Thermally Activated Hole Filling of Dye and Dye-Oligonucleotide Doped into Polymer Films

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We have measured nonphotochemical hole-burned spectra of the 5(and 6)-carboxy-X-rhodamine Nhydroxysuccinimidyl ester (RhSE) and the RhSE molecule bound to an oligonucleotide of three different sizes of guanosine (RhSE-nG) doped in polyvinyl alcohol (PVOH) films. The distribution of barrier heights of TLSs and the linear electron—phonon coupling parameters are measured from temperature-annealingcycling (TAC) hole-filled spectra. Binding an oligonucleotide to a RhSE decreases the hole-burning efficiency, which may be attributed to one degree of freedom of RhSE lost to the oligonucleotide. However, it has little effect on the spontaneous hole filling, the distribution of barrier heights, and the linear electron—phonon coupling. In addition, similar results observed for the burning efficiency, the distribution of barrier heights, and the linear electron—phonon coupling of various sizes of RhSE-nG/PVOH eliminate the possible effect of free volume produced by the large size of the oligonucleotide around the chromophore. These results, along with the identical frequency of satellite holes observed in these samples, also suggest that the chromophore sits outside the large group of the oligonucleotide. Therefore, we believe that binding an oligonucleotide to a chromophore has little effect on the configurational relaxation of chromophore—glass ensembles.

I. Introduction

Nonphotochemical hole-burning (NPHB) spectroscopy has been extensively applied to investigate the configurational relaxation processes of chromophore-glass ensembles at low temperature.¹⁻⁶ The configurational relaxation of glasses is normally described in terms of the two-level systems (TLS) model.^{7,8} Figure 1 shows a schematic diagram of a two-level system. The TLS model represents the interconversion between two wells when the local configuration changes. Because of the presence of various microscopic structural configurations in amorphous glasses, the TLSs characterized by the barrier height (V) and the asymmetry parameter (ϵ) are widely distributed. The coupling between the TLSs and the chromophore may be a result of, for example, the fluctuation of configuration relaxation of the glass and the reorientation of the chromophores with respect to the local environment.⁹ The interconversion among local structures involves nonthermal tunneling and thermally assisted crossing of the barrier separating the product state from the precursor state.¹⁰⁻¹⁶

Friedrich and co-workers have introduced a temperature cycling method to investigate the distribution of barrier heights in various chromophore–glass systems.^{10–13} They observed that the integrated hole area is reduced by cycling the system as a function of annealing temperature. Their results can be described in terms of thermally activated crossing of the barrier from the product state to the original state. Various functions were introduced for the distribution of barrier heights to describe the thermally induced hole filling.^{10–18} A broad distribution of barrier heights on the order of 10^3 cm⁻¹ was found in the



Figure 1. Schematic representation of the double-well potential characteristic of two-level systems: V, barrier height; ϵ , asymmetric energy; d, difference between two minima; q, coordinates. The solid and dashed lines correspond to the transition at low and high temperatures, respectively.

organic glasses.^{10-13,16} Shu and Small¹⁹ suggested that the increase of free volume around the probe could lower the barrier heights because the TLS potential becomes more symmetric. Comparison of the distributions of barrier heights of different systems is important for unraveling the local structure and the dispersive kinetics of the medium.

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Recently, Chang and co-workers²⁰ have demonstrated that temperature annealing-cycling (TAC) hole spectra can provide information on the linear electron-phonon coupling. The TAC hole spectra are taken as follows: a hole-burned (HB) spectrum is obtained at burning temperature (T_b) after a hole is produced; then the temperature is raised to the annealing temperature (T_a) , and the annealing spectrum is taken at T_a ; subsequently, the temperature is cooled back to the cycling temperature (T_c) , and the cycling spectrum is recorded at T_c . It is noted that $T_c = T_b$. They have found that the integrated hole area is reduced when the temperature is raised to T_a . However, part of the holes are recovered when the system is cooled back to the temperature $T_{\rm c}$. The irreversible part of the hole filling at high temperature results from structural relaxation.¹⁰⁻¹³ The reversible change of the hole area is attributed to linear electron-phonon coupling.²⁰⁻²²

The application of the spectral hole-burning method to DNA systems has received less attention. Flöser and Haarer²³ have investigated the hole-burned spectra of two types of DNA, $d(AT)_5$ and $d(GC)_5$, with intercalated daunomycin (Da) in the glycerol-containing buffer solution. The hole-burning efficiency of $Da+d(AT)_5$ is much larger than that of $Da+d(GC)_5$ by a factor of 60. The different burning efficiency is probably due to the stronger interaction in $Da+d(GC)_5$ than in $Da+d(AT)_5$ because GC base pairs possess a larger dipole moment than AT pairs. In addition, AT oligonucleotides are more flexible than GC oligonucleotides because they have two hydrogen bonds rather than three. There are fewer reports, however, of the comparison of the free Da and intercalated Da. To the best of our knowledge, there is no report on the study of how an oligonucleotide bound to a chromophore affects the chromophore-glass interaction at low temperature.

In this work, we have performed the NPHB of the 5(and 6)carboxy-X-rhodamine N-hydroxysuccinimidyl ester (RhSE) and the RhSE molecule bound to the oligonucleotide with three different numbers of bases of guanosine (RhSE-nG, where n =4, 10, 20) and doped in polyvinyl alcohol (PVOH) films. The purpose of this work is to examine whether chemical binding of a chromophore to a large group of an oligonucleotide can affect the configurational relaxation and the chromophore-glass interaction. Linking a large group of an oligonucleotide to a chromophore could affect the chromophore-glass interaction, since one degree of freedom of the chromophore is lost to the oligonucleotide. In addition, a large group of an oligonucleotide could perturb the TLS of the matrix and probably enlarge the free volume around the chromophore. As a result, the configurational relaxation of RhSE-nG/PVOH may be significantly different from that of RhSE/PVOH. This conjecture can be tested by the measurements of the spontaneous filling, the distribution of barrier heights, and the linear electron-phonon coupling from TAC hole spectra of these samples. Varying the number of bases of guanosine binding to a RhSE is important to study the free volume effect and structural configuration. The satellite holes observed in these systems provide information about the local geometry of the chromophore. Since the absorption band is red-shifted while binding an oligonucleotide, we have performed the excitation wavelength dependence study on RhSE/PVOH to measure the burning site effect.^{24,25} This work should provide useful information for a better understanding of the chromophore-glass interaction and the mechanical properties of glass. In addition, we hope to demonstrate that NPHB can provide important information for the study of DNA-dye complexes.

II. Experimental Section

A. Sample Preparation. The oligonucleotide was prepared by solid phase synthesis methods, using an automated DNA



Figure 2. Structure of RhSE-nG adducts.

synthesizer (Applied Biosystems, Inc. Model 391). To facilitate the attachment of 5(and 6)-carboxy-X-rhodamine (Molecular Probes, Inc.) onto an oligonucleotide, an aminohexyl linker (Aminolink 2, Applied Biosystems, Inc.) was coupled onto the 5' end of the oligonucleotide by using a standard DNA synthesis cycle. After the synthesis was complete, the oligonucleotide was cleaved from the solid support (controlled pore glass, CPG) by washing the CPG with concentrated ammonium hydroxide. The eluent was then incubated at 56 °C for 6 h to remove the protection groups on the oligonucleotide. The solution was then transferred to microcentrifuge tubes, and ammonium hydroxide was evaporated dry by spinning the solution in a vacuum centrifuge (Savant, SVC-100H). The salts from the crude oligonucleotide were removed by precipitation. The residue was dissolved in 120 μ L of 1 M NaCl and 140 μ L of 95% ethanol and vortexed. Another 140 μ L of 95% ethanol was added to the sample, which was vortexed again before being incubated at -20 °C for 30 min. After the incubation, the solution was spun in a centrifuge for 10 min, the supernatant was removed, and the sample was spun to dry in a vacuum centrifuge.

To attach the dye to oligonucleotide, 0.2 μ mol of aminelabeled oligonucleotide was dissolved in 80 µL of 0.5 M Na₂-CO₃/NaHCO₃ (pH 9.0 buffer), and 2 mg of 5(and 6)-carboxy-X-rhodamine N-hydroxysuccinimidyl (NHS) ester was dissolved in 24 μ L of DMSO. The dye coupling reaction was performed by mixing the two solutions together at room temperature overnight. Unreacted free dye was removed by a prepacked size exclusion column (NAP-5, Pharmacia). The column was first conditioned by passing 10 mL of 0.1 M triethylammonium acetate (TEAA) buffer through the column. The volume of the sample was brought up to 0.5 mL by adding 0.1 M TEAA buffer before it was pipeted into the column reservoir. The purified dye-labeled oligonucleotide was obtained by adding 1 mL of 0.1 M TEAA, pH 7.0, to the column and collecting the eluent. To remove unlabeled oligonucleotide, an oligonucleotide purification cartridge (OPC) was used. The OPC was flushed with 5 mL of acetonitrile and 5 mL of 2.0 M TEAA (pH 7.0 buffer) before the sample was loaded. The cartridge was then flushed six times with 5 mL of 8% acetonitrile in 0.1 M TEAA buffer. The purified dye-labeled oligonucleotide was then eluted by washing the OPC with 1 mL of 20% acetonitrile in deionized water. Figure 2 shows the structure of dye-oligonucleotide adducts.

The dye molecule (RhSE) and the dye-labeled oligonucleotide (RhSE-*n*G in this work) were introduced into the polymer solution, which was made by dissolving polyvinyl alcohol (PVOH) in purified deionized water.²⁰ The syrupy solution was poured onto a quartz plate. The samples were placed into a desiccator and were allowed to dry into films. Sample thicknesses were $65 \pm 10 \ \mu$ m. The samples were mounted on a brass plate with several drilled holes of ~1 mm diameter and



Figure 3. Typical hole-burning spectra of (a) RhSE/PVOH and (b) RhSE-10G/PVOH at T = 6 K. The insets show their absorption spectra at T = 6 K, respectively. The arrows mark the shallow holes located at ~85 cm⁻¹ to the red of the ZPH.

put into a Janis dewar, where they were cooled by passing cold helium gas over it. The temperature was measured by a calibrated silicon diode temperature sensor (Lake Shore Cryotronics Model). Because of the low thermal conductivity of the polymer samples, all spectra were taken after the temperature was stabilized for at least 1/2 h to ensure the same temperature of the sample as the mount. The deviation of the temperature is ± 0.5 K at low temperature.

It should be noted that the sample of RhSE-10G doped in PVOH was further analyzed by reverse-phase high-performance liquid chromatography (HPLC). It was found that approximately 5% of free dyes remained in the sample.

B. Experimental Setup. The NPHB setup used was fairly conventional in design and has been described elsewhere.²⁰ The absorption spectra were obtained by dispersing the output of a xenon arc lamp (Oriel 6256) through a 1 m Jobin-Yvon THR 1000 spectrometer and then splitting the output into two beams. One beam was used to produce a reference signal and the other beam was focused onto the sample to produce a sample signal. Both signals were recombined and monitored by the same photomultiplier tube whose output was sent to two lock-in amplifiers (Stanford SR530) referenced to the chopping frequencies. The output of the lock-in amplifiers was sent to personal computers, which were used to calculate the logarithmic ratio that provides a signal directly proportional to the sample absorbance. The hole-burned spectrum was burned by using an Ar⁺ laser pumping a dye laser with a band width of ≈ 1 cm^{-1} . The spot size of the burning beam was ~ 1 mm and that of the reading beam was $\sim 200 \ \mu m$. Extreme care was taken to ensure that the cross section of the probe beam at the sample was inside the area of burn laser.

III. Results

Figure 3 shows the hole-burned spectra of RhSE/PVOH and RhSE-10G/PVOH at ~ 6 K. Both HB spectra were obtained from the difference in absorption spectra before and after hole

burning, and they were taken after 10 min of burning with a flux of ~40 mW/cm² at $\lambda_B \approx 581$ nm. A sharp ZPH located at the burning wavelength accompanied by an asymmetric pseudophonon side band hole (PSBH) with tailing on the low-energy side is observed in both HB spectra. It is noted that the depth of the ZPH is saturated for longer burning times. However, the PSBH grows steadily as a function of burning time and eventually dominates the spectral hole in both systems. Figure 3 indicates that the burning efficiency of ~10% in RhSE-10G/PVOH is lower than that of ~35% in RhSE/PVOH. Similar burning efficiency is observed for other bases of RhSE-*n*G/PVOH.

The insets in Figure 3 show absorption spectra for RhSE/ PVOH and RhSE-10G/PVOH at T = 6 K. The peak center of the low-energy absorption band is shifted from ~574 nm in RhSE/PVOH to ~580 nm in RhSE-10G/PVOH. The peak centers of the absorption bands in RhSE-4G/PVOH and RhSE-20G/PVOH are located at ~582 and ~581 nm, respectively. In order to examine whether the lower burning efficiency in RhSE*n*G/PVOH than in RhSE/PVOH is due to the burning wavelength dependence, we have tuned ω_B near the center of the absorption band in RhSE/PVOH. It is found that the depth of ZPH under saturated conditions is ~15% lower at 575 nm than at 581 nm. Therefore, the much lower burning efficiency of RhSE-*n*G/PVOH suggests that an oligonucleotide binding to a RhSE molecule is involved in the process of hole formation.

Figure 3a also shows another shallow hole located at ~ 85 cm⁻¹ to the red of the ZPH. A similar feature has been observed in the HB spectra of rhodamine-640 doped in PVOH.²⁶ However, this shallow hole is not observed in the HB spectra of DODCI/PVOH²⁶ and BODIPY/PVOH.²⁷ Furthermore, a weak vibronic satellite hole located at ~ 230 cm⁻¹, on the lower energy side of the ZPH, can be seen in Figure 3a. Such behavior has been observed for many systems.²⁸ Both features are barely observed in Figure 3b for RhSE-10G/PVOH. However, it should be emphasized that the ~ 230 cm⁻¹ mode in both systems



Figure 4. Typical temperature-annealing-cycling hole spectra of RhSE/PVOH (a-c) and RhSE-20G/PVOH (d-f). The dotted lines present the fit to the experimental data by using the widths of a ZPH, a PSBH, and a shallow hole as 0.6, 6.5, and 7 Å for spectra a,c, 0.8, 6.5, and 7 Å for b, 0.7, 7, and 10 Å for d,f, and 0.9, 7, and 12 Å for e.

was also observed in the CARS spectra.²⁷ We believe that this satellite hole corresponds to a Franck-Condon active excited state vibration of the chromophore. The nearly identical frequency of the satellite holes suggests that the local geometry of the chromophore is not perturbed by binding to the oligonucleotide.

Figure 4 shows temperature annealing-cycling spectra of ZPH for RhSE/PVOH and RhSE-20G/PVOH. The burning times for these HB spectra are 3 and 10 min, respectively, with a flux of ~ 12 mW/cm². The dotted lines present the fit to the experimental data. The details of the simulation approach can be found elsewhere.^{29,30} In brief, the method consists of separately calculating three Gaussian line shapes for a ZPH, a PSBH, and a shallow hole. It is found that the widths of ZPH in the cycling spectra are approximately identical to those in the HB spectra. However, the widths of ZPH in the annealing spectra are slightly broader at higher temperatures for both systems. The reliable measurement of homogeneous line width has been examined by Völker and co-workers.^{31,32} Unfortunately, we are unable to determine the homogeneous line width in our present investigation.²⁰ Nevertheless, the reversible hole width in the TAC spectra implies that the spectral diffusion is unlikely to be the dominant hole broadening mechanism for both systems, because the local structure randomized by the spectral diffusion is an irreversible process.⁵

Figure 4 also shows that part of the hole reduction in area at annealing temperature T_a is recovered when the temperature is cooled back to $T_c = 6$ K. Possible mechanisms for the hole filling involve spontaneous filling and thermally activated processes. We have measured the integrated hole areas of ZPH as a function of waiting time to examine the spontaneous hole filling. Figure 5 shows the plots of hole area as a function of waiting time for RhSE-20G/PVOH under various conditions. Under the burning temperature condition, the ZPH hole area is



Figure 5. Plots of the hole areas of RhSE-20G/PVOH as a function of waiting time under the burning temperature condition for the first 3.5 h, the annealing temperature condition for the next 3 h, and the cycling temperature condition for the last 2 h. In the burning condition, data are obtained at $T_b = 6$ K. The annealing spectra are measured after the sample is raised to $T_a = 20$ K and read at 20 K, and the cycling spectra are obtained after the sample is cooled from $T_a = 20$ K to $T_c = 6$ K and read at 6 K.

reduced by $\sim 15\%$ in the first hour, and then the change of the hole area is insignificant. Under the annealing temperature condition, the integrated areas of ZPHs are nearly identical within experimental error for 3 h of waiting time. Under the cycling temperature condition, the areas of ZPH hole are also approximately identical for more than 2 h of waiting time. In addition, the line width does not change with the waiting time dependence under these conditions. Very similar behaviors have been reported for the system of RhSE/PVOH.³⁰ This establishes that thermally activated hole filling is much more dominant than



Figure 6. Plots of hole area vs annealing temperature under cycling conditions. The burning wavelength is located at \sim 581 nm for all four of the samples. The solid lines are the calculation of eq 4 to fit the experimental data.

spontaneous hole filling. Thus, the contribution from spontaneous hole filling can be neglected for the systems studied in this work.

It is known that the hole reduction in the cycling spectra can provide information about the distribution of barrier heights of low-temperature glasses.^{10–16} Therefore, we measure the integrated hole area in the cycling spectra of RhSE/PVOH, RhSE-4G/PVOH, RhSE-10G/PVOH, and RhSE-20G/PVOH as a function of annealing temperature. In order to eliminate the effect of the initial decay in the hole reduction as shown in Figure 5, the hole area measured in the first cycling spectrum from the cycling of $6 \text{ K} \rightarrow \sim 10 \text{ K} \rightarrow 6 \text{ K}$ is utilized for the normalization. The normalized hole areas as a function of annealing temperature under cycling conditions are plotted in Figure 6. The analysis of these data will be described in the next section.

In our previous work, we have demonstrated that the linear electron-phonon coupling can be measured from the difference in hole reduction between annealing and cycling spectra.²⁰ The data in Figure 7 present the experimental results of the counterpart of $I_{ZPH}(T_a)/I_{ZPH}(T_c)$ as a function of annealing temperature for all four systems. It is noted that the frequency difference between ZPH and PSBH is ~28 cm⁻¹ for all four systems. The deviation of the plots is ~15% at lower temperatures and ~30% at higher temperatures. The analysis of these data will be described in the next section.

In addition, we have tuned the burning frequency to near the center of the absorption band of RhSE/PVOH for examining the excitation wavelength dependence. Figure 8 shows the study of the distribution of barrier heights and the electron-phonon coupling obtained from the TAC results at $\lambda_B = 575$ nm. The importance of this study will be discussed in the next section.

IV. Data Analysis

A. Distribution of Barrier Heights. In general, the initial distribution function in a glass can be viewed as stationary in the sense that the population interconversions among different sites are at equilibrium. After an optical excitation, the initial distribution is perturbed and some types of guest-host interac-

tion processes deplete the population ΔN in the original state to produce a hole in the absorption spectrum.^{33,34} However, this perturbed system tends to return to the initial state because of the population gradient.^{35,36} This is confirmed by the identical absorption spectra taken before hole burning and after thermally activated hole filling at ~120 K. Therefore, we only consider the burnt molecules ΔN that convert from the product state back to the precursor state. Without considering the tunneling effect, the rate of transition classically follows the Arrhenius equation. Since amorphous glasses exhibit a wide variety of local configurations characterized by various barrier heights (V_j), the rate for crossing the barriers is given by

$$R = A \sum_{j} \exp[-V_{j}/kT]$$
(1)

where A is an average frequency factor of molecules in the product state crossing the barrier to the initial state. Considering the distribution of barrier heights, the probability of different sites with barrier heights between V and V + dV is introduced as P(V) dV. The change of ΔN at time t after a hole is burned is given by

$$\Delta N(t) = \int_0^\infty P(V) \, \mathrm{d}V \exp[-Rt] \tag{2}$$

Various distribution functions of inhomogeneities were employed for P(V). Friedrich and co-workers¹⁰⁻¹³ have assumed that the barrier heights V follow a distribution of the form $P(V) \propto V^{-1/2}$ with cutoff at V_{max} . This result agrees with the classic tunneling model.⁷ Fayer and co-workers⁵ have deduced the rate distribution from the time-dependent hole width. They found that a Gaussian form fits the data well. Elschner and Bässler¹⁶ have also employed a Gaussian distribution to describe the distribution of barrier heights in disordered glasses. In this work, we have chosen the Gaussian form for the barrier height distribution,^{16,37} which is given as

$$P_{\rm G}(V) = (2\pi\Gamma_{\rm G}^{2})^{-1/2} \exp[-(V-V_0)^2/2\Gamma_{\rm G}^{2}] \qquad (3)$$



Figure 7. Plots of the ratio of $I_{ZPH}(T_a)/I_{ZPH}(T_c)$ as a function of annealing temperature. The burning wavelength is located at ~581 nm for all four of the samples. The solid lines are the fit to the experimental data using eq 10.



Figure 8. Burning wavelength is located at 575 nm in RhSE/PVOH. (a) The plot of hole area vs annealing temperature under cycling conditions. The solid line is the calculation of eq 4 to fit the experimental data. (b) The plot of the ratio of $I_{ZPH}(T_a)/I_{ZPH}(T_c)$ as a function of annealing temperature. The solid line is the fit to the experimental data using eq 10.

where $\Gamma_{\rm G}$ is the width of the Gaussian distribution. Elschner and Bässler¹⁶ assumed that $V_0 = 0$ for optimizing the fit, and $V \ge 0$ for avoiding the negative activation energy. Without considering the effect of spontaneous hole filling, the change of ΔN resulting from thermally activated processes can be rewritten as

$$\Delta N(T) = (2/\pi \Gamma_{\rm G}^{2})^{1/2} \int_{0}^{\infty} \exp[-(V^{2}/2\Gamma_{\rm G}^{2})] \exp[-At \exp(-V/kT)] \, dV$$
(4)

Unfortunately, eq 4 cannot be analytically solved; it requires a numerical calculation. Three parameters in eq 4 are used to determine the fit to experimental results. Elschner and Bässler¹⁶ have estimated the magnitude of A to be $\sim 10^{12} \text{ s}^{-1}$. Friedrich and co-workers¹⁰⁻¹³ also used the same value for their simulations. We assume that the magnitude of A is $\sim 10^{12}$ s⁻¹ for both cases. Since all the data used in this work were taken after the temperature stabilized for longer than 30 min, the experimental time, t, is given as 10^4 s. The simulation is not sensitive to the change of magnitude of At.^{10-13,16,37} The solid curves in Figure 6 are the fits of eq 4 to the experimental data of RhSE, RhSE-4G, RhSE-10G, and RhSE-20G doped in PVOH films, using $\Gamma_G = 1050 \pm 150$, 1050 ± 150 , 900 ± 150 , and $800 \pm 150 \text{ cm}^{-1}$, respectively. Figure 6 indicates that the distribution of barrier heights is narrower in RhSE-20G/PVOH than in RhSE/PVOH.

We have examined the burning site effect by tuning the burning wavelength from 581 to 575 nm for RhSE/PVOH. The solid curve in Figure 8a fits the experimental data when using $\Gamma = 950 \text{ cm}^{-1}$. It is suggested that the distribution of barrier heights is slightly narrower when the burning wavelength is tuned from the red side to the center of the absorption band of RhSE/PVOH. Therefore, we believe that binding a large group of the oligonucleotide to a chromophore has little effect on the distribution of barrier heights.

B. Electron-Phonon Coupling. In the temperature annealing spectra, the reduction of hole area involves temperature-irreversible and temperature-reversible processes. The former process is attributed to the populations of ΔN_j in the product states with barrier heights of $V_j < kT_a$ crossing the barrier and

TABLE 1: Summary of Experimental Results

	RhSE	RhSE	RhSE-4G	RhSE-10G	RhSE-20G
center of absorption band (nm)	~574	~574	~582	~580	~581
burning wavelength (nm)	\sim 575	~581	~581	~581	~581
burning efficiency (%)	~30	~35	~10	~10	~ 10
frequency of satellite hole (cm ⁻¹)		~ 230	~ 230	~ 230	~ 230
spontaneous hole filling (%)		~ 15		~15	~15
distribution of barrier heights (cm ⁻¹)	~950	$\sim \! 1050$	~ 1050	~ 900	~ 800
linear electron-phonon coupling	~ 0.85	~0.7	~ 0.85	~1.0	~ 1.0

then returning to the initial state. The reversible process is due to the photons absorbed by the thermally activated populations via the linear electron—phonon coupling in the product states, characterized by the asymmetric energies of $\epsilon_a \leq kT_a$. In the temperature cycling spectra, those populations of ΔN_j that returned back to the initial state are temperature-irreversible,^{10–13,16} but the thermally activated populations are retrapped since ϵ_a > kT_b . Thus, the difference of hole reduction between annealing and cycling spectra can be described by the linear electron phonon coupling.²⁰

The theory of linear electron-phonon coupling indicates that the integrated intensity of the ZPH decreases when temperature increases because of the spectral redistribution of the thermal occupation of phonon states.^{2,38-40} The coupling strength can be described by the Debye-Waller factor $\alpha(T)$

$$\alpha(T) = \exp[-S(T)] \tag{5}$$

where S(T) is the coupling constant (Huang-Rhys factor), which is given by^{39,40}

$$S(T) = \sum_{j} P_{j} / \hbar \omega_{j} \coth(\hbar \omega_{j} / 2kT) = \sum_{j} S_{j} \coth(\hbar \omega_{j} / 2kT)$$
(6)

where $P_j = m\omega_j^2 q_j^2/2$ is the half Stokes energy shift for the *j*th mode and q_j is the shift of the equilibrium position of the oscillator. Since it is difficult to determine all the modes involved in the summation of eq 6, an average frequency, $\omega_{\rm P}$, is introduced for reducing the equation to

$$S(T) = \sum_{j} S_{j} \coth(\hbar\omega_{p}/2kT) = S_{0} \coth(\hbar\omega_{p}/2kT) =$$

$$S_{0} (2\tilde{n}(T) + 1) (7)$$

where S_0 is the coupling constant at T = 0 K and $\tilde{n}(T)$ are the thermal occupation numbers at the mean phonon frequency of ω_P , $\tilde{n}(T) = [\exp(\hbar\omega_P/k_BT) - 1]^{-1}$. When temperature increases, the Debye–Waller factor $\alpha(T)$ decreases. In the annealing HB spectra, the hole is burned at a low temperature but is probed at higher temperatures. The Debye–Waller factor $\alpha_{ZPH}(T_a)$ follows

$$\alpha_{\text{ZPH}}(T_{a}) = \alpha(T_{b})\alpha(T_{a}) \approx \exp[-S_{0}(2\tilde{n}(T)+2)] \quad (8)$$

while $T_b \rightarrow 0$. However, the hole is burned and probed at the same low temperature in the cycling spectra, where the Debye–Waller factor $\alpha_{ZPH}(T_c)$ follows

$$\alpha_{\rm ZPH}(T_{\rm c}) = \alpha(T_{\rm b})\alpha(T_{\rm c}) \approx \exp[-2S_0]$$
(9)

The ratio of these two intensities $I_{ZPH}(T_a)/I_{ZPH}(T_c)$ as a function of temperature should follow the relationship

$$I_{\text{ZPH}}(T_{\text{a}})/I_{\text{ZPH}}(T_{\text{c}}) = \alpha_{\text{ZPH}}(T_{\text{a}})/\alpha_{\text{ZPH}}(T_{\text{c}}) = \exp[-2S_0\tilde{n}(T_{\text{a}})]$$
(10)

Two variables, S_0 and ω_P , are used in eq 10 to determine the fit of experimental results. It is known that the ω_P can be measured from the energy difference between ZPH and PSHB.⁴³ In this

work, we have found that the frequency difference between ZPH and PSBH is ~ 28 cm⁻¹ for all four systems. Small and coworkers^{19,28} also found that the ω_P of cresyl violet doped in PVOH is 28 ± 4 cm⁻¹. The solid curves shown in Figure 7 are calculated from eq 10. Using the same average phonon frequency of $\omega_{\rm P} = 28 \ {\rm cm}^{-1}$ and the different electron-phonon coupling parameters of $S_0 = 0.7, 0.85, 1.0, \text{ and } 1.0, \text{ one can fit}$ the experimental data of RhSE/PVOH, RhSE-4G/PVOH, RhSE-10G/PVOH, and RhSE-20G/PVOH, respectively. It is found that the linear electron-phonon coupling is larger in RhSE-20G/PVOH than in RhSE/PVOH. Considering the burning site effect, we have tuned the burning wavelength from 581 to 575 nm for RhSE/PVOH. The fit from eq 10 to the experimental data in Figure 8b by using $\omega_{\rm P} = 28 \text{ cm}^{-1}$ and $S_0 = 0.85 \text{ suggests}$ that linking a large group of an oligonucleotide to a chromophore creates a slight difference in the electron-phonon coupling.

In the following section, we summarize the above results for the comparison in Table 1.

V. Discussion

Table 1 shows that the absorption band is red-shifted and the burning efficiency is lower when a chromophore binds to a group of an oligonucleotide. However, the spontaneous holefilling behavior is similar for both RhSE/PVOH and RhSE-20G/ PVOH. When the results of burning wavelength dependence are considered, the distribution of barrier heights and the linear electron-phonon coupling measured from TAC hole spectra show only a small difference between RhSE and RhSE-*n*G in PVOH. Furthermore, the nearly identical frequency of the satellite holes for them suggests that the local geometry of the chromophore is not perturbed by linking to an oligonucleotide.

Binding a large group of an oligonucleotide to a chromophore may directly affect the properties of the chromophore or influence the configurational structure of the polymer matrix to perturb the chromophore-glass interaction. For example, the large group of oligonucleotide may enlarge the free volume around the chromophore, and then the coupling between chromophore and polymer is perturbed. Nakanishi and coworkers⁴⁴ have studied the HB spectra of Nile blue doped in PVOH and in cross-linked PVOH derivatives. They found that the depth of ZPH decreases as a function of the length of the cross-linker introduced to the PVOH backbone. The variation of the hole depth is attributed to the change of the TLS distribution around the dye entrapped in the void space induced by the cross-linking. In the present work, the large sizes of oligonucleotides could enlarge the void around the chromophore differently. However, the significant difference of the hole depth between RhSE/PVOH and RhSE-4G/PVOH and similar hole depths observed for various bases of RhSE-nG suggest that the change of void space is unlikely to be the dominant mechanism for the lower burning efficiency of RhSE-nG.

Shu and Small¹⁹ suggested that larger free volume around a chromophore could make the TLS_{ext} potential more symmetric and thus lower the barrier heights. In the present work, the small change of the distribution of barrier heights is not significant for examining this effect. In addition, Saiken and co-workers²⁶ have found that in the systems of rhodamine-640

doped in different polymers the electron-phonon coupling is stronger if the side group on the polymer chain is larger and stiffer. They proposed that the linear electron-phonon coupling depends upon the size of the free volume around the chromophore in the polymers. However, similar electron-phonon coupling strengths are found for various bases of RhSE-nG/ PVOH. Therefore, we consider that linking of a chromophore to a large group of an oligonucleotide does not change the TLSext configurations significantly. At this time, we believe that the possible free volume effect due to a large group of an oligonucleotide linking to a chromophore can be neglected in this work.

In our earlier work,³⁰ we proposed that the small difference of linear electron-phonon coupling between RhSE/PVOH and RhSE-10G/PVOH may be due to the coupling between excited chromophore and additional pseudolocal phonon modes resulting from the oligonucleotide. However, the very similar electronphonon coupling between RhSE/PVOH, burned at ~575 nm, and RhSE-4G/PVOH, burned at ~581 nm, implies that this possibility can be neglected. The small difference of electronphonon coupling between RhSE/PVOH and RhSE-nG/PVOH may be closely related to the red shift of the absorption band.⁴⁵

Finally, we speculate that the lower burning efficiency in RhSE-nG/PVOH than in RhSE/PVOH is attributed to one degree of freedom of RhSE lost to the binding of the oligonucleotide. As a result, the effective density of TLSs around the chromophore can be reduced upon optical excitation. This conjecture is further supported by the similar burning efficiencies observed in various bases of RhSE-nG. Furthermore, the similar burning efficiencies in various bases of RhSE-nG imply that the chromophore sits outside the large group of the oligonucleotide. If the chromophore is wrapped inside the large group of the oligonucleotide, the distance between chromophore and the TLS of the matrix may be increased by increasing the length of the oligonucleotide. Therefore, the coupling between chromophore and polymer matrix can be varied as a function of the length of the oligonucleotide. This behavior should also be reflected in the study of the distribution of barrier heights and the electron-phonon coupling. However, these predictions are not observed in this work. Considering the identical frequency of satellite holes observed in these four samples, we believe that the chromophore sits outside the large group of the oligonucleotide.

VI. Summary

We have applied HB methods to investigate how the large group of an oligonucleotide bound to a chromophore affects the configurational relaxation and the chromophore-glass interaction. It was found that binding an oligonucleotide to a chromophore decreases the hole-burning efficiency but has little effect on the hole-filling kinetics. The former is attributed to one degree of freedom of RhSE lost to the binding oligonucleotide. The latter suggests that the configurational relaxation processes of chromophore-glass ensembles are less perturbed by the oligonucleotide. In addition, similar results observed for various bases of oligonucleotide indicate that the free volume effect is not important in this work. Although the details of the structural configuration of RhSE-nG complexes are not clear, the study of TAC hole spectra still provide useful information on the structural configuration of an oligonucleotide-chromophore. We believe that NPHB can provide important information for the study of DNA-chromophore complexes. J. Phys. Chem., Vol. 99, No. 17, 1995 6627

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