides and calf

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thymus DNA are presented as investigated on the basis of single DNA molecule using AFM-correlated CSM-coupled Raman/fluorescence system[4,5] as well as conventional ensemble-averaged spectroscopic techniques.

ENSEMBLE-AVERAGED RAMAN/FLUORESCENCE SPECTRAL PROPERTIES

Interactions of $O=Ti^{IV}(TMPyP)^{4+}$ with poly[d(A-T)$_2$], poly[d(G-C)$_2$] and calf thymus DNA were preliminarily monitored with visible absorption spectroscopy. For $O=Ti^{IV}(TMPyP)^{4+}$, reaction with poly[d(A-T)$_2$] leads to a substantial red shift of the Soret maximum and a large hypochromicity. And changes in the Soret region of $O=Ti^{IV}(TMPyP)^{4+}$ upon interaction with poly[d(G-C)$_2$] are not as large as those observed with poly[d(A-T)$_2$]. There is no shift of the Soret maximum and virtually a small hyperchromicity. The absorption results in the Soret region for $O=Ti^{IV}(TMPyP)^{4+}$ with calf thymus DNA are intermediate between those obtained for the two polynucleotide. For $O=Ti^{IV}(TMPyP)^{4+}$ with calf thymus DNA, there appear to be interactions at both GC and AT sites. In addition to visible absorption spectra, positive induced circular dichroism (CD) bands of all $O=Ti^{IV}(TMPyP)^{4+}$ complexed with polynucleotides were obtained. From results of absorption and CD spectra, we concluded that $O=Ti^{IV}(TMPyP)^{4+}$ interacts with polynucleotides by mode of groove binding.

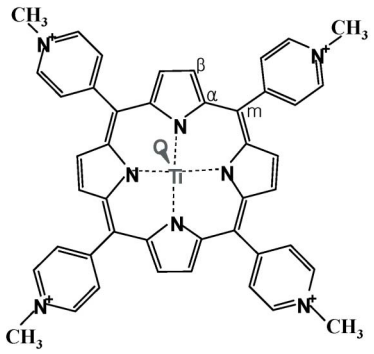


Fig. 1. Molecular structure of $O=Ti^{IV}(TMPyP)^{4+}$.

Fig. 2 shows the ensemble-averaged spectra of $O=Ti^{IV}(TMPyP)^{4+}$ comcomplexed with various DNAs

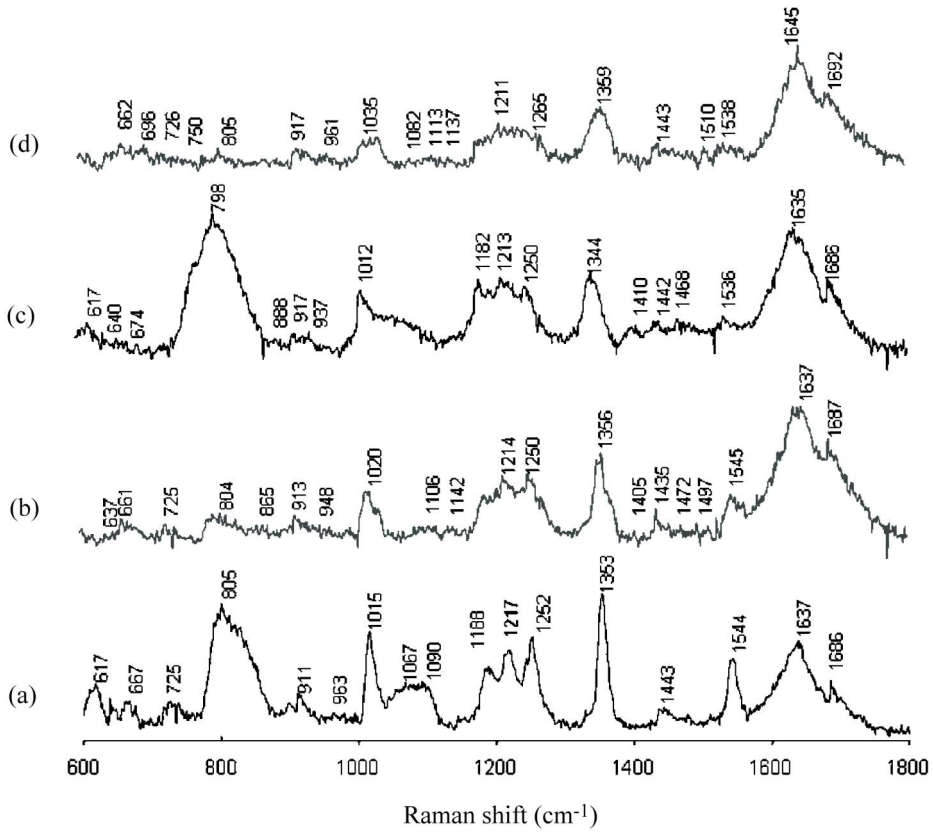


Fig. 2. Ensemble-averaged Raman spectra of $O=Ti(TMPyP)^{4+}$ in the presence of different DNAs in aqueous solution (λ_{ex} = 442 nm) (a) free $O=Ti(TMPyP)^{4+}$ (b) porphyrin-Poly[d(G-C)$_2$] (c) porphyrin-Poly[d(A-T)$_2$] (d) porphyrin-Calf thymus.

measured by conventional resonance Raman techniques. The Raman bands are relatively broad, but readily assigned with reference to the normal mode analysis of similar metallo-$(TMPyP)^{4+}$ and other oxo-titanyl porphyrin. Most of the ensemble-averaged Raman bands measured in the presence of DNAs were observed to exhibit band shifts and intensity variations due to the groove binding of the $O=Ti(TMPyP)^{4+}$ with DNA as expected from the absorption spectral studies. Particularly, the Ti-O stretching band of the free porphyrin complex at 1067 cm^{-1} is significantly down-shifted to overlap with the stretching band of (C_α-C_m) at 1015 cm^{-1} upon interaction with the DNAs, indicating that the groove binding of the porphyrin with DNAs is accomplished by axial ligation of Ti-O with nitrogen at 7 position (N_7) of purine base of DNA. The intensity of the down-shifted Ti-O band is stronger in poly[d(A-T)$_2$] than in poly[d(G-C)$_2$] while it is intermediate in calf thymus DNA. This may be due to difference in the ligation ability of the different base pairs. Actually A-T base pair and G-C pair have two and three hydrogen-bonding, respectively, and the ligation ability of two hydrogen-bonding A-T base pair is expected to be stronger than that of three hydrogen-bonding G-C base pair as inferred from the fact that the axial ligation of protic solvent with Ti-O becomes stronger as the hydrogen bonding ability of the solvent increases. Thus, the axial ligation of the porphyrin should be stronger in poly[d(A-T)$_2$] than in poly[d(G-C)$_2$] as expected from the absorption spectra. Such base-pair dependent Ti-O ligation would lead the porphyrin ring to interact with DNA base pairs differently. As expected, the intensities of the ν_2, ν_4, and $\nu(C_\alpha$-$C_m)$ bands are obviously decreased in the presence of the DNAs in spite of slight band shifts, indicating that polarizability of the porphyrin ring is reduced by binding of the oxo-titanyl porphyrin with hydrophobic environment of DNAs. Interestingly, reduction of the band intensities was observed to be smaller in poly[d(A-T)$_2$] than in poly[d(G-C)$_2$] and calf thymus DNA, indicating that the porphyrin ring in poly[d(A-T)$_2$] still faces hydrophilic aqueous environment as compared to that in poly-[d(G-C)$_2$] or calf thymus DNA. This observation suggests that the porphyrin ring is inserted further into the G-C rich major groove in calf thymus DNA even though the axial ligation with the G-C region is weaker than with A-T region. On the contrary, the porphyrin ring can be inserted into the wider major groove (G-C rich region). Consequently, the porphyrin ring vibration would be also affected differently depending on the base pairs of DNA. If this is the case, the DNA binding effects on vibration frequencies of the porphyrin ring bonds must be clearly observed, but it was difficult to identify the band shifts and band width change correctly because of the line broadening in the ensemble-averaged Raman spectra of the mixed state of free porphyrins and DNA-bound porphyrins. Also the vibration structural changes of the porphyrin ring bound with different DNAs are expected to affect the vibronic structures of the fluorescence spectra differently. However, the spectral features are almost the same due to the ensemble-averaged detection. Therefore, in order to study the vibration structural changes of the porphyrin ring more correctly, we tried to measure the Raman and fluorescence spectra of the single porphyrin-bound DNAs by using the AFM-correlated CSM-coupled Raman/fluorescence techniques.

Fig. 3 shows the AFM-images of different DNAs bound with $O=Ti(TMPyP)^{4+}$ immobilized on cover glass, which exhibit double strand morphologies of about 1 μm length. The morphologies of DNAs seem to be changed upon binding with the $O=Ti(TMPyP)^{4+}$ as Takatoh *et al*. observed for DNA bound with 5,10,15,20-tetrakis[4-trimethyl-amminophenyl]porphyrin-tetra toluenes-4-sulfonate. The shape of double strands of poly[d(A-T)$_2$] seems to be rigid while that of poly[d(G-C)$_2$] or calf thymus DNA looks more or less flexible after binding of the oxo-titanyl porphyrin. This may be due to the base-pair dependent binding modes and strength as discussed above. In order to confirm this, the Raman and fluorescence spectra of the oxo-titanyl porphyrin-bound DNA complexes were measured on a single DNA molecule basis by using the CSM-coupled Raman and fluorescence spectral system.

Fig. 4 shows the CSM-coupled Raman spectra of the single $O=Ti(TMPyP)^{4+}$-bound single DNAs selected from the AFM images and free oxo-titanyl porphyrin powders adsorbed on the quartz glass. In general, the CSM-coupled Raman spectra exhibit more highly resolved bands than the ensemble-averaged Raman spectra, and the vibration bands could be correctly assigned and listed in Table.

The porphyrin macrocyclic bands of free $O=Ti-(TMPyP)^{4+}$ are more or less up-shifted as compared with those of the ensemble-averaged Raman bands. This upshift may be due to lack of water which interacts with the porphyrin ring bonds. On the other hand, the stretching band of Ti-O bond, which is not disturbed by water as described above, is significantly down-shifted to 1022 cm^{-1} from 1067 cm^{-1} measured in diluted aqueous solution, indicating that the highly concentrated oxo-titanyl porphyrins

can be bound with each other by the axial ligation of Ti-O so that Ti-O bond strength is weakened. This intramolecular interaction in solid powder seems to cause the $\nu(C_\alpha\text{-}C_m)$ down-shifted to 1007 cm^{-1} from 1015 cm^{-1} observed in the aqueous solution.

Upon binding with DNAs, the Ti-O stretching band of the free oxo-titanyl porphyrins at 1022 cm^{-1} and one of the porphyrin ring bands, $\nu(C_\alpha\text{-}C_m)$ at 1007 cm^{-1} are merged into a single band at 1024 cm^{-1} due to removal of the intramolecular interaction of the oxo-titanyl porphyrin upon

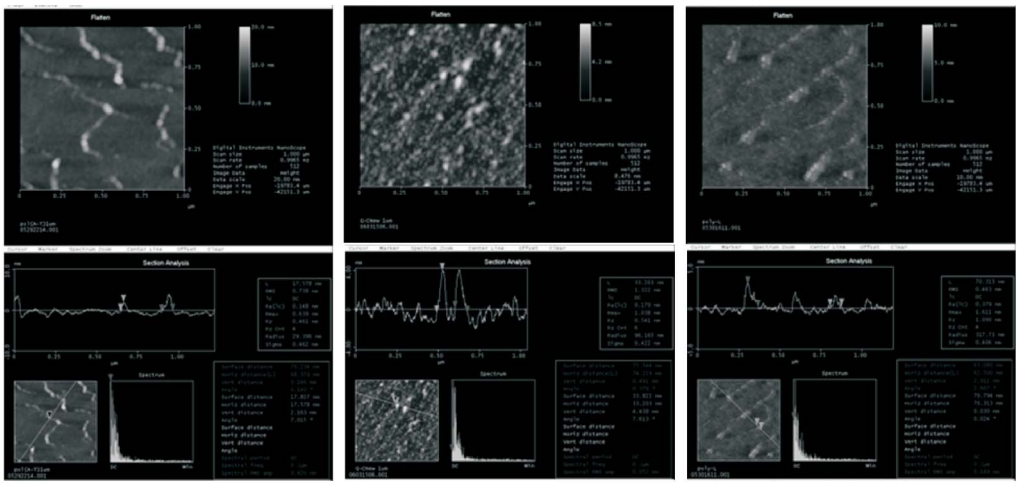
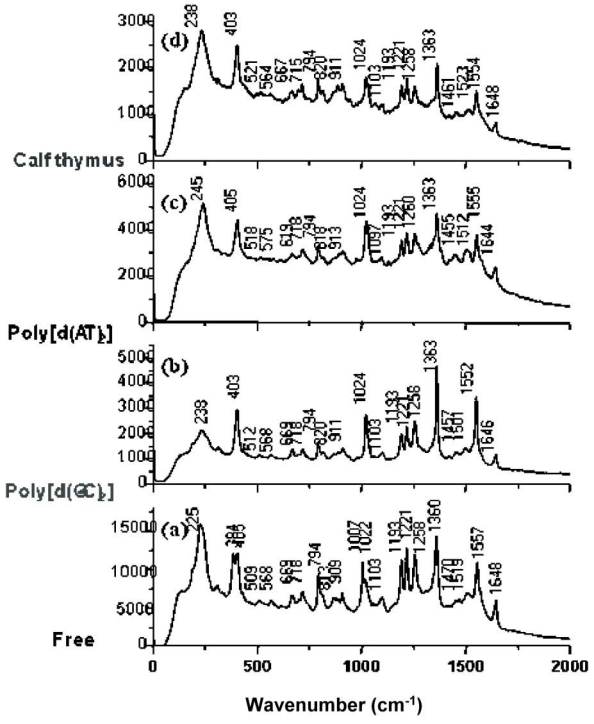


Fig. 3. AFM images of DNAs bound with $O{=}Ti^{IV}(TMPyP)^{4+}$.



Assignment	Free	Poly[d(G-C)$_2$]	Poly[d(A-T)$_2$]	Calf thymus
Φ_4, δ(pyr)	1648	1646	1644	1648
ν_{37}, $\nu(C_\beta\text{-}C_\beta)$	1557*	1552	1555	1554
ν_3, $\nu(C_\alpha\text{-}C_\beta)$	1470	1457	1455	1461
ν_4, $\nu(C_\alpha\text{-}N)$	1360	1363	1363	1363
ν_{11}, $\delta(C_m\text{-pyr})$	1258	1258	1260	1258
δ(pyr)	1221	1221	1221	1221
δ(pyr) + $\nu(N^+\text{-}CH_3)$	1193	1193	1193	1193
ν_{38}, $\delta(C_\beta\text{-}H)$	1103	1103	1097	1103
ν_{28}, $\nu(C_\alpha\text{-}C_m)$	1007			
ν(O=Ti)	1022*	1024	1024	1024
ν(O=Ti) $\cdot$ (H_2O)				
ν_{17}, $\delta(C\text{-}C)_{ip}$	909	911	913	911
$\nu(C\text{-}C)_{ip,t}$ + $\nu(N^+\text{-}CH_3)$	794 813	794 820	794 818	794 820
$(Ti\text{-}N)^4$	405 384	403	405	403
$(N\text{-}C_\alpha\text{-}C_m\text{-}C_\alpha\text{-}N)^4$	225	238	245	238

Fig. 4. CSM-Raman spectra of $O{=}Ti(TMPyP)^{4+}$-nucleic acid complexes on quartz glass.

binding with the DNA base pairs, which of the poly[d(A-T)$_2$] or calf thymus DNA complex is broader than that of the poly[d(G-C)$_2$] complex, indicating the presence of the stronger axial ligation of Ti-O with A-T base pairs as observed from the ensemble-averaged Raman spectra. Such base-pair dependent Ti-O ligation would lead the porphyrin ring to interact with DNA base pairs differently. As expected, the intensities of the ν_2, ν_4, and $\nu(C_\alpha\text{-}C_m)$ bands are obviously decreased in the presence of the DNAs in spite of slight band shifts, confirming that the porphyrin ring faces less hydrophobic environment in poly[d(A-T)$_2$] than in poly[d(G-C)$_2$] and calf thymus DNA as predicted from the ensemble-averaged Raman spectra. Interestingly the folding mode band of N-C_α-C_m-C_α-N was observed at 225 cm^{-1} from the free oxo-titanyl porphyrin, and it was observed to be greatly up-shifted to 245 cm^{-1} with increased intensity in the poly[d(A-T)$_2$] complex as compared to that observed in the poly[d(G-C)$_2$] complex and calf thymus DNA. Also the double stretching modes of Ti-N bond were observed at 384 and 405 cm^{-1} from the free oxo-titanyl porphyrin, and they are merged into one single band (403 cm^{-1}) in the DNA complexes, indicating the loss of vibration energy leading porphyrin ring to be more loosely bound with the G-C rich region than with the A-T rich region in DNA.

In order to confirm this and the DNA-binding effects on photophysical properties of O=Ti(TMPyP)$^{4+}$, the fluorescence spectra of different single DNA-bound complexes were measured by the CSM-coupled fluorescence spectral system as shown in Fig. 5, and they were compared with those of free O=Ti(TMPyP)$^{4+}$ adsorbed on the quartz glass as the DNA-bound O=Ti(TMPyP)$^{4+}$ complexes. Insets in the figure represent the fluorescence images of the single DNA-bound O=Ti(TMPyP)$^{4+}$ complexes, exhibiting the morphologies of DNA strands as observed from the AFM images. The fluorescence of free oxo-titanyl porphyrin powder exhibits the 0-0, 0-1 and 0-2 vibronic emission bands respectively at 550 nm, 640 nm and 680 nm. The 0-0 and 0-1 emissions are quenched as compared to that of 0-2 emission by self absorption due to high concentration in the powder state. However, these emissions are significantly enhanced in the DNA-complexes due to dilution of the oxo-titanyl porphyrin by binding with base pairs. The emission enhancement is the greatest in poly[d(A-T)$_2$] complex whereas it is similar in both poly[d(G-C)$_2$] and calf thymus DNA complexes. This confirms that O=Ti(TMPyP)$^{4+}$ is bound preferably with the G-C rich region in calf thymus DNA. Nevertheless, the vibronic bands are not highly resolved in poly[d(A-T)$_2$] complex as compared to those in both poly[d(G-C)$_2$] and calf thymus DNA complexes, indicating that the porphyrin ring is deeply inserted into the

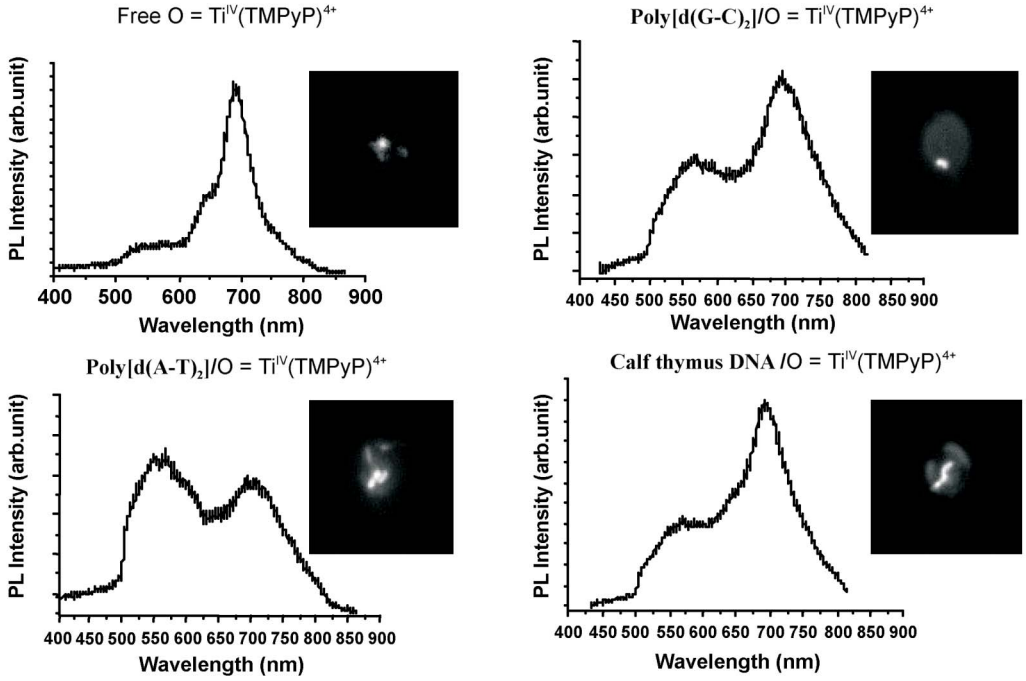


Fig. 5. Comparison of confocal fluorescence spectra of O=Ti^{IV}(TMPyP)$^{4+}$ DNA complexes.

G-C rich major groove in calf thymus DNA as discussed previously. Thus, DNA probably deforms after binding with porphyrin to access more hydrogen bonding environment.

We also measured the fluorescence lifetimes of the free oxo-titanyl porphyrin and its DNA complexes by monitoring two vibronic emission at 613 nm and 664 nm. The free oxo-titanyl porphyrin exhibits a single decay component with lifetime of 1.0 ns for both vibronic emissions, indicating that both emissions originates from the first electronically excited singlet state (S_1). However, in the poly-[d(A-T)$_2$] complex, a long lifetime component (~9.0 ns) was observed in parallel with enhancement of the vibronic emission. This must be due to formation of the tightly bound complex with the A-T base pairs, which reduces vibration energy loss resulting in the inhibition of nonradiative transition. This long lifetime component is also observed in the poly[d(G-C)$_2$] complex, but its relative portion is decreased, indicating again that the oxo-titanyl porphyrin is loosely bound with the G-C base pairs. Instead, another new shorter lifetime component (~2.0 ns) was observed, implying that an excited charge transfer state of the oxo-titanyl porphyrin may be formed more easily in the flexible G-C environment than in A-T environment.

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REFERENCES

1. Jeon, K.-S.; Park, T. S.; Suh, Y. D.; Yoon, M. *J. Photochem. Photobiol. A: Chem.* **2009**, in press.
2. Fiel, R. J.; Howard, J. C.; Mark, E. N. Dattagupta, N. *Nucleic Acids Res.* **1979**, *6*, 3093.
3. Cho, D. W.; Jeoung, D. H.; Ko, J. H.; Kim, S. K.; Yoon, M. *J. Photochem. Photobiol. A.* **2005**, *174*, 207.
4. Suh, Y. D.; Schenter, G. K.; Zhu, L.; Lu, P. *Ultramicroscopy* **2003**, *97*, 89.
5. Kim, H.-H.; Song, N. W.; Park, T. S.; Yoon, M. *Chem. Phys. Lett.* **2006**, *432*, 200-204.