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Satellite Hole Investigation of the Vibrational Modes of 9-Aminoacridine upon Binding to DNA

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The spectral features of satellite holes are used to investigate 9-aminoacridine-DNA interactions. The hole depths of the outer ring vibronic modes are reduced more than that of the inner ring vibronic modes, implying that inner ring motion is less perturbed than outer ring motion. As a result, the mode coupling between the inner ring and outer ring is reduced upon binding to DNA. However, similar hole frequency and width of the satellite hole corresponding to the NH_2 mode upon binding to DNA imply that the amino group of 9-aminoacridine sits outside the DNA.

INTRODUCTION

The satellite holes (SHs) in spectral hole burning (HB) spectrum have been introduced to investigate hydrogen bonding in chromophore-oligonucleotides interactions.[1,2] Very intriguing HB spectra were observed when a 4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-s-indacene-3-propionic acid, succinimidyl ester (BODIPY) molecule is chemically bound to oligonucleotides of different base compositions, implying that specific base pairs interact with BODIPY differently. The observation of hole broadening and shifting of the specific satellite holes is attributed to the hydrogen-bonding formation between the BODIPY and oligonucleotides. However, the similarity of other SHs reveals that not all vibrational modes of BODIPY are perturbed by the hydrogen bonding; instead, only those modes associated with the hydrogen bonding contribute to the change in their respective SHs. This site specificity study of the hydrogen bonding formation in SHs motivates our interest to apply the HB method for examining drug-DNA interactions. Very recently, Hayes and coworkers combined fluorescence and the HB method to study the thiazole orange derivative, TO-PRO-3 and its dimeric analogue, TO-TO-3, binding to DNA.[3]

In order to study the coupling sites of guest-host interactions, we chose 9-aminoacridine (9AA) because vibrational frequencies of 9AA have been studied by means of Raman, infrared, and fluorescence spectra.[4-6] In addition, the interaction of 9AA and DNA has been extensively examined by Raman,[4] fluorescence,[6] X-ray crystallography,[7] and NMR.[8] Recently, we have studied HB spectra of 9AA doped in glycerol:water (Gl:H_2O) glasses with different pH values[9] and in polyvinyl alcohol film.[10] The high resolution of SHs can be used to distinguish different tautomeric forms, amino and imino, of 9AA, specifically the absence of 514 cm^{-1} SH and the frequency shift from 1368 cm^{-1} SH to the 1338 cm^{-1} SH in the imino form of 9AA. Furthermore, strong mode coupling between the inner and outer rings observed in the amino form of 9AA suggests that the in-plane structure favors electronic resonance on the acridine ring. In this work, we have performed the HB spectrum of 9AA bound to DNA, a designed oligonucleotide: d-$(GCATGCTAGCATGC)_2$ duplex (GC14), doped in Gl:H_2O glass. The purpose of this work is to study the local interactions of drug-DNA complexes by monitoring the active modes characterized by their SHs.

EXPERIMENTAL

A home-made intracavity frequency-doubled OPO system pumped by a frequency doubled output from a Q-switched and mode-locked Nd:YAG laser (Quantronix 416) was utilized to produce holes. The laser emitted trains of ~ 15 pulses separated by 13 ns at a repetition rate of 500 Hz. The bandwidth was found to be less than 2 cm^{-1} with the pulse energy of ~ 10 μJ at 425 nm. The wavelength was tunable in the region of 380-480 nm. The experimental setup of HB has been described elsewhere.[11] The absorption spectrum was obtained by dispersing the output of a xenon arc lamp (Oriel 66083) through a home made double beam spectrometer with a spectral resolution of ~ 0.03 nm. The

spot size of the burning beam was ~ 1 mm and that of the reading beam was ~ 200 μm. 9AA was purchased from Acros and used without further purification. The designed oligonucleotide of the purified GC14 was purchased from Perkin-Elmer. 9AA-GC14 was physically mixed and further examined by NMR before the HB experiments. Sample was made by dissolving 9AA (or 9AA-GC14) in 5:4 Gl:H_2O solution. Clear glasses were normally formed by introducing the sample into a Janis dewar from room temperature to 6 K.

RESULTS AND DISCUSSION

Fig. 1 shows the NMR spectra, indicating that 9AA indeed interacts with GC14 upon mixing. Fig. 1a shows the spectrum of pure GC14 dissolved in D_2O. Fig. 1b shows the spectrum of 9AA-GC14 complex with a ratio of 1:7 between 9AA and the DNA base pairs. Fig. 1c presents the spectrum of 9AA dissolved in the mixed solution of 93.7% D_2O and 6.3% CD_3OD. The triplet farthest right is due to some impurity, which fortunately is used as an internal marker. Considering the linewidths and chemical shifts of the peaks of 9AA, but lack of difference on the linewidth and chemical shift of the triplet of the internal impurity, the possibility of magnet inhomogeneity can be disregarded. In addition, some resonances of GC14 are changed after mixing with 9AA. Clearly, 9AA and GC14 interact with each other.

Figs. 2a and 2b show the preburn and postburn spectra of 9AA/Gl:H_2O, 9AA-GC14/Gl:H_2O taken at λ_B = 427 and 431 nm, respectively. The 9AA is bound to GC14 with a ratio of 1:7 between 9AA and the DNA base pairs. Similar pulse energy of ~ 8 μJ was used to burn for 90 min for each spectrum. The difference between each set of spectra gives the HB spectrum as shown in Figs. 2c and 2d. The red-shift from ~ 424 nm to ~ 428 nm in the absorption spectrum of 9AA-GC14 is an evidence for 9AA-DNA interactions.[12] However, a comparison of Figs. 2c and 2d shows that virtually no frequency shifts in SHs are detected within the experimental deviation. An NMR study of 9AA-GC14 indicates that the residue of free 9AA is very small and can be ignored. The lack of shift in hole frequency implies that the interaction between 9AA and GC14 is not dramatic in the bound form compared to the BODIPY-oligonucleotide interactions.[2]

Fig. 2c shows a sharp zero phonon hole (ZPH) with burning depth of 10% associated with a pseudo-phonon side band hole (PSBH) located at red-side of the ZPH. In addition, three prominent SHs at 390, 1368, and 1756 cm^{-1} and two weak SHs at 1032 and 1166 cm^{-1}, accompanied by their PSBHs, are present at blue-side of the ZPH. Fig. 2d shows a sharp ZPH with burning depth of ~ 7% and three prominent SHs at 390, 1368, and 1758 cm^{-1} associated with their PSBHs. The reduction of burning depth upon binding to DNA is consistent with the previous study,[13] indicating that one degree of freedom of 9AA is lost to the binding GC14. Furthermore, it is known that the Franck-Condon (FC) factor can be estimated from the ratio of the integrated hole ar-

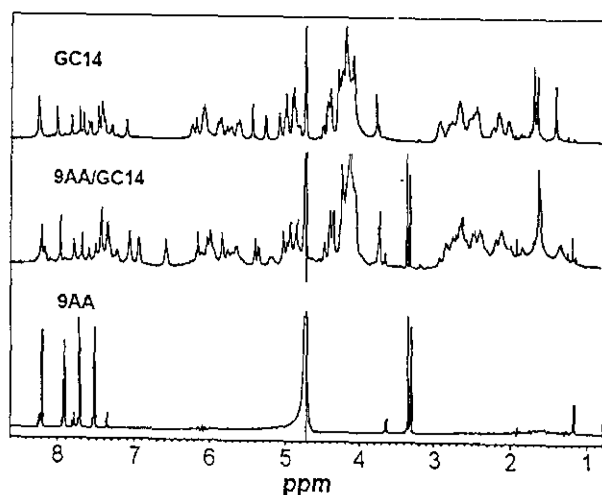

Fig. 1. NMR spectra of GC14 (a), 9AA-GC14 (b), and 9AA (c).

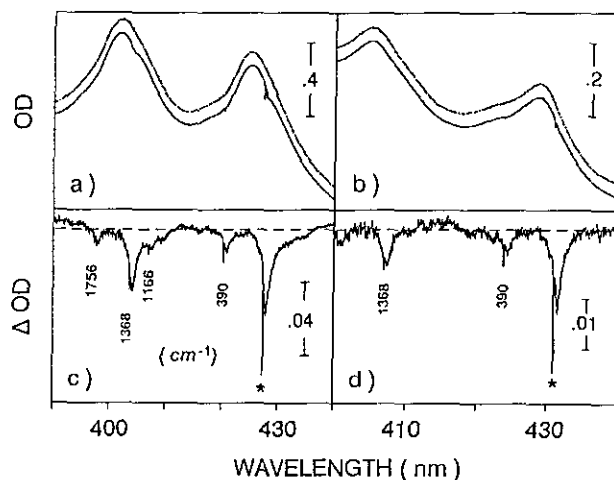

Fig. 2. Preburn (dot line) and postburn (solid line) spectra of free 9AA (a) and GC14 bound 9AA (b) doped in Gl:H_2O glasses and the corresponding HB spectra (c) and (d), respectively. A star (*) denotes the burning position. Prominent SHs are labeled with excited-state vibrational frequencies.

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eas between blue-side SH and ZPH.[9,14,15] The FC factors of the 390 and 1368 cm^{-1} modes estimated from Fig. 2c are ~ 0.28 for each mode.[9] However, the FC factors of the 390 and 1368 cm^{-1} modes measured from Fig. 2d are ~ 0.26 and ~ 0.18, respectively. The FC factor of the 1368 cm^{-1} mode of 9AA is much larger than that of 9AA-GC14. In addition, the FC factor of the 1166 cm^{-1} SH is smaller in Fig. 2d than in Fig. 2c. In our previous work,[9] the 390 cm^{-1} SH is attributed to the inner ring motion; the 1166 cm^{-1} SH is assigned predominantly to the C-H bending mode; and the 1368 cm^{-1} SH is most likely related to the totally symmetric mode. In comparison to 9AA, the similarity of the FC factor of the 390 cm^{-1} mode and the reduced FC factors of 1166 and 1368 cm^{-1} modes in 9AA-GC14 suggest that the inner ring motion is less perturbed than the outer ring motion when binding to GC14.

In order to examine how the NH_2 mode of 9AA is perturbed upon binding to DNA, Figs. 3a-3c show the HB spectra of 9AA/Gl:H_2O, 9AA/Gl:D_2O and 9AA-GC14/Gl:H_2O taken at λ_B = 410, 414, and 414 nm, respectively. The pulse energies are ~5, ~ 5, and ~ 8 μJ with the same burning time of 60 min. An NMR study of 9AA dissolved in D_2O indicates that the two protons of the amino group on 9AA are replaced by deuterons via exchange with D_2O. Deuteration of the amino group of 9AA can clearly verify the corresponding motions of the acridine ring and amino group. In addition, the result of the deuteration effect provides strong evidence for the mode coupling. Careful examination of the frequencies of SHs in Fig. 3a indicates that the SHs appearing at 124, 260, and 300 cm^{-1} are due to negative couplings between the 390 cm^{-1} mode and the 514, 652, and 690 cm^{-1} modes.[9] A comparison between Fig. 3a and 3b shows that the SHs at 124 and 514 cm^{-1} of 9AA/Gl:H_2O are red-shifted to 112 and 502 cm^{-1} of 9AA/Gl:D_2O, respectively. The same frequency shift supports that the 124 and 112 cm^{-1} SHs are due to negative couplings of the 514 and 502 cm^{-1} modes to the 390 cm^{-1} mode, respectively. The red-shift of these frequencies can be ascribed to the heavy atom effect of the amino group. In addition, it is clear that the 514 cm^{-1} SH is closely related to amino group motion. The approximately identical frequencies of 390, 652, and 690 cm^{-1} SHs upon deuteration imply that these modes are determined mainly by the ring motion of 9AA. Normal mode calculations of vibrational frequencies of 9AA suggest that the 652 and 690 cm^{-1} modes involve the C-C-C planar deformation and outer ring motion.[9]

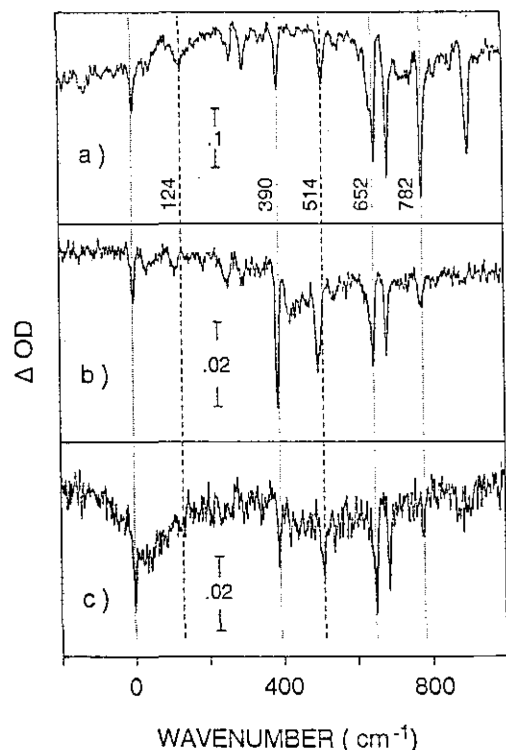

Fig. 3. HB spectra of 9AA/Gl:H_2O glass (a), 9AA/Gl:D_2O glass (b) and 9AA-GC14/Gl:H_2O glass (c). The burning wavelengths are located at 410 nm (a), 414 nm (b), and 414 nm (c). The dashed and dotted lines are for visual guides.

Similar hole frequency and hole width of the 514 cm^{-1} SH are observed in Figs. 3a and 3c, implying that this mode is not perturbed significantly upon binding to GC14. Based on the significant spectral difference of SHs between the free and DNA bound BODIPY,[1,2] we propose that the direct hydrogen-bonding formation between the NH_2 mode of 9AA and nucleic acid base is negligible. Consequently, it is possible that the NH_2 group sits outside the oligonucleotide. In addition, the depths of the 652 and 690 cm^{-1} SHs are reduced more than that of the 390 cm^{-1} SH upon interaction with GC14. This feature agrees with the previous suggestion that inner ring motion is less perturbed than outer ring motion. Different reductions of hole depths for various SHs indicate that mode-specific interactions are involved upon binding to GC14. Another distinct feature is that those low-frequency SHs resulting from negative coupling are much weaker or even vanish in Fig. 3c, indicating that the coupling between the inner and outer rings in 9AA is reduced. In conclusion, a tentative mechanism is proposed: the skeleton of the acridine ring is perturbed by the interactions between the periphery of the acridine moiety and the GC14. Slight distortion of the ring skeleton is very likely if 9AA is intercalated in DNA base pairs. As a result, the electronic resonance on the acridine ring is disturbed and the coupling between the inner and outer rings is then reduced.

Sakore and coworkers[6] found two distinct intercalative binding modes of a 9AA in a crystalline complex with 5-iodocytidylyl(3′-5′)-guanosine. One involves a pseudo-symmetric stacking interaction between 9AA and GC base pairs. The other one is an asymmetric interaction, mainly determined by stacking forces between acridine and guanine ring. They further reported that the amino group of 9AA points toward either the major or the minor groove of the double helix. Their results support our proposed mechanism.

In summary, our HB results suggest that the acridine ring of 9AA interacts with GC14, but the NH_2 group sits outside the GC14. More experiments are necessary for further addressing the conformational structure and the details of the interactions between the 9AA and DNA, especially the relationship between functional group and structural activity. This is of particular importance for the study of drug-DNA interactions since many antibiotic drugs are derivatives of acridine at the 9th position. Our HB spectroscopic finding should not be overinterpreted; however, it points to an interesting direction, namely, the possibility of measuring the conformational structure of drug-DNA complexes. In addition, the mode-specificity characterized by their sharp SHs should provide useful information about local interactions of drug-DNA complexes.

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Key Words

Hole burning; Satellite holes; 9AA-DNA interactions; Mode coupling; Conformational structure.

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