Low-Temperature NMR Studies on the Structure of Uridine Dimers

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The genetic information of DNA as well as secondary and tertiary structural elements of nucleic acids relies on the formation of specific hydrogen bonds between the four nucleobases. However, due to the presence of multiple proton donor and acceptor sites, configurations other than Watson−Crick T(U)A and CG base pairs may be formed under appropriate conditions. Indeed, Hoogsteen, reverse Hoogsteen, and reverse Watson−Crick hydrogen bonds are observed in systems like triple-helical or parallel-stranded oligonucleotides. To examine association of bases without interference from steric constraints and base stacking interactions, NMR studies have been performed in apolar solvents on suitably derivatized free bases in the past.[1,2] However, information on the detailed structure of the associates is limited due to their fast exchange on the NMR time scale at ambient temperatures.

Noncanonical U-U base pairs occur in several RNAs.[3−5] Generally, three different structures with two cyclic hydrogen bonds are conceivable for the two uracil bases (Figure 1a). In symmetric dimers **I** and **II**, the carbonyl groups at positions 2 and 4 are engaged in hydrogen bonds, respectively. Dimer **III** having no axis of symmetry consists of two nonequivalent uracil bases. Structures **I** and **II** can interconvert only via complete dissociation and recombination of the monomers. In contrast, structure **III** can rearrange to **I** or **II** by a 180° rotation of one uracil base around an axis through its N3 and C6 ring atoms breaking only one of the two hydrogen bonds. Measurements on the concentration-dependent ^{13}C NMR chemical shifts and on the IR stretching bands of the two carbonyl groups in 1-cyclohexyluracil indicated preferential binding at O-4 although the existence of dimers **I** and **III** in solution could not be ruled out.[6,7] Dimeric structures of type **II** have also been found in the solid state by X-ray studies on 1-methyluracil.[8]

Herein we describe the first direct evidence of dimeric uridine species coexisting in solution by NMR. For this purpose, we have performed low-temperature NMR measurements of 3′,5′-diacetyl-2′-deoxyuridine specifically ^{15}N labeled at the 3-position to reduce the NH imino proton line width upon ^{15}N decoupling especially at lower temperatures.[9] Furthermore, a deuterated
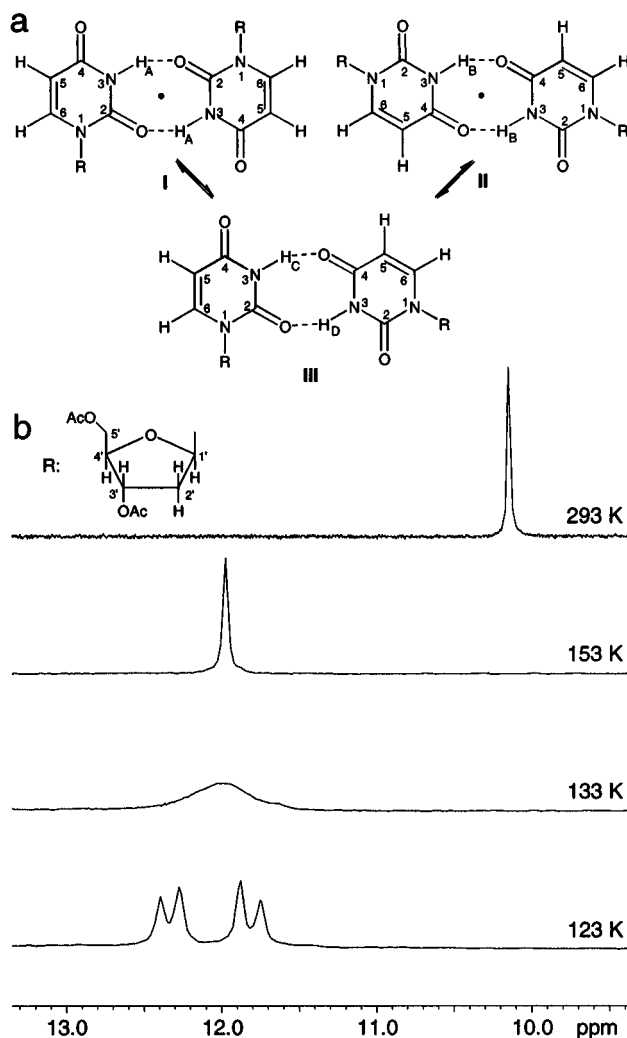
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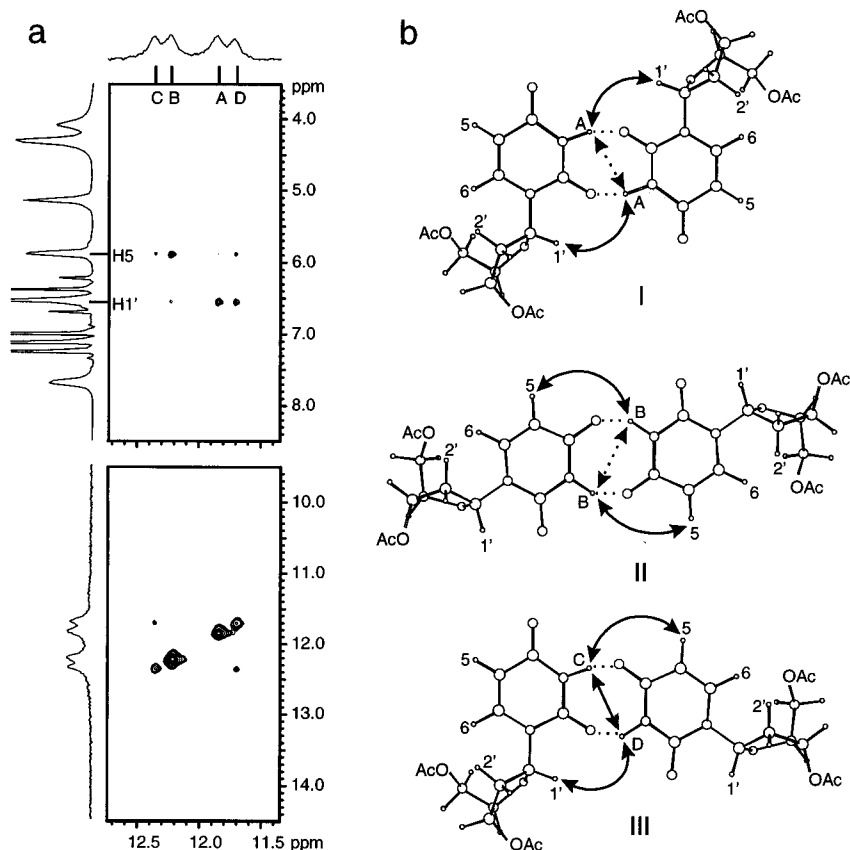

Figure 1. (a) Structures of the dimeric uracil derivatives. (b) Temperature-dependent ^1H {^{15}N} NMR spectra showing the imino proton resonances of [3-^{15}N]-3′,5′-diacetyl-2′-deoxyuridine in CDClF$_2$/CDF$_3$ (c = 88 mM).

freon mixture CDClF$_2$/CDF$_3$ was used as solvent which allows measurements at temperatures low enough for the observation of individual dimers in slow exchange.[10]

In Figure 1b, imino proton resonances of the uridine derivative are plotted as a function of temperature. By lowering the temperature, the imino signal shifts downfield due to increased dimer formation and considerably broadens at about 133 K. Below 133 K four signals emerge at 12.36, 12.25, 11.85, and 11.72 ppm. Apparently, these resonances are associated with imino protons of the three different dimeric species.

To assign the four signals to the dimers of Figure 1a, a homonuclear 2D NOE experiment was aquired at 113 K. Regions showing imino−imino and imino−H5/H1′ contacts are plotted in Figure 2a. At this temperature no cross peaks due to chemical exchange are observed. However, there are prominent cross peaks between imino protons at 12.36 and 11.72 ppm, between both downfield-shifted imino protons and H5, as well as between both upfield-shifted imino protons and H1′ protons.

NOE connectivity patterns expected for the three dimers in their anti conformation are illustrated in Figure 2b.[11] As can

(11) Internucleoside distances between imino protons and the anomeric H1′ proton of the hydrogen-bonded nucleoside depend on the O4′−C1′−N1−C2 glycosidic torsion angle κ. However, intranucleoside NOE contacts observed between H6 and H2′ and missing contacts between H6 and H1′ protons clearly indicate an anti conformation with glycosidic torsion angles in the 180° range for the uridine derivative at low temperatures (data not shown).

Figure 2. (a) ^1H {^{15}N} 2D NOE spectrum of [3-^{15}N]-3′,5′-diacetyl-2′-deoxyuridine in CDClF$_2$/CDF$_3$ (c = 88 mM). The spectrum was acquired at 113 K with a 60 ms mixing time. Portions of the spectrum show imino-imino (bottom) and imino–base, sugar proton cross peaks (top) with corresponding one-dimensional spectra at the top and to the left. (b) Models of the three cyclic dimers of 3′,5′-diacetyl-2′-deoxyuridine in their anti conformation. Arrows indicate short interproton distances (<4 Å) to the imino protons with dashed arrows connecting two equivalent imino protons that give no observable NOE contact.

be seen, only internucleoside connectivities of the imino proton hydrogen bonded to the 4-carbonyl with H5, of the imino proton hydrogen bonded to the 2-carbonyl with H1′, and of the two nonequivalent imino protons in dimer **III** are expected to give rise to strong NOE cross peaks with distances of about 3.6, 3.3, and 3.0 Å, respectively. Thereby resonance assignments for the imino protons become straightforward. NOE contacts between the most downfield- and most upfield-shifted signals connect the two iminos of the asymmetric structure **III**. Cross peaks to H5 protons for the two downfield-shifted resonances identify imino protons hydrogen bonded to the 4-carbonyl whereas cross peaks to H1′ protons for the upfield-shifted resonances allow assignment of imino protons bonded to the 2-carbonyl. Additional weak cross peaks observed between downfield-shifted imino protons and H1′ as well as between upfield-shifted imino protons and H5 can be attributed to intranucleoside contacts involving distances greater 4 Å.

Having unambiguously assigned all of the four imino resonances, we deconvoluted the signals at 123 K by spectral simulation and determined the integral ratio to be 1.2:1.3:1:1 for resonances A, B, C, and D, respectively. Because C and D arise from the two nonequivalent imino protons within the same structure **III**, relative populations of the dimers at 123 K amount to 27% for structure **I**, 29% for structure **II**, and 44% for structure **III**.

Taking into account the statistic advantage of forming the asymmetric structure **III**, none of the two uracil carbonyl oxygens appears to be predominantly engaged in hydrogen bonding within the homodimer. Rather, both carbonyl groups are comparable in their proton acceptor strength and the particular base pair scheme in a nucleic acid should mostly be determined by steric constraints and stacking effects. However, more downfield-shifted imino proton chemical shifts indicate stronger hydrogen bonds involving the 4-carbonyl as compared to the 2-carbonyl group.

Knowledge of the properties of nucleobases is crucial not only for a better understanding but also for the prediction of nucleic acid structure. These studies open up the way for further investigations on the preferred mode of nucleoside homo- and heteroassociation. Also, base analogues designed for various biochemical and clinical applications may initially be tested for their binding affinity and specificity toward natural bases or base pairs before their incorporation into oligonucleotides.