Drug Binding to DNA: Observation of the Drug-DNA Hydrogen-Bond-Stretching Modes of Netropsin Bound to DNA via Raman Spectroscopy

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Raman scattering and normal mode calculations have been used to study the low frequency vibrations of NaDNA, netropsin, and the NaDNA·netropsin complex. Vibrational modes at about 60 and 180 cm^{-1} are observed from the DNA·netropsin complex but not from either pure DNA or pure netropsin. Theoretical calculations show that these are stretching modes of the hydrogen bonds connecting netropsin to DNA. Identification of these modes in this and other systems may facilitate the study of biomolecule·ligand binding dynamics. [S0031-9007(98)05502-1]

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The interactions of DNA and RNA with other molecules are of central importance in biology [1,2]. Knowledge of ligand binding to nucleic acids is critical for a complete understanding of replication and transcription processes. DNA is a major target of anticancer and antibiotic drugs. Single-stranded short DNA sequences are structurally modified and designed as antisense drugs to bind and facilitate the cleavage of disease-related messenger-RNAs by ribonuclease H. The binding dynamics of both antisense and DNA-bound drugs primarily involve low frequency modes characteristic of weak drug-DNA hydrogen bonds and nonbonded interactions. Therefore an understanding of the relevant binding dynamics is of importance in facilitating the design of a wide variety of drugs.

X-ray and NMR studies have provided detailed structural information about DNA·ligand binding [1,3]. This permits one to identify the binding sites and to develop a theoretical understanding of the properties of the complex. Several calorimetric studies have also appeared which provide useful information about the energetics and thermodynamics of the binding [3]. In addition, vibrational spectroscopies (Raman scattering and infrared absorption) and normal mode calculations have been used to probe the binding induced conformation distortion in DNA and DNA binding drugs through the study of high frequency modes [4–6]. Despite this progress, the low frequency collective motions that are most relevant to the binding dynamics between DNA and ligands are yet to be probed.

The investigation of collective motions may be facilitated by the study of low frequency hydrogen bond (H-bond) stretch modes. DNA·ligand H bonds have been found in many DNA·ligand systems. The collective motions relevant to binding involve the stretching of these bonds. Therefore a study of the stretching modes of these bonds can shed light on the relevant collective motions. In earlier studies low frequency Raman spectroscopy has been used to examine the interbase H-bond breathing modes in DNA [7–10]. The same technique can be extended to the study of DNA·ligand binding dynamics.

In this Letter we report the observation of the vibrational modes in which the hydrogen bonds connecting netropsin to DNA are stretched. This is the first observation of a DNA·ligand stretching mode for any ligand. Because of the large mass oscillating and the relatively weak bonds involved, these stretching modes are low in frequency. This observation of the DNA·netropsin stretching modes, when combined with numerical calculations, provides the first direct measure of the effective force constants of the bond between DNA and any ligand.

Netropsin is a powerful drug with antiviral, antitumor, and antibiotic properties. Though this drug is too toxic for clinical use, it has been studied intensively since it is a model of base-specific but nonintercalative DNA-binding drug molecules [4,5,11–21]. Netropsin binds tightly in the minor groove of adenine/thymine-rich regions. Single crystal x-ray studies [14,18,21] of netropsin bound to oligonucleotides show that three nitrogen atoms of the netropsin form four hydrogen bonds with oxygen and nitrogen atoms in the bases of the DNA. The geometry of the netropsin is significantly distorted by the formation of the complex: the two pyrrole rings make an angle of 33° with respect to each other (rather than 20° as in the free molecule) and the two ends of the crescent-shaped netropsin move towards each other. In contrast, the geometry of the DNA is virtually unchanged by the formation of the complex.

Raman scattering experiments were performed on netropsin in solution, and on wet-spun films of either NaDNA or NaDNA·netropsin. The spectra were excited using less than 10 mW of either the 514.5 or 488.0 nm lines of an Ar^+-ion laser. The low laser power was used to avoid heating effects [22]. A 90° scattering geometry was used to collect the Raman signal which was dispersed in a double monochromator (ISA U-1000). A thermoelectrically cooled photomultiplier tube was used to detect the scattered light with standard photon-counting electronics. Highly oriented films of calf-thymus NaDNA were prepared by the wet-spinning technique

[23]. Some of these films were also soaked in ethanolic solutions of netropsin for three weeks in order to produce DNA·netropsin films in which there were 0.22 netropsin molecules per base pair. Small samples were mounted in sealed cuvettes in which the relative humidity (RH) was maintained by placing a small amount of the appropriate saturated salt solution in the bottom of the cuvette [24]. Since netropsin stabilizes the B conformation in calf-thymus DNA [20], experiments were performed at 93% and 95% RH. Both the DNA and DNA·netropsin were in the B conformation for these experiments, as confirmed by detecting the presence of the 835 cm^{-1} B-form marker band [25]. Data were also taken at 75% RH since the Raman signal has been found empirically to be relatively strong at that RH. Raman spectra from the DNA·netropsin samples at this RH displayed the 835 cm^{-1} B-form marker band while Raman spectra from pure DNA samples displayed the 807 cm^{-1} A-form marker band.

The B-DNA polymer Polyd(GATATC)·Polyd-(GATATC) (which is an infinitely long double helix with a six base pair repeating sequence of GATATC) was used to carry out normal mode calculations. The coordinates of this DNA polymer is generated from the fiber data using the helical symmetry of the standard B conformation [26]. The coordinates of the netropsin-bound polymer are generated from the crystal x-ray coordinates of a netropsin·d(CGCGATATCGCG) complex [27]. As this complex adopts a B conformation, the effect of CGC and GCG caps on the structure of GATATC is relatively small and thus can be neglected. In our model of the netropsin-bound DNA polymer there is one netropsin drug bound to every GATATC sequence. The sequences are then connected by helical symmetry with a per unit cell helical rise of 20.6 Å and twist angle of 216°. The details of the formalism and force fields for both systems are described elsewhere [5]. The intensity of the normal modes is calculated based on an empirical function derived for modeling that of the interbase H-bond breathing motions [28].

The low frequency region (below about 250 cm^{-1}) of the vibrational spectrum of DNA has been studied by Raman scattering. Urabe and Tominaga [7] first showed that a broad band around 90 cm^{-1} is an intramolecular mode of the double helix. Solution studies showed that the frequency of this mode softened to zero as the double helix was disrupted by increasing the temperature. Later work by Weidlich *et al.* showed that this broad band is actually composed of three modes [10]. These three modes are all believed to involve vibrations of the two helices against one another and their intensities depend on the conformation. A fourth low-frequency mode has been observed in B-DNA at about 25 cm^{-1}. This mode has been shown to be intermolecular in nature (i.e., a solid state effect due to vibrations of one DNA molecule against its neighboring molecules) and dominates the spectrum below about 40 cm^{-1} [9,10]. Since our interest is the vibrations within the complex, this mode is not of interest.

Figure 1(a) shows the low frequency Raman spectra of both DNA and DNA·netropsin at 93% RH. The relative intensities of these two spectra were determined by normalizing the data so that the mode at 1095 cm^{-1} has the same intensity, as shown in Fig. 1(b). This mode is due to a symmetric stretch of the PO_2 group and the intensity of this mode has been shown to be independent of the conformation [29,30]. The DNA spectrum shows the broad features due to vibrations of the double helix itself. The DNA·netropsin spectrum is similar, showing that the vibrations of the two DNA helices against each other are still present. The DNA·netropsin spectrum has a higher intensity in this region, suggesting that Raman scattering is also occurring by the additional modes present in the complex.

The three lines in Fig. 2 show the difference spectra obtained by subtracting the spectrum of DNA from that of the DNA·netropsin complex at 95%, 93%, and 75%

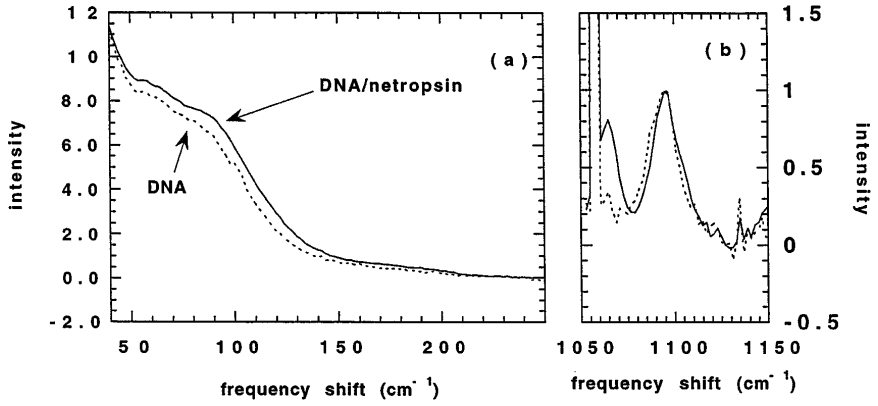

FIG. 1. The Raman spectra from wet-spun films of DNA·netropsin (solid line) and DNA (dashed line) at 93% RH for frequencies between (a) 40 and 250 cm^{-1} and (b) 1050 and 1150 cm^{-1}. The intensity scales of the two data sets have been normalized so that the intensity of the 1095 cm^{-1} mode is the same.

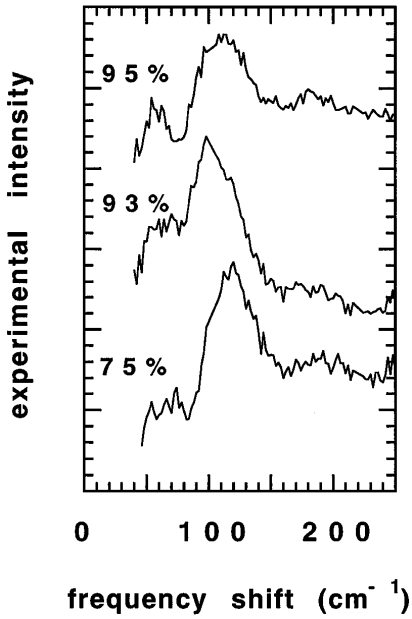

FIG. 2. The difference Raman spectra for DNA·netropsin films at 95%, 93%, and 75% RH. The 93% and 95% RH data have been offset for clarity.

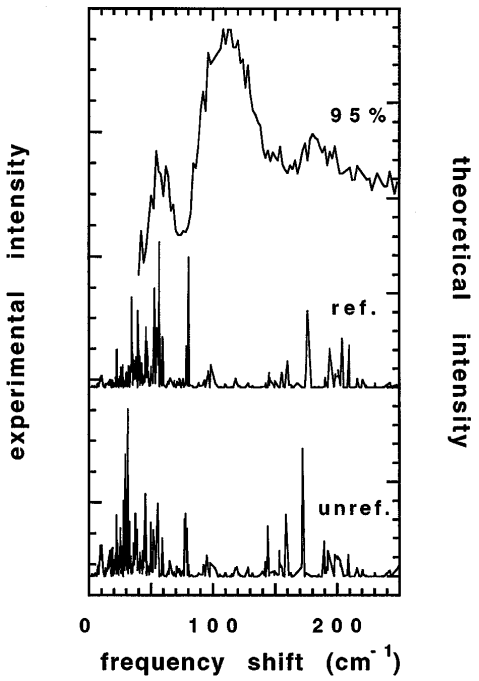

FIG. 3. The difference Raman spectrum for DNA·netropsin films at 95% RH is shown as the top line ("95%"). The bottom two lines show the calculated stretching of the hydrogen bonds connecting the netropsin to the DNA with refined ("ref.") and unrefined ("unref.") force constants.

RH. To obtain the difference spectra, the intensities of the spectra from pure DNA and from the DNA·netropsin complex at the same RH are scaled until the 1095 cm^{-1} modes have the same intensity, as shown in Fig. 1, and the intensities are subtracted at each frequency [31]. Three modes are evident in these difference spectra: a relatively intense mode at about 110 cm^{-1} and weaker modes at about 60 and 180 cm^{-1}. The 110 cm^{-1} mode is also observed in Raman experiments on solutions of netropsin, showing that this mode is an internal mode of the netropsin molecule itself [32]. Because of its very low frequency (for an internal mode), this mode is probably torsional in nature. The 60 and 180 cm^{-1} modes are not observed from either DNA by itself or netropsin by itself. This indicates that they are due to excitations which exist only in the DNA·netropsin complex.

Figure 3 shows the difference spectrum obtained at 95% RH along with the results of two normal mode calculations. The bottom line (unref.) shows the result using the unrefined force constants (determined by using the x-ray data of Refs. [14,18,21] with the Lippincott-Schroeder model of the hydrogen bond [33]) for the hydrogen bonds connecting the netropsin to the DNA. The height of the theoretical curve is proportional to the amount of stretching of the DNA·netropsin hydrogen bonds weighted by their respective charge transfer along each bond for each frequency [28]. The calculated modes cluster in two regions centered at about 40 and 170 cm^{-1}, close to the experimental observations. The x-ray data have shown that the lengths of the hydrogen bonds connecting netropsin to DNA cluster in two ranges: about 2.7 and 3.2 Å. The calculated modes cluster near two frequencies because of the clustering of the hydrogen bonds. Based on the standard H-bond (Morse) potential, the force constant reduces with increasing length, which in turn gives rise to lower frequencies [33]. Our calculations show that the shorter hydrogen bonds (which are stronger than the longer ones) give rise to the higher frequency modes at about 170 cm^{-1} in the calculations while the longer hydrogen bonds give rise to the lower frequency modes at about 40 cm^{-1}. The theoretical intensities are also consistent with the experimental observations: many more modes are predicted in the lower frequency range than in the higher frequency range. These results show that these are stretching modes of the hydrogen bonds connecting the netropsin to the DNA.

The agreement between theory and experiment can be improved by varying the force constants of these hydrogen bonds. The middle line in Fig. 3 (ref.) shows results of the calculation with the refined force constants. The frequencies of the predicted modes are now about 60 and 180 cm^{-1}, in excellent agreement with the experimental data. Both the refined and unrefined force constants are given in Table I. Our refined force constants represent the first direct measurement of the force constants of a ligand attached to DNA. In this manner, such experiments and calculations permit the determination of atomic-level interactions between double-stranded DNA and its ligands. Moreover the combined spectroscopic and normal mode

TABLE I. Comparison of unrefined and refined force constants for netropsin-DNA H bonds in netropsin bound Polyd(GATATC)·Polyd(GATATC). NT denotes netropsin. The nomenclature for DNA bases is G1-A2-T3-A4-T5-C6 in the first strand and C12-T11-A10-T9-A8-G7 in the second strand.

H bond	Length (Å)	Force constant (mdyn/Å) Unrefined	Refined
N6(NT)-H-N3(A10)	3.169	0.0261	0.0640
N4(NT)-H-O2(T3)	3.219	0.0157	0.0227
N10(NT)-H-O2(T5)	2.704	0.6483	0.7883
N10(NT)-H-O2(C6)	2.707	0.6335	0.7735

analysis facilitates the study of the low frequency collective motions and underlying binding dynamics in this and other biomolecule·ligand systems.

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