Persistent Spectral Hole Burning for a Free-Base Porphyrin Doped in a DNA-Lipid Complex Film

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ABSTRACT: We performed persistent spectral hole burning for a free-base porphyrin (TMPyP) doped in a DNA-lipid complex film. The microscopic environments of the TMPyP intercalating or outside-binding to the double helix of the DNA-lipid complex is evaluated. The real inhomogeneous width for the TMPyP/DNA-lipid complex is similar to that for a TMPyP/poly(vinyl alcohol) system. In temperature cycling experiments the hole begins to broaden at 40 K, whereas the hole area does not show marked decrease below 100 K. The irreversible hole broadening is attributed the relaxation of the alkyl chain in the lipid part. No decrease in hole area during the temperature cycle is supposed to reflect the binding structure of the TMPyP molecules to the double helix in the DNA-lipid complex.

KEY WORDS Tetra(4-*N*-methylpyridyl)porphine / DNA-Lipid Complex / Intercalation or Outside-Binding / Persistent Spectral Hole Burning / Temperature Cycle / Irreversible Changes of Hole Profiles/

We have utilized persistent spectral hole burning (PSHB) as a spectroscopic technique to study various aspects of dye/polymer systems for more than ten years.^{1,2} PSHB is a phenomenon where a persistent decrease or "a hole" is produced in an absorption spectrum of a dye embedded in a solid matrix by its site-selective excitation at low temperatures.³ Figure 1 illustrates the principle of PSHB. At low temperatures each dye molecule shows a sharp absorption band. However, the surrounding matrix gives a different microscopic environment for each dye, leading to an inhomogeneous distribution of resonant frequencies (Figure 1(a)). When a laser is irradiated to a certain frequency of the inhomogeneous band, only the molecules which are resonant to the laser frequency are excited. If the excited molecules react and become to other species, the absorption at the laser frequency decreases and "a spectral hole" is produced (Figure 1(b)). From the PSHB measurements we can obtain various information about the dye-matrix systems such as dephasing, low-energy excitation, electron-phonon interaction, and structural relaxation.¹⁻³

In this paper, we report the results of PSHB of a *p*-toluenesulfonate salt of TMPyP doped in a DNA-lipid complex film. DNA can interact with cationic porphyrins in several manners.^{4,5} Typical binding ways between porphyrin and DNA is intercalation and outside groove binding. A free-base porphyrin, α , β , γ , δ -tetrakis(4-*N*-methylpyridyl)porphine (TMPyP, see Figure 2) is one of the aromatic dyes known to be an intercalator or an outside-binder to DNA with high binding constants.^{4,5} Because some porphyrin compounds inhibit cancer or AIDS, the interactions of porphyrins with DNA have been paid great attention.

In these years DNA has attracted interests also as a functional material. This is because DNA has high molecular weight, quite ordered structure, and unique properties such as anisotropic electron transfer between the intercalating aromatic molecules. Since porphyrin has high functional activities such as electron transport and nonlinear optical

susceptibility, the combination of DNA with TMPyP is also attractive for the field of applied science.

When we consider to use DNA for functional materials, it is important to make it to solid transparent films and to align the double helix. Recently, a new type of nano-organized systems using DNA, namely, a DNA-cationic lipid has been reported.⁶⁻⁸ Figure 2 shows the structure and a schematic illustration of the DNA-lipid complex. In this DNA-lipid complex, counter cations of phosphate anions are exchanged to cationic lipophilic amphiphiles. This complex can be dissolved in most organic solvents and can form a transparent aligned film by casting and drawing. The intercalation behavior of the aromatic molecules known as intercalators to DNA is also observed for the DNA-lipid complex film. Therefore, this DNA-lipid complex opens up the possibility of applying DNA to organic functional materials such as a light polarizer or an adsorber of cancer suspect agents.⁸

Figure 1. Principle of PSHB.

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Figure 2. Schematic illustration of DNA-lipid complex.

The main interest for us in this study is how the binding of the TMPyP to the double helix of the DNA-lipid complex influences on the microscopic environments around the dye. There are PSHB studies for a few dyes binding to oligonucleotide^{9,10} or DNA^{11,12} systems. In these studies nonphotochemical mechanisms are used for hole burning. Generally nonphotochemical holes are thermally less stable than photochemical ones. Here we measured thermally-induced irreversible changes of hole profiles up to 100 K using the TMPyP which has a photochemical mechanism for hole burning. The inhomogeneous distribution of the absorption lines of TMPyP in the DNA-lipid complex is also measured. We compare the results of PSHB for the TMPyP/DNA-lipid complex with those of TMPyP with a raw DNA in water-glycerol matrix to see the effects of the lipid part. A TMPyP-doped poly(vinyl alcohol) (PVA) film without DNA was also used as a typical amorphous polymer system. Thermally-induced structural relaxation and the extent of heterogeneity around the TMPyP was discussed in relation to the particular structure of the DNA-lipid complex.

EXPERIMENTAL

Materials and Sample Preparation

The DNA-lipid complex was prepared by the method reported previously.⁸ As a raw DNA, the sodium salt of DNA from Salmon testes (ca. 2000 bp, average Mw: 1.3×10^6 , from Sigma) which is the same one as we prepared for the DNA-lipid complex was used. The DNA-lipid complex film doped with TMPyP was prepared by solvent casting on a quartz plate from the $\text{CHCl}_3/\text{MeOH}$ (1:1) solution of the DNA-lipid complex and TMPyP. The obtained film were about 0.2 mm thick and contain about 1.0×10^{-2} mol/kg of TMPyP. The sample of TMPyP with raw DNA in H_2O -glycerol (4:6) was measured in a cell made of two acrylic-resin plates bonded by an epoxy resin with a silicon spacer (1 mm). The concentration of the TMPyP in H_2O -glycerol (4:6) is about 2.0×10^{-3} mol/L. The TMPyP/PVA film was prepared by solvent casting from the water solution and contains about 4.0×10^{-3} mol/kg of TMPyP. For the measurement of CD spectrum a ~5 mm thick TMPyP/DNA-lipid complex sample containing about 1×10^{-2} mol/kg of

Figure 3. Experimental setup for PSHB measurement.

TMPyP was prepared.

Measurements

Figure 3 shows the experimental setup for PSHB measurements. The samples were placed in a cryostat with a cryogenic refrigerator (Sumitomo, SRD204) which can cool the samples to 20 K. A ring dye laser (Coherent, 699-01) with DCM dye pumped by an argon ion laser (Coherent, Innova 70) was irradiated to the samples from the direction of 45° to the sample plane for burning holes. The burned holes were detected through the change in transmittance from the direction perpendicular to laser irradiation with a halogen lamp, a 1 m monochromator (JASCO, CT100C), a photomultiplier (Hamamatsu, R943-02), and a lock-in amplifier (JASCO, LA126W). Hole profiles obtained in transmittance were normalized by the transmission profiles without samples to get the hole profiles in absorbance. CD spectrum of the sample was measured using a JASCO J-500 spectropolarimeter.

RESULTS AND DISCUSSION

Figure 4 shows the absorption spectra of a TMPyP/DNA-lipid complex film measured at room temperature (dotted line) and at 20 K (solid line). Four bands called "Q-bands" are observed between 500 nm and 700 nm. Spectral holes were burned in the lowest band around 640 nm. The peak wavelengths of the four bands, particularly in the case of the lowest band, shift to shorter wavelength as the sample is cooled from room temperature to 20 K. This behavior is also observed for other samples.

Figure 5 shows CD spectra from 380 nm to 480 nm for a phosphate buffer (pH 6.5) solution containing 4.8×10^{-2} base pair mol/l of raw DNA and 7.3×10^{-4} mol/L of TMPyP (a), a $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$ (4:1:0.05 volume ratio) solution containing 3.3×10^{-2} base pair mol/l of DNA-lipid complex and 5.1×10^{-4} mol/l of TMPyP (b), and a TMPyP/DNA-lipid complex film containing 1.0×10^{-2} mol/kg of TMPyP (c). The molar ratio of the TMPyP to the base pair for these

Figure 4. Absorption spectra of a TMPyP/DNA-lipid complex film measured at room temperature (dotted line) and at 20 K (solid line).

three samples is similar (about 1/65). The induced CD spectra in the region of the Soret band give a convenient assignment of the binding modes of the porphyrins to nucleic acids.^{4,5} Intercalating porphyrin to the double helix shows negative Cotton effect and outside-binding porphyrin shows positive Cotton effect. The peak of the positive Cotton effect observed for the outside-binding TMPyP appears at shorter wavelength than the negative peak observed for the intercalating one.⁵ The induced CD spectra for the TMPyP with DNA in buffer (Figure 5 (a)) and TMPyP with DNA-lipid complex in $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$ (Figure 5 (b)) show only a negative peak at 440 nm, suggesting that most TMPyP intercalates to the DNA helix in these solutions. On the other hand, the induced CD spectra for TMPyP/DNA-lipid complex film (Figure 5 (c)) shows both negative and positive peaks. Therefore, some of the TMPyP molecules changes the binding mode from intercalation to outside-binding during the evaporation of the solvent. As for the intensity of the induced CD spectra, it seems difficult to discuss because the samples containing DNA-lipid complex ((b) and (c), especially for the film (c)) show considerable light scattering which decreases the apparent ellipticity. Since the TMPyP is soluble in water and insoluble in most organic solvents, the TMPyP is not likely to enter the nonpolar lipid part in the DNA-lipid complex film. Therefore we assume that most TMPyP molecules in the DNA-lipid complex film either intercalate or outside-bind to the double helix.

The profiles of a growing hole burned at 20 K for a TMPyP/DNA-lipid complex film is shown in Figure 6. Traces (a), (b), (c), and (d) are difference spectra measured after 0.8 min, 2.5 min, 10.5 min, and 29.1 min irradiation, respectively, of 0.75 mW/cm² dye laser at 653.7 nm. The hole width ($\sim 1 \text{ cm}^{-1}$) and the quantum efficiency of hole burning ($\sim 1 \times 10^{-3}$) are similar to those for other free-base porphyrin/polymer systems,¹³ which indicates that no aggregation or interaction at the excited state occurs between the TMPyP molecules.

The inhomogeneous distribution of an absorption band of a doped dye reflects the extent of heterogeneity of microscopic environments around the dye. However, from the apparent absorption spectra we can obtain only the convolution of several absorption components, namely, transitions to vibronic states or phonon levels. In free-base porphyrin/polymer systems only the purely electronic zero-phonon transition becomes sharp at 20 K.¹⁴ Therefore, the distribution of saturated hole depths expresses the

Figure 5. CD spectra from 380 nm to 480 nm for (a): phosphate buffer (pH 6.5) solution containing 4.8×10^{-2} base pair mol/l of raw DNA and 7.3×10^{-4} mol/l of TMPyP, (b): $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$ (4:1:0.05 volume ratio) solution containing 3.3×10^{-2} base pair mol/l of DNA-lipid complex and 5.1×10^{-4} mol/l of TMPyP, and (c): a TMPyP/DNA-lipid complex film containing 1.0×10^{-2} mol/kg of TMPyP.

inhomogeneous distribution profile of a single transition component of the dye in each matrix.¹⁵ Figure 7 shows the distribution of the saturated hole width for TMPyP/DNA-lipid complex film. The obtained distribution profile can be fitted by the Gaussian function well. Although there should be two binding modes for TMPyP in the DNA-lipid complex film, the inhomogeneous distribution profile does not show any splitting. We measured the distribution of saturated hole depths also for the samples of TMPyP-DNA/ H_2O -glycerol and TMPyP/PVA. Table I lists the inhomogeneous width, w_i , and the peak frequency of the inhomogeneous distribution, n_{max} , for the measured samples. The DNA-lipid complex film shows a similar inhomogeneous width to the PVA system. Furthermore, the TMPyP-DNA/ H_2O -glycerol sample shows even larger inhomogeneous width than the PVA sample. This result means that the binding of TMPyP to the DNA helix does not provide a uniform microenvironment for the dye. This is in contrast to a free-base substituted hemeprotein which shows much smaller inhomogeneous width than polymer systems.¹⁶ According to the statistical theory,¹⁷ the inhomogeneous width depends on the strength of intermolecular interactions. Recently, we found a linear correlation between the inhomogeneous width and the solubility parameter of host polymers which expresses the strength of intermolecular interactions.¹⁸ The large inhomogeneous width observed for DNA/ H_2O -glycerol

Figure 6. Profiles of a growing hole burned at 20 K for a TMPyP/DNA-lipid complex film. Traces (a), (b), (c), and (d) are difference spectra measured after 0.8 min, 2.5 min, 10.5 min, and 29.1 min irradiation, respectively, of 0.75 mW/cm² dye laser at 653.7 nm.

system may be due to the large strength of intermolecular interactions of H₂O and glycerol (solubility parameters are; 16.5 for glycerol, 23.2 for H₂O, and 12.6 for PVA¹⁹). In Table I the DNA-lipid complex and DNA/H₂O-glycerol systems show smaller peak frequency of the inhomogeneous distribution, $\nu_{\max}$, than the PVA sample. The statistical theory also predicts that the peak of the inhomogeneous distribution depends only on the strength of van der Waals interaction. Thus, the strength of the van der Waals interaction should be large when the TMPyP binds to the DNA helix.

Next we discuss the results of temperature cycle experiment. Figure 8 illustrates the scheme of temperature cycle experiment. In the temperature cycle experiment, the temperature of the sample is elevated to a certain excursion temperature, and then the sample is cooled again to the initial burning temperature and the hole profile is measured. This temperature cycle is repeated with increasing elevated temperature. From the hole profiles during the temperature cycles, hole broadening (i.e., increase in hole width) and hole filling (i.e., decrease in hole area) were evaluated. Figure 9 illustrates the increase in hole width and the decrease in hole area induced by temperature cycling. The profile of a spectral hole corresponds to the remaining absorption of the dyes which have not reacted by the laser irradiation. When the resonant frequency of each dye is changed, the hole profile changes although the absorption profile of each dye does not change at a constant temperature. The increase in hole width, which is called spectral diffusion, means small frequency shifts (or small environmental change) of each dye molecule (Figure 9(a)). Slight changes of matrix conformation can induce this phenomenon. Large conformational changes which occur apart from the dye should also result in small frequency shifts leading to hole broadening. The decrease in the hole area, called hole filling, corresponds to relatively large shifts of resonant frequencies of dye molecules (Figure 9(b)). The backward reaction of hole burning processes or large changes of microscopic environments of the dye molecules can induce the hole filling phenomenon. Because the thermally-induced hydrogen tautomerization of TPP does not occur below 100 K²⁰, the decrease in hole area below 100 K in the present study reflects

Figure 7. Absorption profile and the distribution of the saturated hole depth for TMPyP/DNA-lipid complex film. The dashed line represents the Gaussian function fitted with the distribution of the saturated hole depths.

Table I. The real inhomogeneous width, ω_i , and the peak frequency of the inhomogeneous distribution, $\nu_{\max}$, for the measured samples.

Sample	ω_i (cm ⁻¹)	$\nu_{\max}$ (cm ⁻¹)
TMPyP/DNA-lipid complex	352	15320
TMPyP with DNA in CHCl ₃ /MeOH/H ₂ O	465	15310
TMPyP/PVA	360	15550

large changes of microscopic environments caused by some relaxation of matrix polymers.

Figure 10 shows the excursion temperature dependence of the increase in hole width for the TMPyP/DNA-lipid complex and the TMPyP-DNA/H₂O-glycerol. Although the TMPyP-DNA/H₂O-glycerol system shows no marked hole broadening below 125 K, the hole width for the TMPyP/DNA-lipid complex begins to increase at 40 K. The sudden increase in hole width at 40 K was observed also for tetraphenylporphine (TPP) doped in polymers containing more than three serial methylene chains such as low-density polyethylene (LDPE) and poly(normalpropyl methacrylate) (PnPMA).²¹ The hole broadening for these systems was attributed to the relaxation of the serial methylene chains. Therefore, we conclude that the hole broadening for the DNA-lipid complex system is caused by the relaxation of the lipid part. This is consistent with the fact that no marked hole broadening is observed for TMPyP-DNA/H₂O-glycerol system.

The hole area for the TMPyP/DNA-lipid complex normalized by the initial hole area at 20 K as a function of excursion temperature is shown in Figure 11. For comparison the results of the same experiments measured for TPP/LDPE and TPP/PnPMA²¹ are also shown in Figure 11. The TMPyP/DNA-lipid complex keeps more than 70% of its initial hole area at 100 K. On the other hand, the hole area for TPP/LDPE and TPP/PnPMA at 75 K is less than 40% of the initial values. Although the hole width for TMPyP/DNA-lipid complex shows quite similar behavior with that for TPP/LDPE and TPP/PnPMA, the result of the hole area is quite different.

Figure 8. Procedure of temperature cycle experiment.

Figure 9. Schematic illustration of spectral diffusion (a) and hole filling (b) caused by temperature cycles.

In other words, though the DNA-lipid complex matrix induces small environmental changes above 40 K as well as LDPE and PnPMA do, the porphyrin does not "feel" large changes of microscopic environments in the DNA-lipid complex matrix as does in LDPE and PnPMA.

We suppose that this difference arises from the binding structure, in particular the intercalation mode, of the TMPyP to the DNA helix. The CD spectrum of the TMPyP/DNA-lipid complex shown in Figure 5 indicates that considerable amount of TMPyP molecules are still intercalating to the double helix. Figure 12 shows a schematic illustration of the microscopic environments around the doped dyes for random polymer systems with alkyl chains (a) and for the TMPyP/DNA-lipid complex (b). For both cases small environmental changes are induced by the relaxation of alkyl chain. In usual dye/polymer systems (Figure 12(a)), the dye molecule should "feel" a large environmental change when the adjacent alkyl chains undergo relaxation. On the other hand, in the DNA-lipid complex system the intercalating dyes are "shielded" by the DNA helix and thus there are few alkyl chains adjacent to the dye (Figure 12(b)). Even for the TMPyP molecules which do not intercalate but outside-bind to the double helix, the dyes should be partly covered with the double helix. Consequently, the dye molecule is not likely "feel" large environmental changes even when the outside alkyl chain undergoes relaxation.

Figure 10. The excursion temperature dependence of the increase in hole width for the TMPyP/DNA-lipid complex (□) and the TMPyP-DNA/H₂O-glycerol (△).

Figure 11. The excursion temperature dependence of hole area normalized by the initial value at 20 K for the TMPyP/DNA-lipid complex (□), TPP/LDPE²¹ (▽), and TPP/PnPMA²¹ (○).

CONCLUSION

In conclusion, we measured persistent spectral hole burning for a porphyrin (TMPyP) binding to the double helix of a DNA-lipid complex. The microscopic environments of the TMPyP are evaluated and discussed from the aspect of the binding modes and the structure of the DNA-lipid complex. The extent of heterogeneity of microscopic environments for the DNA-lipid complex system is similar to that for random polymers. However, the DNA-lipid complex system showed a different behavior of irreversible change in hole area from random polymer systems. This property is attributed to the particular binding structure of the TMPyP/DNA-lipid complex system.

PSHB spectroscopy has been used mainly for studying amorphous glasses including polymers, inorganic or molecular crystals, and a few biological systems.¹⁻³ In addition to these materials, nano-organized systems such as unimolecular micelles²² and dendrimers²³ are quite attractive because they provide a well-defined structure and the

Figure 12. A schematic illustration of the microscopic environments around the doped dyes for a polymer system with alkyl chains (a) and for the TMPyP/DNA-lipid complex (b). In usual dye/polymer systems (a), the dye molecule should "feel" a large environmental change when the adjacent alkyl chains undergo structural relaxation. On the other hand, in the DNA-lipid complex system (b) the intercalating (or outside-binding) dyes are "shielded" by the double helix and thus there are few alkyl chains adjacent to the dye.

possibility to understand the dye-matrix interactions more in detail. The present DNA-lipid complex system should be an example of nano-organized systems which is suitable for PSHB studies.

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