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INVESTIGATION OF THIONIN INTERACTING WITH POLYNUCLEOTIDES BY SATELLITE HOLES

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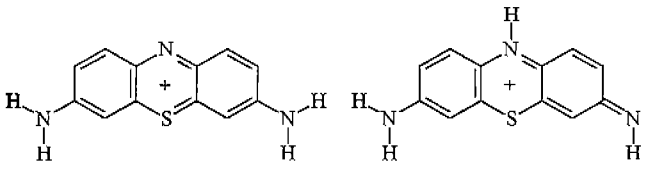
Abstract–We have introduced the nonresonant holes in the satellite hole spectrum to examine the interaction of two tautomers of thionin to various polynucleotides, including $[d(GC)_7]_2$, $[d(AT)_7]_2$ and calf thymus. The saturated depths of nonresonant holes are much reduced upon interacting with $[d(GC)_7]_2$ and calf thymus than with $[d(AT)_7]_2$, implying that the binding affinity of thionin to $[d(GC)_7]_2$ and calf thymus is stronger than $[d(AT)_7]_2$. In addition, the less perturbation on the exocyclic amino group of the amino-form of thionin upon interacting with $[d(AT)_7]_2$ suggests that a direct hydrogen bond formed between the amino group of thionin and the phosphate of nucleic acids is unlikely.

INTRODUCTION

The distinct nonresonant holes (NRHs) in the satellite hole (SH) spectra are very useful to identify the tautomeric structures of dye molecules[1-3] and to examine the recognition and the binding modes of dye molecules to various polynucleotides.[4-8] The advantage of the SH method oer other spectral methods is the amplification of the intensities of the active modes by inverse Frank-Condon (FC) factors when the excitation is tuned in congested vibronic transitions. This is because each NRH can be viewed as an individual electronic transition of its own vibronic transition at laser wavelength. In addition, the SH spectrum is a selective method with background free. Recently, Weng *et al.*[3] have identified two tautomeric structures of thionin appeared in glycerol:water (Gl : H_2O) glass based on the two sets of vibrational modes in the SH spectra. The two structures were proposed by Marek *et al.*,[9] as shown in scheme 1.



Scheme 1.

Thionin is a tricyclic heteroaromatic molecule that is known to interact with DNA by intercalation.[10] Tuite and Kelly[11] reported that the binding constants of thionin with [poly(dG-dC)$_2$ and [poly(dA-dT)]$_2$ were of the same order of magnitude, although binding of the thionin to [poly(dG-dC)]$_2$ is a little stronger than to [poly(dA-dT)]$_2$. However, they suggested that the interaction of the thionin to [poly(dG-dC)]$_2$ is intercalative, but the interaction of the thionin to [poly(dA-dT)]$_2$ is more than one binding mode. They further proposed that the exocyclic amino group of thionin can form a hydrogen bond with the phosphate group of nucleic acids to stabilize binding. Note that only the amino-form of thionin was discussed in their work. In this work, we apply the SH spectra to examine the interactions of two tautomeric structures of thionin to various polynucleotides, including $[d(AT)_7]_2$ (abbreviated as AT), $[d(GC)_7]_2$ (GC) and calf thymus (CT-DNA).

MATERIALS AND METHODS

The experimental setup for the SH spectrum has been described elsewhere.[12] Holes were produced by using a home-made dye laser pumped by a mode-locked and Q-switched Nd:YAG laser (Quantronix 416). The laser emitted trains of ~10 pulses separated by 13 ns at a repetition rate of 500 Hz. The output of the dye laser provides several μJ of pulse energy with a bandwidth less that 2 cm^{-1}. The absorption spectra were obtained by dispersing the output of a xenon arc lamp (Oriel 66083) through a home-made double beam spectrometer with a resolution of ~ 0.33 nm. Extreme care was taken to ensure that the cross-section of the probe beam at the sample was inside the burning area.

Thionin was purchased from Aldrich and used without further purification in this work. Note that no appreciable difference on the two sets of NRHs of thionin is observed after purification.[3] Oligonucleotides of AT and GC were purchased from Applied Biosystems and CT-DNA was purchased from Sigma. The ratio between dye and DNA base pair 1:7 in the glycerol:water (5:4 v/v) solution with the dye concentration of ~1 $\times 10^{-4}$ M. Clear glass was normally formed by introducing the

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sample directly into a Janis dewar from room temperature to 6 K.

RESULTS AND DISCUSSION

Fig. 1 shows the absorption and fluorescence spectra of the free thionin and the thionin mixed with each of the oligonucleotides of AT and GC at room temperature. The red-shift of the absorption maxima upon interacting with AT and GC has been reported previously and has been attributed to dye-nucleotide base stacking interactions by Tuite and Kelly.[11] Fig. 1 shows that fluorescence is quenched by a factor of ~70% upon binding with AT and greatly quenched by a factor of > 99% upon binding with GC. Such base dependent

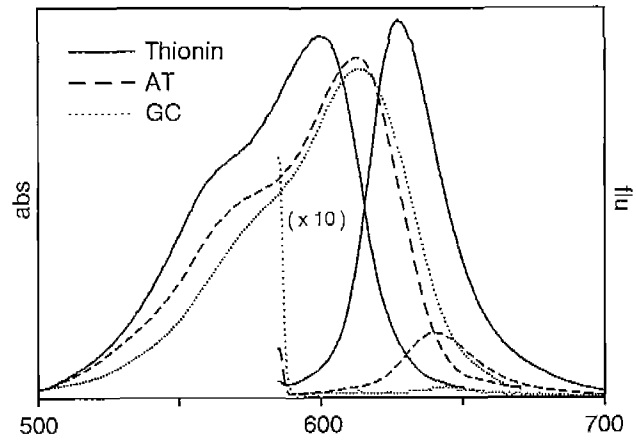

Figure 1. Absorption and florescence spectra of the free thionin and the thionin mixed with each of the oligonucleotides of AT and GC at room temperature. The excitation of the fluorescence is located at 585 nm.

effects has been attributed to stacking of the dye heterocycle next to a G than an A residue since G base is more polarizable, giving a larger perturbation of dye optical properties.[10] The large fluorescence quench is consistent with the sandwich model for thionin intercalated in the base pairs of GC.

Fig. 2 shows the low temperature preburn and postburn SH spectra of thionin and thionin mixed with AT, GC and CT-DNA doped in Gl:H_2O glass taken at λ_B~600 nm. It is found that the two absorption bands of thionin at ~613 and ~625 nm upon interacting with GC and CT-DNA. The absence of these two bands at room temperature is probably due to thermal broadening effect. The ~603 and ~617 nm bands have been assigned to the amino-form and imino-form of thionin, respectively.[3] Fig. 2 shows that the relative intensity of the absorption band of the imino-form to that of the amino-form of thionin decreases appreciably upon interacting with GC and CT-DNA than with AT. Furthermore, significant reduction of the hole depths of thionin upon interacting with GC than AT implies that binding of the thionin to GC is stronger than AT.

Figs. 3a-3d show the corresponding SH spectra obtained

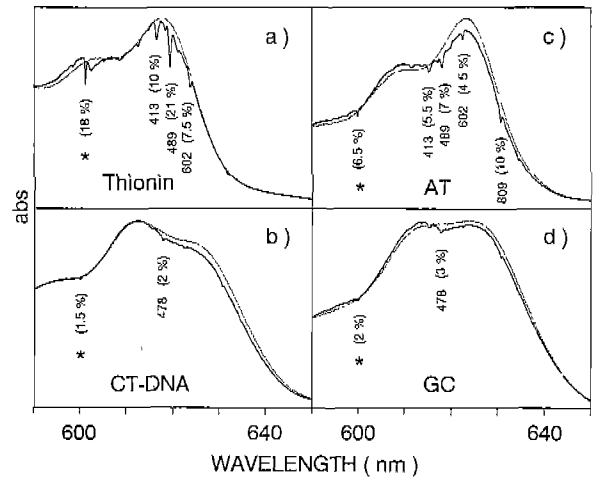

Figure 2. Preburn and postburn spectra of thionin (a) and the thionin mixed with each of the oligonucleotides of CT-DNA (b), AT (C), and GC (d) dissolved in 5:4 glycerol:water solution at T ~ 6 K. Prominent satellite holes with the ratios of hole depths are labelled with excited-state vivrational frequencies.

from the difference between each set of spectra in Figs. 2a-2d. It is important to observe different NRHs at 478 and 489 cm-1 in Fig. 3, indicating that the appearance of 478 cm^{-1} NRH in Figs. 3b and 3d is not due to residues of the free thionin. In addition, the occurrence of the 908 cm-1 NRH in Figs. 3b-3d results from the thionin-DNA interaction since it is not observed in Fig. 3a. When the burning wavelength is tuned to 605 nm, the 809 cm^{-1} NRH disappears in the spectrum of free thionin, but appears in the spectra of thionin upon interacting with these polynucleotides, as shown in Figs. 4a and 4d. We believe that the contribution from residues of the free thionin to the NRHs appeared in Figs. 3b-3d can be neglected.

In order to investigate the binding sites of thionin to AT, mode assignment is required for comparing the relative changes of the hole depths. In our previous study,[3] the normal mode calculations suggested that the (478, 798) and (489,

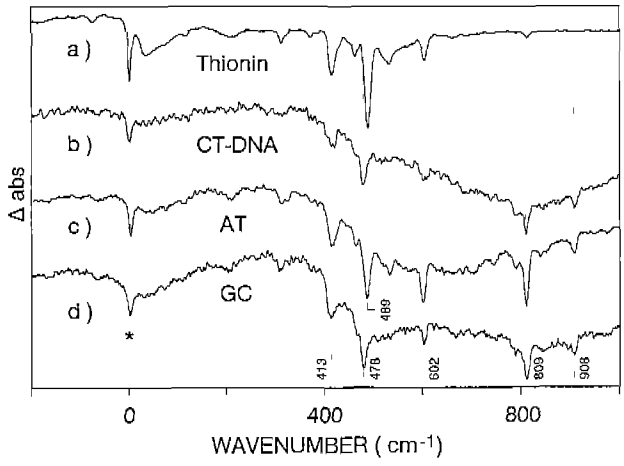

Figure 3. The corresponding SH spectra obtained from the difference between each set of spectra in Figs. 2a-2d.

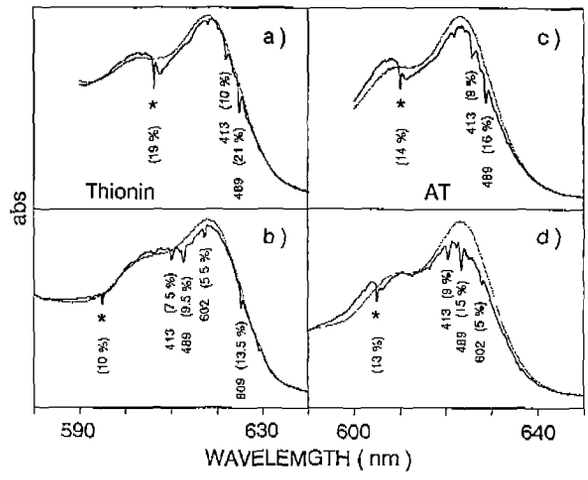

Figure 4. Preburn and postburn spectra of thionin taken at $\lambda_B \sim 605$ nm (a) and $\lambda_B \sim 595$ nm (b) and the thionin mixed with AT taken at $\lambda_B \sim 610$ nm (c), and $\lambda_B \sim 605$ nm (d) at T ~ 6 K. Prominent satellite holes with the ratios of hole depths are labelled with excited-state vibrational frequencies.

809) cm^{-1} NRHs are due to the inner ring motion of the amino-form and the imino-form of thionin, respectively. The 602 cm^{-1} NRH is related to ring skeleton motion. Deuteration of the amino group was conducted to distinguish the amion group from ring moiety. Our SH results showed that the 413 cm^{-1} NRH disappears but the 388 cm^{-1} NRH appears upon deuteration (data not shown). Normal mode calculation and deuteration study allow us to assign the 413 cm^{-1} NRH to NH_2 motion of the amino-form.

Since the absorption spectrum contains at least two absorption components, only a qualitative comparison of the reduction of the hole depths is given. Considering the red side of the absorption spectrum is less complicated than the blue side of the absorption spectrum, Fig. 4 shows preburn and postburn spectra taken at $\lambda_B \sim 605$ and ~ 595 nm for free thionin and $\lambda_B \sim 610$ and ~ 605 nm for thionin mixed with AT. Since the 478 and 798 cm^{-1} NRHs are not observed at red side of the absorption spectrum, we compare relative changes of the hole depths among the 413, 602, 489 and 809 cm^{-1} NRHs upon interacting with AT. Taking into account the ~5 nm red-shift of the absorption band upon interacting with AT, the SH spectrum taken at $\lambda_B \sim 600$ nm for free thionin is compared to the spectrum taken at $\lambda_B \sim 605$ nm for thionin interacting with AT. Figs. 2 and 4 show that the hole depths of 489 and 809 cm^{-1} related to the imino-form are reduced to a similar ratio fo ~ 0.7. In addition, the hole depth of 602 cm^{-1} related to the amino-form is also reduced to a similar ratio of ~0.7. However, thehole depth of 413 cm^{-1} NRH slightly decreases about 10%. The less reduction of the 413 cm^{-1} NRH indicates that the hydrogen bonding between the exocyclic NH_2 mode of thionin and the OH mode of matrix is less perturbed upon interacting with AT. It further implies that a direct hydrogen bond formed between the amino group of thionin and the phosphate group of DNA is unlikely. However, an indirect hydrogen bonding *via* water between them cannot be eliminated.

Although the comparison of the hole depths of NRHs upon interacting with GC and CT-DNA is more difficult, the hole depths of the 478 and 489 cm^{-1} NRHs are found to be ~ 2% and ~ 1.5% upon interacting with CT-DNA, respectively. Thus, we consider that the binding affinity for the tautomers are similar to both polynucleotides.

The lack of shift in hole frequency implies that the interaction between thionin and these polynucleotides is not dramtic in the bound form compared to the BODIPY-oligonucleotide interactions.[7,8] However, it is consistent with the intercalation model since the frequency of the in-plane vibrational modes of chromophore is less shifted by the stacking between the ring moiety of dye molecule and the DNA base pairs. Although our spectral results suggest silmilar binding affinity for the two tautomers of thionin to GC, different binding geometries can occur because of their different dipole moments.[3] In addition, we suspect that the amino-form of thionin is partially intercalated with AT or external stacking with AT because of the steric hindrance from the backbone in the minor groove and the methyl group of thymine in the major groove of the B-form DNA structure. The binding site appears to be from the major groove of AT because of the less perturbation on the exocyclic amino group. Hwever, the imino-form can intrude into the base pairs AT with the long axis at certain orientation to the axis of nucleic acids.

In summary, we have demonstrated that two tautomers of thionin exist upon interacting with these polynucleotides. Our results suggest that the binding affinities of these two tautomers are similar to each polynucleotide. Significant reduction of the hole depths upon interacting with GC than AT suggests that either the optical properties of thionin is more perturbed or the thionin molecule is more isolated form the matrix by GC than AT. In addition, we have shown that the exocyclic amino group is less perturbed by AT. Circular dichroism may be useful to probe the details of binding geometries be useful to probe details of binding geometries between two tautomers of thionin and these polynucleotides.

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