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WHITE LIGHT HOLE BURNING OF TO-PRO-3 BOUND TO OLIGOMERIC DNA

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Abstract Binding of the dye TO-PRO-3 iodide to alternating GC and AT oligomers of DNA is investigated by absorption and hole burning spectroscopies. This dye is one of a series extensively used for nucleic acid staining. In addition to narrow laser induced holes, the dye/oligomer complexes also exhibit white light induced hole burning. These holes are indicative of a dye/DNA binding mode in addition to the previously identified externally bound and intercalative modes. Possible structures of this mode are considered.

INTRODUCTION

Previously, we reported on low temperature absorption, fluorescence, and hole burning studies of the dye TO-PRO-3 bound to double-stranded and single-stranded calf thymus DNA[1]. TO-PRO-3 is the reddest absorbing and fluorescing member of a series of dyes based on thiazole orange and oxazole yellow. These dyes are characterized by high binding constants to DNA which have been ascribed to the formation of intercalated dye-DNA complexes. The dyes also have high molar extinction coefficients and, in fluid solution, the bound dye has a high fluorescence quantum yield while the quantum yield for the unbound dye is low. In rigid media at low temperatures, the quantum yield is high for both free and bound dye, implying that the fluorescence quenching is dynamical in nature.

In our previous work, we demonstrated that the small red shift and broadening of the dye's absorption band which occurs on binding to DNA can be understood in terms of two binding modes for the dye-DNA complex. In one of these modes, the dye has an environment similar to that of free dye and, consequently, a free-dye-like absorption. For the other binding mode, the absorption is red shifted. The observed spectrum is the sum of the two contributions. In agreement with other work[2, 3], we assigned the two binding modes to be an externally bound mode in which the dye is accessible to solvent and

experiences an environment not dissimilar to that of free dye, and an intercalated mode in which the dye is situated between the DNA bases. In the intercalated mode, the dye-DNA interaction is stronger, and the dye is not accessible to solvent, causing the red shift in absorption. Based on the hole burning data, a reasonable approximation to the absorption shape of the intercalated dye can be determined from a broad lower energy hole that occurs when saturated holes are burned in the region in which externally bound dye absorbs. This hole was interpreted as being due to energy transfer from the externally bound dye to the intercalated dye, followed by non-photochemical hole burning of the intercalated species. The shape of this hole was taken as being close to the absorption profile for these molecules, with the failure to observe sharp holes from intercalated dye being due to the strong electron-phonon coupling for the intercalated dye.

Previous room temperature absorption and dichroism studies of the related dyes, YO and YOYO, had indicated that this dye also can bind by both intercalation and in external complexing[3]. In that work, it was reported that the intercalated binding is the preferred mode, with externally bound species being observed only at dye/DNA ratios greater than 0.2. In contrast, from the relative intensities of the external and intercalated contributions to the absorption, our results indicated that for TO-PRO-3, external binding is competitive with intercalation, and may even be the preferred binding geometry.

To further investigate the dye-DNA interaction, we have used DNA oligomers, of length 20 base pairs, with alternating GC or AT sequences, ($d(GC)_{20}$ and $d(AT)_{20}$). The oligomer results were compared with results for high molecular weight poly-GC or poly-AT and with calf thymus DNA samples. For all samples, absorption, fluorescence and hole burning studies were performed. A full account of the results will be presented elsewhere. In this report, we focus on a unique white light hole burning observed for these samples and its implications regarding the dye-DNA binding.

EXPERIMENTAL

TO-PRO-3 iodide was purchased from Molecular Probes, Inc., as a 1mM solution in DMSO and was used without further purification. $d(GC)_{20}$:$d(GC)_{20}$ and $d(AT)_{20}$:$d(AT)_{20}$ were obtained from Iowa State University DNA Synthesis Facility as single strands. Duplexes were formed by heating the oligomers 4-5 °C beyond their respective melting temperatures (T_m=80 °C for $d(GC)_{20}$:$d(GC)_{20}$ and T_m=40 °C for $d(AT)_{20}$:$d(AT)_{20}$). The oligomers were then allowed to cool to room temperature overnight, after which they were stored at -20 °C. All samples were prepared immediately before use in 40% glycerol/60% phosphate buffer. For absorption measurements, TO-PRO-3/oligomer

samples were prepared in 0.05 and 0.2 dye/DNA base pairs, (bp), ratios. Dye/bp concentrations were 20 mM/400 mM and 40 mM/200 mM for both TO-PRO-3/d$(GC)_{20}$:d$(GC)_{20}$ and TO-PRO-3/d$(AT)_{20}$:d$(AT)_{20}$.

Absorption spectra were obtained as described previously[1]. Laser-selective hole burning was done by irradiating the samples for one minute at λ_B=627.7 nm with an intensity of 39 mW cm^{-2}. Post laser-selective hole burned and white light hole burned absorption spectra were obtained at regular intervals while continuously irradiating the samples with the tungsten light source from the Fourier transform spectrometer for up to 135 min. The intensity for the white light source was 37 mW cm^{-2}.

RESULTS

Fig. 1 shows 4.2 K absorption spectra of TO-PRO-3 bound to the GC-oligomer for dye/bp ratios of 0.05 and 0.2. The absorption maximum is at 646.9 nm for the more dilute sample and shifts to 644.2 nm for the more concentrated sample. There is also a vibronic absorption band at ~588 nm. Similar spectra for the dye bound to the AT-oligomer are shown in Fig.2, with the absorption maximum at 639.2 nm, independent of concentration, and a vibronic band at 588 nm. These spectra and others will be discussed in more detail in a forthcoming publication[4], but at this point suffice it to say that the spectra are generally consistent with the previous observation that the spectra have contributions from both externally bound and intercalated dye. From the absorption maxima and concentration shifts, the external complex dominates the AT-oligomer spectra, while for the GC-oligomer, there seems to be more intercalated dye at low dye/bp ratios. As before, the absorption of the intercalated dye is assigned to the red side of the absorption, causing some asymmetry on that side of the band, noticeable particularly in the AT-oligomer, Fig. 2, while the externally bound complex has an absorption whose peak is near the free dye absorption maximum (640.7 nm at 4.2 K). Note also that the AT-oligomer shows an additional low energy band at 677 nm which is concentration dependent, indicating that it is probably due to dimerization of the dye.

The top spectrum of Fig. 3, shows the hole spectrum of the dye/GC-oligomer following burning at 627.7 nm. Similar spectra are observed for the dye bound to the AT oligomer. At the laser burn frequency, there is a narrow zero-phonon hole with a pseudo-phonon sideband hole and pseudo-vibronic holes to the red. In more expanded spectra, there is an increase in absorption, (an anti-hole), to the blue of the zero phonon hole. A blue shifted anti-hole is consistent with the hole burning mechanism being due to non-photochemical hole burning[5, 6]. Overlapping the pseudo-vibronic holes is a broad hole

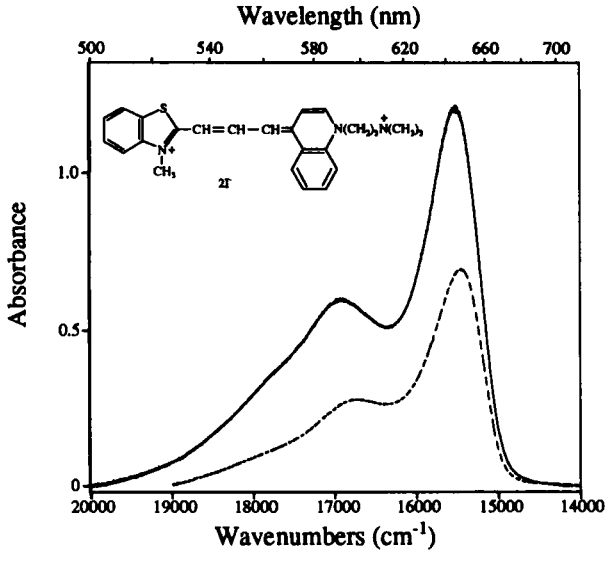

FIGURE 1 Absorption of TO-PRO-3 bound to $d(GC)_{20}$:$d(GC)_{20}$ at 4.2 K. The structure of the dye is also shown. The solid line is for 0.2 dye/bp, the dashed line for 0.05 dye/bp.

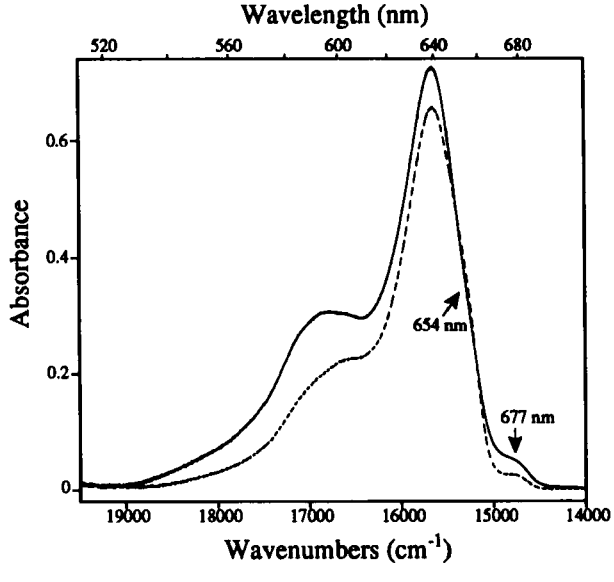

FIGURE 2 The absorption spectrum of TO-PRO-3 bound to $d(AT)_{20}$:$d(AT)_{20}$ at 4.2 K. The solid line is for 0.2 dye/bp, the dashed line for 0.05 dye/bp.

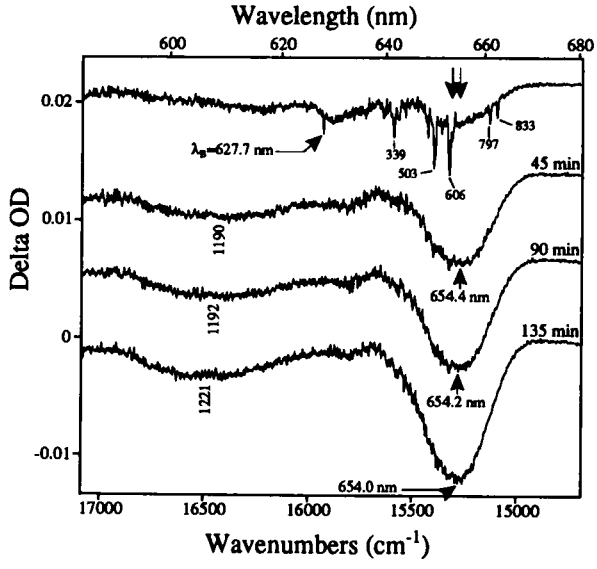

FIGURE 3 (Top) Hole spectrum of TO-PRO-3 bound to $d(GC)_{20}$•$d(GC)_{20}$ at 4.2 K. The hole was burned at 627.7 nm for one minute with 39 mw cm^{-2}. Pseudo-vibronic holes are labeled with their shift in wavenumbers from the burn wavelength. Spectra labeled 45, 90, and 135 min, show the changes in the hole spectrum as a function of time after the top spectrum. The sample was continuously illuminated during this period.

peaked at ~654 nm, indicated by the double arrow. Previously, for dye bound to DNA, we have assigned this as burning of the intercalated complex excited either by energy transfer from the externally bound dye or directly through vibronic absorption, with the width being indicative of relatively strong electron-phonon coupling for the intercalated dye. The additional spectra of Fig 3., show the evolution of the hole shape over a period of 135 min. after hole burning. During this time period, the sharp holes disappear while the broad hole deepens considerably, and slightly blue shifts. As these spectra are measured in a Fourier- transform spectrometer, the spectral source is continually irradiating the sample. The spectral range of the source is from 8,000 to 25,000 cm^{-1}, peaked at 10,000 cm^{-1}. If the sample is shielded from the source except during actual measurements, the changes shown in Fig 3 do not occur, i.e., the hole spectra are persistent. However, the light source alone can also cause burning of a broad hole as shown in Fig. 4. To obtain this figure, absorption spectra were measured every 45 minutes and the time dependent spectra were each subtracted from the first spectrum

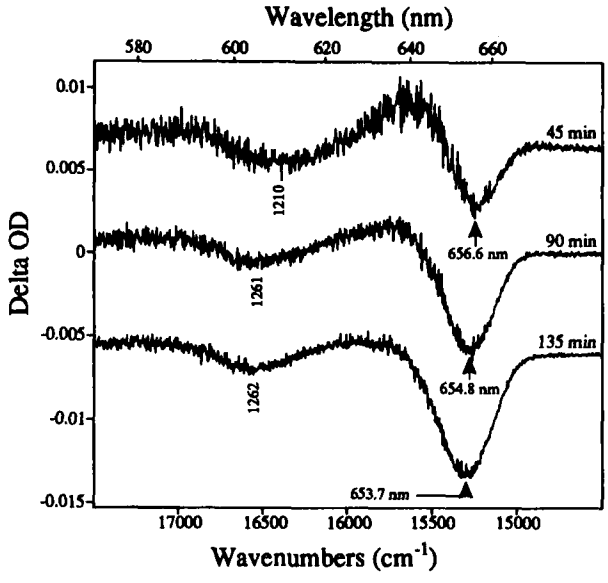

FIGURE 4 White light induced changes in the TO-PRO-3/d(GC)20•d(GC)$_{20}$ absorption spectrum as a function of time. The spectra were obtained at 4.2 K for a 0.05 dye/bp sample. The white light intensity was 39 mW cm^{-2}.

measured. As can be seen from the Figure, the broad holes observed in Figure 3 are also produced by the source over the same time period. Experiments were also performed using filters on the source so that its spectrum contained energy only in the TO-PRO-3 absorption band. These experiments showed that the observed effect is caused only by light in the TO-PRO absorption region. For the GC-oligomers, the white light induced hole approaches saturation after ~150 minutes at ~6% change in Optical density at the peak of the hole. For the AT-oligomer, the saturated white light induced hole is ~2%.

DISCUSSION

We consider first the implications of the observed white light hole burning for the previous results on TO-PRO-3 bound to single- and double-stranded DNA[1]. In that work, laser hole burning on the red side of the absorption band produced spectra similar to the top spectrum in Figure 3, i.e., a narrow zero phonon hole at the burn wavelength accompanied by pseudo-vibronic holes superimposed on a broad hole to the red side of the band. The zero phonon hole and the vibronic holes were assigned to burning of externally bound dye characterized by weak electron-phonon coupling (S ~ 0.7), while the broad hole was assigned to intercalated dye which is burned either by energy transfer

from the externally bound dye or by vibronic absorption. We have re-examined those samples and find that they also undergo the white light hole burning shown here for dye-oligomer samples. However, in both the DNA samples and in the oligomer samples, the broad holes from white light burning appear to be distinct from the broad holes accompanying laser burning. In all cases, the laser induced broad holes have maxima that are to the red of the white light induced holes (by 0.5 to 1.0 nm). Also, the broad laser induced holes appear with much lower fluence than is required to burn holes with white light. (Although the white light and laser intensities are similar, the use of filters shows that only excitation into the absorptionis effective in producing the white light hole. The intensity in this region is 100 times less than the laser intensity.) Thus, the holes produced by white light appear to be different from those which accompany laser burning. Therefore, the previous explanation for the laser induced holes being due to burning of intercalated dye is still reasonable.

What then is the origin of the white light induced holes? At this point, we lack sufficient data to provide a definitive answer to this question and must be somewhat speculative. At 4.2 K, it is doubtful that there is sufficient translational mobility for the dye to dis-intercalate. Thus, we favor interpreting the hole spectra as indicating that there are three distinct binding modes of the dye to DNA oligomers. The absorption of intercalated dye can still be approximated by the broad hole observed with laser hole burning, and there is an externally bound mode which has an absorption quite similar to that of free dye. In addition, there is another binding mode whose absorption maximum is intermediate between these two and given approximately by the maximum of the white light induced hole. There are numerous possibilities for this binding mode, including partially intercalated and externally bound with a different point of binding than the free-dye-like externally bound molecules. Conformational isomers of the dye are also possible. Whatever the details of binding, electronic excitation can induce the molecule into altering its binding so that the molecule now has an absorption similar to that of the free-dye-like, externally bound molecules, (based on the maximum of the antihole observed with white light hole burning, see Figure 4). As shown in Figure 4, both the broad hole and its associated antihole blue shift with increasing burning time.

That there may be multiple externally bound conformations of the dye with DNA should be of interest to those using this dye for conventional DNA staining applications and for electrophoresis applications. If there is a predominance of externally bound dye in the dye-DNA complex, the binding constant for the dye will be much less than if the dye is predominantly intercalated and the dye will be more apt to be removed during

electrophoresis. We are continuing to investigate TO-PRO and TOTO series of dyes by hole burning.

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