Hydrogen Bond Effect on the Chromophore-Oligonucleotide Conformational Structure Studied by Nonphotochemical Hole Burning Spectroscopy

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Here we illustrate that the nonphotochemical hole burning (NPHB) method can be utilized to study the chromophore-oligonucleotide conformational structure. We have studied the NPHB spectra of the 4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-s-indacene-3-propionic acid succinimidyl ester (BODIPY) and the BODIPY molecule bound to two different lengths of oligomers of guanosine deoxyribonuceotides (BODIPY-$(dG)_n$) doped in poly(vinyl alcohol) (PVOH) films. Our experimental results show that the reduction of the hole burning efficiency depends upon the length of oligonucleotide. The coupling between the chromophore and glass can be reduced if the chromophore is isolated from the polymer matrix by the oligonucleotide. Since the oligonucleotide can form an intramolecular hydrogen bond with BODIPY, it is suggested that the BODIPY is wrapped inside the oligonucleotide. Considering the previous study of a different chromophore bound to an oligonucleotide,[1,2] we believe that the chromophore-oligonucleotide conformation can be drastically perturbed by the intramolecular hydrogen bond.

NPHB spectroscopy has been extensively applied to investigate the configurational relaxation processes of chromophore-glass ensembles at low temperature.[3] Recently, Chang and coworkers[1,2] applied the NPHB method to study how chemical binding of a chromophore to a large group of an oligonucleotide affects the chromophore-glass interaction. Figure 1, parts a and b, show the preburn and postburn hole spectra of 5-(and 6-)carboxy-X-rhodamine *N*-hydroxy succinimidyl esters (RhSE) and the RhSE molecule bound to an oligonucleotide of 10 bases of guanosine deoxyribonucleotide (RhSE-$(dG)_{10}$) doped in PVOH films at 6 K. A hole was burned by using an Ar^+ laser pumping a ring dye laser (Coherent 899-01) with a photon flux of ~12 mW/cm^2 and a burn time of 10 min for both postburn spectra. A sharp zero phonon hole (ZPH) located at the burning wavelength, accompanied by an asymmetric pseudophonon side band hole with tailing on the low-energy side, is observed in the postburn spectra. The burning efficiency of the ZPH measured from the ratio of the hole depth to the absorbance is ~10% for RhSE-$(dG)_{10}$/PVOH and ~35% for RhSE/PVOH.[2] The lower burning efficiency in RhSE-$(dG)_n$/PVOH than in RhSE/PVOH is thought to be due to one degree of freedom of RhSE being lost to the binding of the oligonucleotide.[2] The chemical structure of RhSE is shown at the top of Figure 1a.

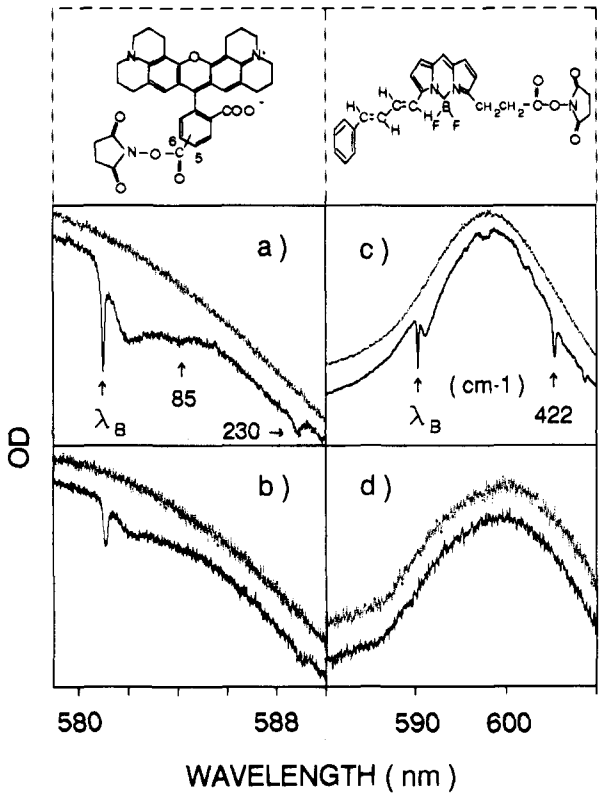

Figure 1. Preburn and postburn spectra of RhSE/PVOH (a), RhSE-$(dG)_{10}$/PVOH (b), BODIPY/PVOH (c), and BODIPY-$(dG)_{10}$/PVOH (d). The structure of RhSE is presented at top left, and the structure of BODIPY is shown at top right.

Very recently, we have examined the intermolecular hydrogen bond effect on the satellite holes (vibronic ZPH) in the HB spectra of the BODIPY doped in PVOH films.[4] Figure 1c shows the preburn and postburn hole spectra of BODIPY/PVOH at 6 K. A hole was burned by using a homemade sync pump dye laser with a photon energy of ~2.5 μJ and a burn time of 15 min. The dye laser with pulse width of ~30 ps, pumped by a mode-locked and Q-switched Nd-YAG laser, emitted trains of ~10 pulses separated by 13 ns at a repetition rate of 500 Hz.[4] It is found that the burning efficiency of the ZPH is ~40%. Furthermore, an intense satellite hole is located at 422 cm^{-1} to the red side of the ZPH. With reference to the bending frequency of the BF_3 molecule at ~440 cm^{-1} [5] and the HB spectra of the derivatives of BODIPY, we proposed that the 422 cm^{-1} satellite hole corresponds to the B-F bending mode.[4] The BODIPY molecule contains a BF_2 functional group; the strong electronegativity of the F atom allows it to form a hydrogen bond with PVOH. The motion of the B-F mode, enhanced by the excitation of the BODIPY, makes the rearrangement of the configurations of the host more efficient via intermolecular hydrogen bond. As a result, the 422 cm^{-1} satellite hole is enhanced by the intermolecular hydrogen bond.

Since the oligonucleotide can possibly form a hydrogen bond with BODIPY, it is important to examine how the intramolecular

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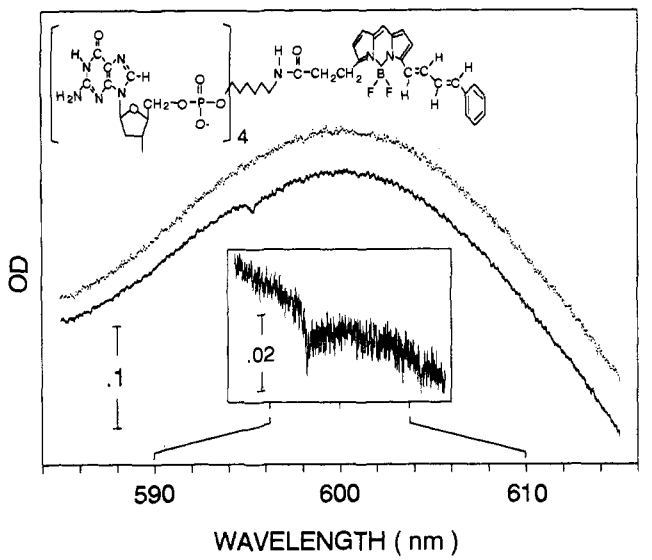

Figure 2. Preburn and postburn spectra of BODIPY-$(dG)_4$/PVOH. The upper corner shows the chemical structure of BODIPY-$(dG)_4$. The inset shows the hole-burned spectrum obtained from the difference between preburn and postburn spectra.

hydrogen bond between BODIPY and the oligonucleotide perturbs the chromophore−glass interaction and the chromophore−oligonucleotide conformational structure. In this work, we have performed NPHB of BODIPY bound to two different lengths of oligomers of guanosine deoxyribonucleotides (BODIPY-$(dG)_n$, where $n = 4$ and 10) doped in PVOH at 6 K. Sample preparation for BODIPY-$(dG)_n$/PVOH is identical to that for RhSE-$(dG)_n$/PVOH, which has been previously described in detail.[2] The chemical structure of BODIPY-$(dG)_4$ is shown in the upper corner of Figure 2. The experimental setup for the NPHB of BODIPY/PVOH can be found elsewhere.[4] Figure 1d shows the preburn and postburn hole spectra of BODIPY-$(dG)_{10}$ doped in PVOH at 6 K. The burning wavelength of the sync pump dye laser is 590 nm, with a photon energy of ~7 μJ and a burn time of 60 min. It is surprising that no hole is observed in Figure 1d. It is important to determine the mechanism responsible for the significant difference of hole burning efficiency between BODIPY/PVOH and BODIPY-$(dG)_{10}$/PVOH.

The hole formation is closely related to the coupling between the chromophore and glass.[3] The excited chromophore could interact with some isoenergetic configurations of the host molecules, which is normally described by the tunneling model associated with a distribution of asymmetric intermolecular double-well potentials,[6,7] the so-called two-level systems (TLSs). Because of phonon-assisted tunneling or thermally activated processes, some types of guest−host interactions deplete the population of the burning site, which produces a persistent hole in the absorption spectrum.[8] Given these facts, we suggest that the absence of the ZPH in BODIPY-$(dG)_{10}$/PVOH is due to the reduction of the coupling between BODIPY and the PVOH matrix. Considering the mechanism of the lower burning efficiencies in RhSE-$(dG)_{10}$/PVOH than in RhSE/PVOH, the absence of the ZPH in BODIPY-$(dG)_{10}$ can be due to more degrees of freedom of BODIPY being lost to the binding of the oligonucleotide. If an intramolecular hydrogen bond is formed between BODIPY and the oligonucleotide, the BODIPY can probably be wrapped inside the oligonucleotide. Consequently, the TLSs of the glass are hardly perturbed while exciting BODIPY.

Furthermore, if the chromophore is wrapped inside the oligonucleotide, the coupling strength between the chromophore and the polymer matrix may depend upon the length of the oligonucleotide. Figure 2 shows the preburn and postburn spectra of BODIPY-$(dG)_4$/PVOH at 6 K. Taking the difference of the two spectra gives the hole-burned spectrum, as shown in the inset in Figure 2. The burning wavelength is 595 nm, with a photon energy of ~7 μJ and a burn time of 60 min. The ZPH is exhibited with burning efficiency of <5%. Our results indeed show that the reduction of the chromophore−glass coupling depends upon the length of the oligonucleotide. We believe that the BODIPY sits inside the oligonucleotide. Further examination of BODIPY-$(dG)_2$/PVOH will be performed.

In addition, similar hole burning efficiency and the identical frequency of the satellite hole at 230 cm^{-1} of RhSE-$(dG)_4$, RhSE-$(dG)_{10}$, and RhSE-$(dG)_{20}$ doped in PVOH reported in our previous work[2] suggested that RhSE sits outside the oligonucleotide. In this work, no prominent satellite hole is observed in the system of BODIPY-$(dG)_4$/PVOH. It should be emphasized that the 422 cm^{-1} mode in BODIPY/PVOH is observed in the CARS spectra.[9] However, we are not able to detect the CARS signal of the 422 cm^{-1} mode in BODIPY-$(dG)_4$/PVOH. The disappearance of the 422 cm^{-1} satellite hole implies that the intermolecular hydrogen bond effect between BODIPY and the polymer matrix is not prominent. This is probably also due to the intramolecular hydrogen bond formed between BODIPY and the oligonucleotide. We believe that the different behavior of the two chromophores is due to the intramolecular hydrogen bond.

At the present time, our NPHB spectroscopic finding should not be overinterpreted; however, it points to an interesting direction, namely the possibility of measuring the conformational structure of chromophores bound to oligonucleotides. The intramolecular hydrogen bond between a chromophore and an oligonucleotide can cause significant changes in conformational structure. Different structures can exhibit different genetic dynamics. For instance, we conjecture that single linear and circular strands of oligonucleotide should exhibit different behavior during the duplication of DNA. Furthermore, the study of the satellite hole may open a new door for the microscopic study of DNA−chromophore interactions.

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