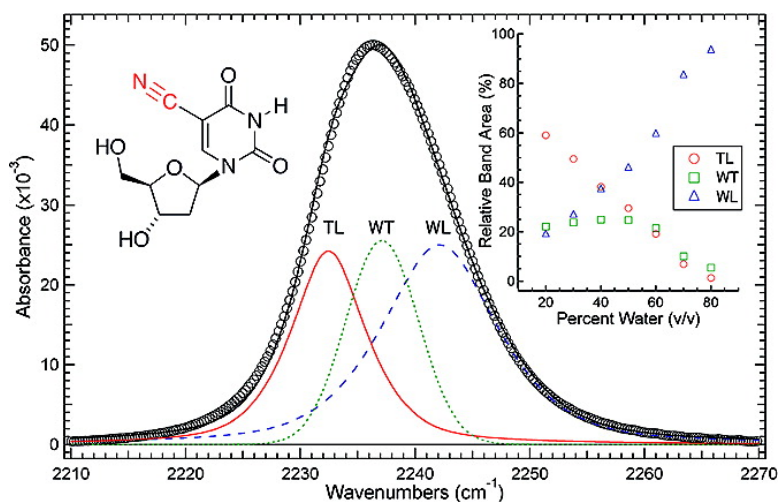


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A Vibrational Probe for Local Nucleic Acid Environments: 5-Cyano-2'-deoxyuridine

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Nitriles have been shown to be effective vibrational probes of local environments in proteins but have yet to be fully utilized for the study of nucleic acids. The potential utility of 5-cyano-2'-deoxyuridine (**1**) as a probe of local nucleic acid environment was investigated by measuring the dependence of the IR nitrile stretching frequency ($\tilde{\nu}_{\text{C}\equiv\text{N}}$), line shape, and absorbance on solvent and temperature. The $\tilde{\nu}_{\text{C}\equiv\text{N}}$ was found to be sensitive to solvent with an observed blue shift of 9.2 cm^{-1} in going from THF to water. The dependence of the nitrile IR absorbance band was further investigated in water–THF mixtures. Global line shape analysis, difference FTIR spectroscopy, and singular value decomposition (SVD) were used to show the presence of three distinct local environments around the nitrile group of **1** in these mixtures. A modest blue shift in $\tilde{\nu}_{\text{C}\equiv\text{N}}$ was observed upon a hydrogen-bond-mediated heterodimer formation between **2** (a silyl ether analogue of **1**) and 2,6-diheptanamido-pyridine (**3a**) in chloroform. The intrinsic temperature dependence of the $\tilde{\nu}_{\text{C}\equiv\text{N}}$ was found to be minimal and linear over the temperature range studied. The experimental studies were complemented by density functional theory (DFT) calculations on the dependence of the nitrile stretching frequency on solute–solvent interactions and upon heterodimer formation with model systems.

Introduction

Nitriles ($\text{R}-\text{C}\equiv\text{N}$) have proven to be useful vibrational probes of biomolecules.^{1–9} Several factors contribute to this, including the following: (1) the nitrile IR absorption is narrow, relatively intense and in a clear region of the spectrum; (2) nitriles are sensitive to changes in the local environment; (3) nitriles contain only two atoms, making them smaller than many other probes; (4) the nitrile group is stable.^{1,5} Proteins have been studied using nitrile vibrational probes for over two decades.² The vibrational Stark effect (VSE) of a nitrile-containing inhibitor was recently used to measure the electric field of the active site of human aldose reductase.³ Nitriles have also been inserted into proteins by incorporation of 4-cyanophenylalanine (4-CN-Phe) either synthetically^{5–7,10–12} or by *in vivo* nonsense suppression.⁸ This latter modification was studied by vibrational^{5–8} and fluorescence spectroscopy.^{10–12} The nitrile IR absorption frequency of 4-CN-Phe was shown to depend upon the solvent. For instance, in H_2O it was 2237.2 cm^{-1} and in relatively nonpolar THF the stretch of Fmoc–4-CN-Phe was 2228.5 cm^{-1} .⁵ Thus, a model for typical absorptions in hydrophilic and hydrophobic environments was established and used to study peptide structure.^{5,6} Thiocyanate probes have also been site-specifically incorporated into proteins.⁴

Nitriles have been incorporated into nucleic acids over the past two decades as well,^{13–22} but the idea to employ them as DNA/RNA vibrational probes is more recent. Last year, Silverman et al. published the Stark tuning rates of a number of nitrile-containing nucleosides.²³ This work laid the foundation

for the use of the VSE of nitriles as a new experimental method to measure electric fields in nucleic acids. More recently, Krummel and Zanni²² demonstrated that the vibrational coupling of ¹⁴N- and ¹⁵N-labeled 5-cyano-2'-deoxyuridines can be used to measure distances and angles in a DNA oligomer. Interpretation of these nitrile vibrational studies on biomolecules has been enhanced by a host of detailed and extensive experimental and theoretical studies on smaller aliphatic nitriles.^{24–33}

When incorporated into duplex DNA, the nitrile group of 5-cyano-2'-deoxyuridine (**1**) is in the major groove and N2-nitrile-2'-deoxyguanosine is in the minor groove.²³ Here, the utility of **1** (and its silyl ether analogue, **2**) as a probe of local environment was investigated by measuring the dependence of the nitrile stretching frequency, line shape, and absorbance on solvent, structure, and temperature. The dependence of the nitrile IR absorption band was investigated using a series of water–THF mixtures. These solvents provide a large range of dielectric constants that cover the dielectric environments found in DNA.³⁴ The effect of heterodimer formation in chloroform between **2** and **3a** was investigated as a model of the hydrogen-bonding interactions between nucleic acid base pairs. These experimental studies were complemented with density functional theory (DFT) calculations to probe the impact of hydrogen bonding with water and heterodimer formation on the nitrile stretching frequency, in addition to the structural and energetic impact of the nitrile group on heterodimer formation. Lastly, the temperature dependence of the nitrile IR absorbance band was measured to show the feasibility of using this IR probe to investigate temperature-induced structural changes in nucleic acids upon melting.

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Results and Discussion

The solvent-dependent IR absorbance band corresponding to the nitrile stretching frequency $\tilde{\nu}_{\text{C}\equiv\text{N}}$ of **1** was investigated in THF, water, and mixtures of these two solvents. The $\text{C}\equiv\text{N}$ stretching frequency in THF was 2232.7 cm^{-1} , while the absorbance band broadened and blue-shifted by 9.2 cm^{-1} to 2241.9 cm^{-1} in water.³⁵ The frequency shift and broadening of the band in water is likely due in part to solute–solvent interactions including hydrogen bonding. DFT calculations were carried out on a model system, 5-cyanouracil, to determine the effect of hydrogen bonding on the nitrile stretching frequency in the gas phase.

The DFT calculated $\tilde{\nu}_{\text{C}\equiv\text{N}}$ in 5-cyanouracil shifts from 2356.1 to 2362.3 cm^{-1} when an explicit water molecule is included in the model to interact with the nitrile at the B3PW91/6-31++G(d,p) level. Prior to the frequency calculation, the model of 5-cyanouracil and the one water molecule was geometry optimized at the same level of theory. The resulting 6.2 cm^{-1} blue shift in $\tilde{\nu}_{\text{C}\equiv\text{N}}$ is due to hydrogen bonding between the nitrogen atom of the nitrile group and the hydrogen atom of water. This interaction effectively strengthens the nitrile bond, resulting in an increase of $\tilde{\nu}_{\text{C}\equiv\text{N}}$. This result suggests that at least part of the experimental blue shift in $\tilde{\nu}_{\text{C}\equiv\text{N}}$ going from THF to water as the solvent is due to hydrogen bonding between the aqueous solvent and the nitrile group of **1**. This model is clearly limited by the calculations being performed in the gas phase and not accounting for different potential hydrogen-bonding configurations between 5-cyanouracil and water, such as more than one water molecule interacting with the nitrile moiety. The results of this simple model are, however, in agreement with the observed solvent dependence of the $\tilde{\nu}_{\text{C}\equiv\text{N}}$ of **1** and nitrile-modified amino acids.⁵

The solvent dependence of $\tilde{\nu}_{\text{C}\equiv\text{N}}$ for **1** was further investigated by a series of water–THF mixtures. Figure 1A shows the $\tilde{\nu}_{\text{C}\equiv\text{N}}$ IR absorbance band of **1** for solvents ranging from 20 to 80% water in THF prepared by volume (v/v), normalized to the same maximum absorbance. These spectra show that the nitrile stretching frequency increases from 2233.9 to 2241.6 cm^{-1} in transitioning from 20 to 80% water, with a simultaneous broadening of the band up until $\sim 60\%$ water. The blue shift in $\tilde{\nu}_{\text{C}\equiv\text{N}}$ occurs over the entire solvent range, with a more dramatic increase in the 50–70% water range.³⁵ This illustrates the dependence of $\tilde{\nu}_{\text{C}\equiv\text{N}}$ on a wide range of local environments presented by the solvent.

Three line shape functions, each composed of a weighted sum of a Lorentzian and Gaussian function with the same band position, were used to globally fit the IR absorbance band in the nitrile stretching region of **1** in the water–THF mixtures. The result of the global fit for the 50% absorbance spectrum is shown in Figure 1B. Due to the position and band widths of the three bands and drawing from a previous literature study on nitrile-modified amino acids in water–THF mixtures,⁵ the three bands were labeled THF-like (TL), water–THF (WT), and water-like (WL), as shown in Figure 1B. This model is in agreement with a previous study that suggests a ternary nature of water–THF mixtures consisting of free THF molecules, water–THF complexes, and free water molecules for the majority of the mixtures studied here.^{36,37} However, at high THF or high water percentages, the mixtures are more binary in nature, consisting of free THF or free water molecules, respectively, and water–THF complexes.³⁷ The WL and TL bands arise exclusively from interactions between water and THF solvent molecules with **1**, respectively, while the WT band

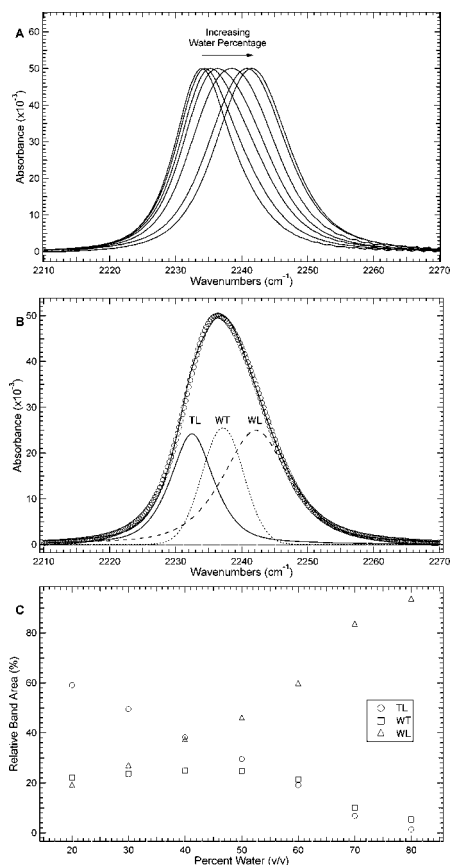


Figure 1. (A) FTIR absorbance spectra of **1** in water–THF mixtures ranging from 20 to 80% water (v/v) in approximately 10% increments recorded at 293 K. The spectra were normalized to the same maximum absorbance. The concentration of **1** was 50 mM. (B) FTIR absorbance spectrum (open circles) of **1** in a 50% water solution modeled by three line shapes corresponding to a THF-like (TL, solid curve), water–THF (WT, short dashed curve), and water-like (WL, long dashed curve) band. (C) Dependence of the relative band area of the three components on percent water of the solvent.

is due to solute–solvent interactions involving both water and THF solvent molecules.

The relative band area percentage of the TL band from the global line shape analysis decreases as the percentage of water in the solvent increases, while the relative band area of the WL band increases over the same range (Figure 1C). The relative area of the WT band gradually increases with increasing water percentage to a maximum of $\sim 40\%$ and then rapidly decreases with further increases in water content. The exact nature of the solvent–solute interactions in the WT band is unclear; however, the experimental results clearly highlight the sensitivity of $\tilde{\nu}_{\text{C}\equiv\text{N}}$ to its local environment.

Difference FTIR spectroscopy in combination with singular value decomposition (SVD) further supports the existence of three distinct local environments around the nitrile of **1** in the mixed water–THF solvent system. Figure 2A shows the difference spectra of **1** in the nitrile stretching region generated by subtracting the 60% water absorbance spectrum from the 20–80% water absorbance spectra. The spectra were normalized by area prior to the subtraction. The difference spectra show a first derivative feature due to the shift of $\tilde{\nu}_{\text{C}\equiv\text{N}}$ with solvent and two distinct isosbestic points at $\sim 2236.8\text{ cm}^{-1}$ for the low water percentages and at $\sim 2239.4\text{ cm}^{-1}$ for the higher water percentages. The presence of these isosbestic points underscores the need for at least a three-state system to adequately describe the

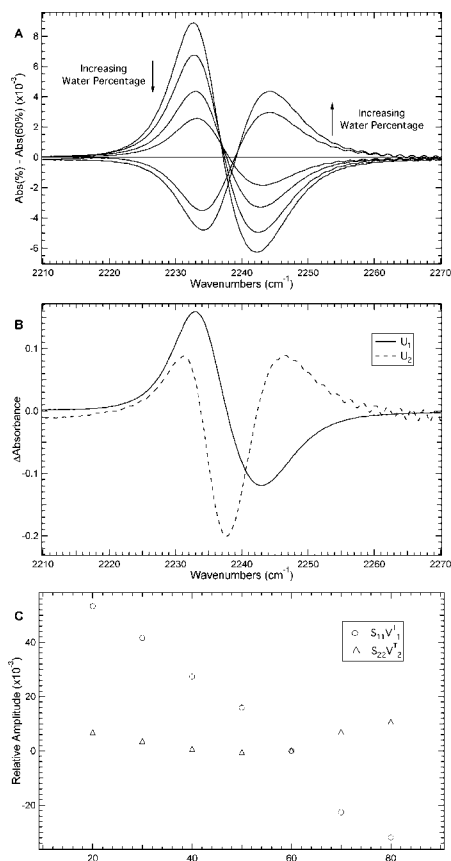


Figure 2. (A) Difference FTIR spectra of **1** formed by the subtraction of the FTIR spectrum corresponding to 60% water, 40% THF (v/v) from the FTIR absorbance spectra corresponding to 20–80% water in approximately 10% increments. Prior to subtraction, the FTIR absorbance spectra were normalized by area. (B) The first two spectral components from the U matrix resulting from an SVD analysis of the difference FTIR spectra in panel A. (C) The corresponding weighted solvent dependence (SV^T) of the basis spectra in panel B.

dependence of the $\tilde{\nu}_{C\equiv N}$ line shape in this mixed solvent system with each state characterized by different solute–solvent interactions.

An SVD analysis of the difference spectra reveals that only two basis spectra (Figure 2B) are required to represent >97% of the experimental data. The first basis function (U_1) illustrates the shift in the $\tilde{\nu}_{C\equiv N}$ with increasing amounts of water in the solvent, while the second basis function (U_2) highlights the change in the bandwidth that occurs simultaneously with the shift in the vibrational frequency. The solvent dependence of the first basis spectrum weighted by the appropriate singular value ($S_{11}V_1^T$, open circles) shown in Figure 2C is similar to the change in the relative band areas of the TL and WL components from the global line shape analysis of the FTIR absorbance spectra in Figure 1C. The solvent dependence of the second basis spectrum weighted by its singular value ($S_{22}V_2^T$, open triangles) shows an initial decrease in amplitude with a minimum at ~50% water and then an increase in amplitude with increasing water percentage in the solvent. This solvent dependence qualitatively agrees with the dependence of the relative band area of the WT band and the overall spectral bandwidth of the raw data with percentage of water in the solvent. Hence, the SVD analysis further supports the three-state model used in the global line shape analysis to characterize the IR absorbance band of the nitrile stretching frequency of **1** in water–THF mixtures.

CHART 1

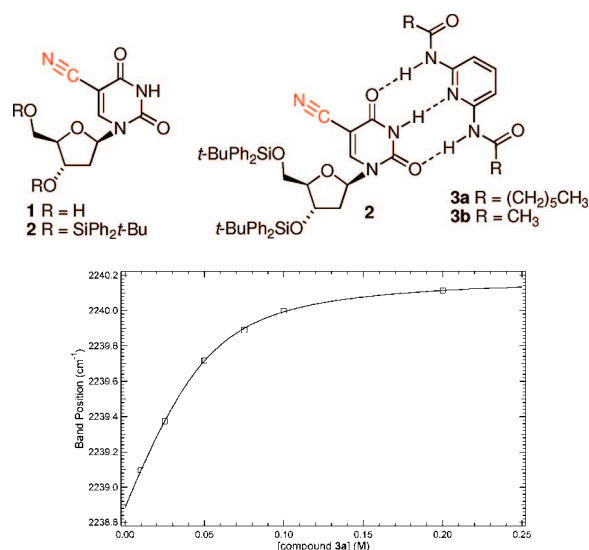


Figure 3. Dependence of the nitrile stretching frequency of **2** (open squares) on the concentration of **3a** fit to the appropriate binding constant equation³⁵ (solid curve).

The dependence of $\tilde{\nu}_{C\equiv N}$ on the formation of a heterodimer between **2** and **3a** (Chart 1) in chloroform similar to DNA base pairs is shown in Figure 3. The $\tilde{\nu}_{C\equiv N}$ of **2** undergoes a modest blue shift upon increasing concentration of **3a**, as shown in Figure 3, due to heterodimer formation. The increase in the concentration of **3a** shifts the equilibrium in favor of the heterodimer, illustrating the sensitivity of $\tilde{\nu}_{C\equiv N}$ to “base pairing” in addition to solvent effects. The calculated association constant, K_a , is $\sim 90 \text{ M}^{-1}$, which is in agreement with literature reports of similar heterodimers.^{38–41}

DFT calculations at the B3PW91/6-31++G(d,p) level on a model heterodimer formed between 5-cyanouracil and **3b** were performed to verify that the experimentally observed blue shift of $\tilde{\nu}_{C\equiv N}$ of **2** upon addition of **3a** was due to heterodimer formation. Initially, the structures of both monomeric 5-cyanouracil and its heterodimer with **3b** were geometry optimized. The gas phase DFT calculations show that the nitrile stretching frequency blue shifts 2.6 cm^{-1} upon heterodimer formation, which is in agreement with the experimental measured shift in the $\tilde{\nu}_{C\equiv N}$ of **2** with increasing amounts of **3a** in solution. This suggests that heterodimer formation is the origin of the shift.

The utility of the nitrile group as a vibrational probe of DNA structure partially depends on the structural and energetic effects due simply to the presence of the nitrile functional group. The DFT calculated potential energy surface (PES) at the B3PW91/6-31++G(d,p) level, formed by modulating the hydrogen-bond distance between 5-cyanouracil and **3b** in the gas phase, shows that the energy of interaction is -62.1 kJ/mol with a hydrogen-bond distance of 1.91 \AA for the geometry optimized dimer.³⁵ A similar PES was calculated for the interaction of 5-uracil with **3b** and showed an energy of interaction of -64.2 kJ/mol with an optimal hydrogen-bonding distance of 1.96 \AA .³⁵ Thus, the presence of the nitrile group destabilized the dimer by only ~3%. This is in agreement with DNA melting experiments of duplexes containing **1**.¹⁴ Consequently, the nitrile group is a nonintrusive probe of local environment and structure.

The solvent and hydrogen-bonding sensitivity of **1** and **2** suggests that the nitrile moiety can be used as a vibrational probe to study DNA structural changes with site-specificity. Since temperature is commonly used to modulate DNA structure, the intrinsic temperature dependence of the nitrile IR absorbance

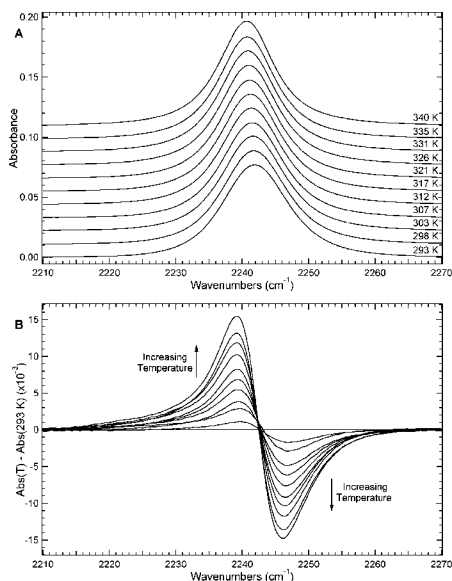


Figure 4. (A) Temperature-dependent FTIR absorbance spectra of **1** in water from 293 to 340 K in ~ 5 K increments. (B) Temperature-dependent difference FTIR spectra of **1** in water formed by subtracting the FTIR absorbance spectrum recorded at 293 K from the spectra recorded at higher temperatures.

band of **1** was investigated in water, as shown in Figure 4A (the solvent needed for DNA studies). Both the $\tilde{\nu}_{\text{C}\equiv\text{N}}$ and the bandwidth $\Delta\tilde{\nu}_{\text{C}\equiv\text{N}}$ decrease linearly from 2242.0 cm^{-1} and $\Delta\tilde{\nu}_{\text{C}\equiv\text{N}}$ at 293 K to 2240.6 cm^{-1} and $\Delta\tilde{\nu}_{\text{C}\equiv\text{N}}$ at 340 K.^{35,42} This frequency shift is manifested as a first derivative feature in the temperature-dependent difference spectra resulting from subtracting the lowest temperature spectrum from the higher temperature spectra (Figure 4B). This modest linear intrinsic temperature dependence³⁵ of the nitrile IR absorbance band of **1** should permit changes in the IR absorbance band due to DNA conformational changes induced by melting or ligand (protein, drug) binding to be observed.

Conclusions

The nitrile group of **1** and **2** was shown to be a responsive vibrational probe. The IR absorbance band corresponding to the nitrile stretching vibration was shown to be sensitive to solute–solvent interactions and heterodimer formation with a modest linear intrinsic temperature dependence in water. In regards to solvent and temperature dependence, **1** is quite similar to 4-CN-Phe, which has been an extremely useful tool for studying local protein environments.^{5–8} This suggests that, upon incorporation into DNA oligomers,^{14,17–19,22} **1** will prove to be an effective, site-specific, and relatively nonintrusive probe with a range of potential applications in studying nucleic acid structural changes upon melting or ligand (protein, drug, metal) binding. IR studies of DNA oligomers containing **1** are currently being pursued.

NMR spectroscopy can also be utilized to monitor the local environment of the nitrile group. Analysis of **1** and **2** by ¹³C NMR and the corresponding C \equiv N¹⁵-labeled compounds by ¹⁵N NMR is currently under investigation. The details of these NMR studies will be reported in a future manuscript.

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Supporting Information Available: Experimental details and references for the synthesis and characterization of **1**, **2**, and **3a**, FTIR spectra acquisition, global line shape fitting, the determination of the binding constant for complex **2:3a**, DFT calculations, and the SVD analysis. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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