Geometry and Strength of Hydrogen Bonds in Complexes of 2′-Deoxyadenosine with 2′-Deoxyuridine

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Abstract: NMR studies have been performed on the association of 3′,5′-di-O-acetyl-2′-deoxyuridine with a 2′-deoxyadenosine derivative in solution. Using a CDClF/CF3 solvent mixture, measurements at very low temperatures allowed the observation of individual complexes in the slow-exchange regime. From 2D NOE connectivities of the imino proton resonances at 125 K the predominant species could be assigned to a Watson–Crick geometry. However, concentration-dependent 15N chemical shifts of specifically labeled uridine and adenine nucleosides indicate a considerable population of Hoogsteen base pairs at ambient temperatures. These results together with a more downfield chemical shift of the Watson–Crick bound imino proton point to a stronger hydrogen bond in Watson–Crick when compared to Hoogsteen base pairs.

Introduction

The specific association of nucleobases driven by hydrogen bonding is not only fundamental to such important biological processes such as replication and transcription but also a major determinant of nucleic acid structure. Thus, Watson–Crick pairing between the adenine (A) and the uracil (U) or thymine (T) base constitutes a structural key element in most double-stranded RNA and DNA. However, owing to their multiple proton donor and acceptor sites, base pairing schemes involving two hydrogen bonds other than a canonical Watson–Crick geometry are conceivable for A and U(T). In fact, duplex DNA with reversed Watson–Crick1 or Hoogsteen hydrogen bonding2a–d has been shown to form under appropriate conditions. But knowing the relative stability and the preferred conformation of such complexes is important not only for understanding nucleic acid structure but also for the rational development of new technologies which are based on molecular recognition. These include the use of DNA specific ligands such as antisense and antigene oligonucleotides3 or the design of self-assembling molecules and synthetic host compounds which utilize AU(AT) base pairs as organizational or recognition elements.4a–e

An early crystal structure of 9-ethyladenine and 1-methyluracil demonstrated that the bases are not arranged in a Watson–Crick geometry as is mostly found in DNA and RNA oligomers but are rather hydrogen bonded in a Hoogsteen configuration.5 A more stable Hoogsteen orientation was also obtained from calculations in the gas phase,6a,b but other theoretical investigations yielded four nearly isoenergetic orientations for the A–T base pair.6c However, because of the specialized environment of the crystal or in the gas phase, no conclusions can be drawn as to the preferred geometry under solution conditions. Previous studies on the association of A with U or T nucleobases have considerably contributed to our current understanding of base pair stabilities in nonpolar or moderately polar solvents.7a–c However, they failed to unambiguously determine the preferred geometries due to their inherent limitations. Thus, IR measurements often suffer from poor resolution of vibrational bands and possible vibrational coupling. Likewise, with coexisting complexes in fast exchange and without a precise knowledge of the hydrogen-bonding perturbation on the electron distribution, NMR chemical shift data do not permit a rigorous analysis in terms of specific geometries at ambient temperatures. Consequently, preferred configurations of AU or AT base pairs could only be indirectly inferred from the available solution data and have provided a qualitative picture at most.

We have recently employed a NMR technique at very low temperatures with a deuterated Freon mixture CDClF/CF3 as solvent to study the various self-association modes of an uridine nucleoside and some of its analogues.5,9 With measurements in the liquid state as low as 113 K individual hydrogen-bonded complexes of nucleobases in slow exchange could be

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observed and unambiguously characterized in solution for the first time. We now present NMR studies on the association of adenosine and uridine derivatives at low and ambient temperatures to further explore the strength and preference of hydrogen bonding in the formed heterocomplexes.

Methods

NMR experiments were performed on a Bruker AMX500 spectrometer. Temperatures were adjusted by a Eurotherm Variable Temperature Unit to an accuracy of \( \pm 1.0 \) °C. \(^1\)H chemical shifts in chloroform at 293 K were referenced relative to CHCl\(_3\) (\( \delta_H = 7.24 \) ppm) and in a Freon mixture relative to CHClF\(_2\) (\( \delta_H = 7.13 \) ppm). For \(^{15}\)N chemical shifts an external reference of \(^{15}\)NHCl in 10% HCl was used (\( \delta_N = 0 \) ppm). Signal integrals were determined with a Lorentzian curve fitting routine of the UXNMR software package. Concentration-dependent chemical shifts were fitted with an appropriate equation by employing the Marquardt–Levenberg algorithm. Electrostatic potentials were calculated with PC SPARTAN Pro V1.0.1.

Materials

Reagents of the highest quality available were purchased from Sigma-Aldrich, Deisenhofen, Germany. [3-\(^{15}\)N]-3,5'-Diacetyl-2'-deoxyuridine was prepared from unlabeled 2'-deoxyuridine as described previously\(^{10,11}\) and 7-deaza-2'-deoxyadenosine (2'-deoxytubercidin) was obtained from Aldrich, Deisenhofen, Germany. [3-\(^{15}\)N]-3'-Deoxy-4-thiouridine (thiO-U) we measured concentration-dependent imino proton chemical shifts of the pyrimidine base in a mixed chloroform solution (Figure 2). The sulfur atom in the 4-thio analogue was shown to be inferior to oxygen as hydrogen bond acceptor and is not expected to be significantly involved in hydrogen bonding.\(^3\) While the formation of U\(_2\) (thioU) homodimers and AU\(_2\) (A\(^{thio}\)U\(_2\)) trimers can be neglected by using a large excess of adenosine, the association constant for adenosine self-pairing was initially determined by concentration-dependent measurements of the adenine amino proton chemical shift and found to be 2.3 M\(^{-1}\) (not shown). Although small, this self-association was explicitly taken into account for fitting the experimental data of the mixture. Also, knowing the limiting chemical shift of the imino proton in a monomer at \( c \rightarrow 0 \) from the self-association studies on the uridine derivatives leaves only two adjustable parameters for the fitting procedure, namely the association constant for uridine and 2'-deoxy-4-thiouridine (thioU).


the heterodimer formation and the limiting chemical shift of the imino proton in a heterodimer at infinite concentration. The association constant thus obtained from the least-squares fit to the imino proton in a heterodimer at infinite concentration. The concentration-dependent $^1H$ chemical shift of the imino proton does not allow any evaluation of predominant pairing geometries because of fast exchange between individual complexes at 293 K. However, using specifically $^{15}N$-labeled nucleosides, more information about the association mode of base pairs at room temperature can be obtained. In Figure 3, the $^{15}N$ chemical shift of a 1:1 mixture of di-O-acetylated [7-$^{15}N$]-2'-deoxyadenosine and 3-$^{15}N$-2'-deoxyuridine in chloroform is plotted as a function of nucleoside concentration. As expected for an imino group involved in a hydrogen bond, the uridine $^{15}N$ resonance is shifted downfield upon increasing the temperature. As has been observed for the homoassociation of the uridine derivatives, the imino signal shifts downfield upon cooling due to increased formation of hydrogen-bonded complexes. Below the coalescence point at 163 K individual imino resonances in slow exchange on the chemical shift time scale appear in the $^1H$ NMR spectrum. Apparently, these include signals of imino protons in AU complexes $H_A$ and $H_B$ at 15.1 and 14.3 ppm and resonances $H_C$ at higher field, which can be attributed to the homodimers of excess uridine by comparison with previous experiments. Signal integration at 138 K gives an intensity ratio $H_A/H_B$ of about 4.5:1.

Substituting acetyl with triisopropylsilyl protecting groups has been found advantageous for enhancing adenosine solubility in this solvent especially at lower temperatures. Nevertheless, upon decreasing the temperature of a 1:1 mixture of 3',5'-di(triisopropylsilyl)-2'-deoxyadenosine and the protected 2'-deoxyuridine, the purine nucleoside partially precipitates leaving the highly soluble uridine in excess. In Figure 4, imino resonances of an A + U mixture in Freon are plotted as a function of temperature. As has been observed in the homoaasociation of the uridine derivatives, the imino signal shifts downfield upon cooling due to increased formation of hydrogen-bonded complexes. Below the coalescence point at 163 K individual imino resonances in slow exchange on the chemical shift time scale appear in the $^1H$ NMR spectrum. Apparently, these include signals of imino protons in AU complexes $H_A$ and $H_B$ at 15.1 and 14.3 ppm and resonances $H_C$ at higher field, which can be attributed to the homodimers of excess uridine by comparison with previous experiments. Signal integration at 138 K gives an intensity ratio $H_A/H_B$ of about 4.5:1.

Assignment of the AU imino protons to Watson–Crick or Hoogsteen geometries should be easily accomplished through $^1H$-$^1H$ NOE contacts of the imino resonances at low temperatures. From Figure 1 it becomes apparent that for the Watson–Crick configuration NOE cross-peaks are expected between the hydrogen-bonded imino and $H_2$ and amino protons of adenine.

On the other hand, in addition to the imino–amino contact there is a close spatial proximity between the imino and the adenine $H_8$ proton in a Hoogsteen base pair. Note that $^1H$-$^1H$ NOE’s do not easily allow one to distinguish between normal and reverse geometries. As seen from Figure 5 (left), adenosine $H_2$ and $H_8$ protons are clearly resolved at higher temperatures. Unfortunately, the two signals broaden and merge around the coalescence temperature preventing an unambiguous resonance assignment at lower temperatures. Thus, to exclude any potential NOE contact between $H_8$ and Hoogsteen bound uridine imino protons, the adenosine derivative was selectively deuterated at its $\beta$-position. No residual $H_8$ resonance was observed in the $^1H$ NMR spectrum at 203 K after deuteration as illustrated in Figure 5 (bottom right).

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homodimers HC clearly exhibit strong exchange cross-peaks at K is shown in Figure 6. Whereas imino resonances of uridine deoxyuridine with the 8-deuterated adenosine nucleoside at 125 °C, H2, H8 and uracil H6 protons indicated.

Figure 5. 1H NMR spectra of a mixture of 3′,5′-di-O-acetyl-2′-deoxuryridine with nondeuterated (left) and 8-deuterated (right) 3′,5′-di(triisopropylsilyl)-2′-deoxyadenosine in Freon. Base and sugar proton spectral regions are shown at 138 (top) and 203 K (bottom) with adenine H2, H8 and uracil H6 protons indicated.

Figure 6. Portion of a 2D NOE spectrum of 3′,5′-di-O-acetyl-2′-deoxuryridine and 8-deuterated 3′,5′-di(triisopropylsilyl)-2′-deoxyadenosine in Freon showing cross-peaks of imino and base protons. The spectrum was acquired at 125 K with a 50 ms mixing time.

A portion of a 2D NOE spectrum of a mixture of 2′-deoxuryridine with the 8-deuterated adenosine nucleoside at 125 K is shown in Figure 6. Whereas imino resonances of uridine homodimers Hc clearly exhibit strong exchange cross-peaks at this temperature, no exchange cross-peaks for the two downfield shifted imino resonances HA and HB are observed. However, a strong cross-peak of resonance HA must originate from a NOE contact to H2 and unambiguously identify an imino proton engaged in a AU Watson–Crick hydrogen bond. Additional weaker cross-peaks connect this imino signal with the two adenine amino resonances whose identification is based on deuterium exchange experiments. Corresponding cross-peaks having the same intensity within experimental error are also observed in a 2D NOE experiment with a nondeuterated adenine nucleoside (spectrum not shown). Although an additional NOE contact to adenine H8 is expected for a Hoogsteen bound imino proton in the nondeuterated sample, no NOE cross-peak to the imino resonance HB at 14.3 ppm could be observed possibly due to its low intensity. Also, low-temperature NMR measurements on N6-methyl-2′-deoxyadenosine suggest a significant line broadening at decreasing temperature of the adenine H8 resonance when compared to adenine H2 further obstructing observation of a potential Hoogsteen imino-H8 contact.

A tentative assignment of resonance HB to a Hoogsteen bound imino proton is supported by low-temperature measurements on a mixture of 2′-deoxuryridine with 7-deaza-2′-deoxyadenosine (Figure 7). Again, separate imino signals between slowly exchanging hetero- and homodimers appear in the spectra below the coalescence temperature at 173 K. However, only a single imino resonance at 15.5 ppm downfield shifted with respect to the signals of the homodimers is observed in the 1H NMR spectrum for the heterodimer between the 7-deaza-analogue and uridine. Clearly, substituting N7 with CH in 7-deaza-2′-deoxyadenosine eliminates the Hoogsteen hydrogen bond acceptor site leaving only the possibility of forming a Watson–Crick base pair.

Discussion

Geometry of AU Complexes. The results obtained from the one- and two-dimensional NMR experiments described above unambiguously show that the Watson–Crick AU base pair predominates at low temperatures with a population of about 80%. Although there is no observable NOE contact, assignment of the low-intensity resonance HB to an AU imino proton in a Hoogsteen configuration is clearly indicated by its absence for the associates formed by U with 7-deaza-A. Note also that changing a Watson–Crick for a Hoogsteen base pair requires the disruption of both hydrogen bonds in the cyclic dimers whereas homodimers of uridine can interconvert via the asymmetric O2−O4 homodimer by breaking only one hydrogen bond at a time. Consequently, the temperature-dependent imino proton spectra in Figure 4 illustrate that homodimers of uridine are in much faster mutual exchange at a given temperature than AU heterocomplexes which are already in slow exchange at T ≤ 138 K as evidenced by signals HA and HB. This is also demonstrated by the presence and absence of exchange cross-peaks for the homo- and heterodimers in the 2D NOE spectrum acquired at 125 K.

Population differences between Watson–Crick and Hoogsteen geometries appear to diminish at higher temperatures. The 15N chemical shift of adenine N-7 used as a probe for Hoogsteen hydrogen bonding in the fast exchange limit depends on both the population of Hoogsteen base pairs and the nitrogen chemical shift difference in a monomer and a N-7 hydrogen-bonded dimer. Thus, without knowledge of the N-7 limiting
chemical shifts the Hoogsteen population can be only qualitatively evaluated on the basis of the concentration-dependent $^{15}$N chemical shift of the purine base. The available data at 293 K point to a significant contribution of N-7 as hydrogen acceptor in AU complexes in line with previous studies suggesting that both Watson–Crick and Hoogsteen sites have about equal affinity for uracil binding.\textsuperscript{14,16,17}

There is no easy way to discriminate between AU base pairs with the 2-carbonyl or the 4-carbonyl oxygen of the pyrimidine base engaged in hydrogen bonding by $^1H$–$^1H$ NOE experiments. Previous $^{13}$C NMR studies pointed to a participation of both carbonyl groups as proton acceptor,\textsuperscript{18} but $^1O$ NMR chemical shifts in AT base pairs were interpreted in favor of reverse geometries.\textsuperscript{19} In the present studies no additional imino proton resonances which may be attributed to coexisting normal and reverse geometries are observed at low temperatures for the AU complexes. This may result from (i) the participation of only one uridine carbonyl in hydrogen bonding forming solely one type of geometry or (ii) to corresponding resonances being isochronous and therefore not resolved. The latter possibility is not only supported by the equal participation of both uridine carbonyls as proton acceptor in the uridine homodimers\textsuperscript{8,9} but also by the association constant found for base pair formation between adenosine and uridine as well as adenosine and 4-thiouridine. Thus, the equilibrium constant for adenosine–uridine dimerization as found by concentration-dependent chemical shifts is larger by a factor of 2 when compared to the association constant as determined for the adenosine–4-thiouridine complex. Disregarding large differences in stability for the individual base pairs, the statistical disadvantage of forming only reverse base pairs with the 4-thio analogue may account for the different association constants, provided that both carbonyls of uridine participate about equally in hydrogen bonding to adenosine. In fact, the slightly distorted line shape as is observed for the imino signal $H_2$ at 125 K (see Figure 4) indicates the superposition of nearly isochronous resonances. These may not only include Watson–Crick imino protons in normal and reverse geometries but also imino protons in AU base triplets. The formation of such ternary complexes by the simultaneous occupation of both Watson–Crick and Hoogsteen sites was already demonstrated in 1957 by the observation of three-stranded polyuridinates containing one adenine and two uracil residues\textsuperscript{20} and was later shown to also occur for the free bases in nonaqueous solutions based on NMR experiments and on a combination of near-infrared spectroscopy with vapor pressure osmometry.\textsuperscript{14,16}

Hydrogen Bond Strength. Comparison of our data at low and ambient temperatures indicates an increasing preference of the Watson–Crick configuration upon cooling. Such a situation must arise from a larger heat of formation $-\Delta H^\circ$ for a Watson–Crick as compared to a Hoogsteen arrangement of AU complexes. Strictly speaking, this heat of formation is the entire enthalpy difference between solvated dimers and monomers, but assuming only small differences between the enthalpy of solvation for the AU base pairs, differences in $-\Delta H^\circ$ can be attributed to the relative enthalpy of hydrogen bond formation. Using the proton chemical shift as indicator for the relative strength of hydrogen bonds, observation of a more deshielded Watson–Crick imino proton $H_2$ agrees with its expected

### Table 1

<table>
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<tr>
<th>homodimer *</th>
<th>heterodimer with A</th>
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<tr>
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\* From ref 9. \textsuperscript{a} Data of AT base pairs in an intramolecular DNA triple helix were taken from ref 24.

participation in a stronger hydrogen bond.\textsuperscript{21} Based on a simple electrostatic model, the hydrogen bond energy should increase as the donor becomes more acidic and the acceptor becomes more basic due to an increase in partial positive and partial negative charge on the donor and acceptor, respectively.\textsuperscript{22} Correspondingly, the observed order of $^1H$ NMR chemical shifts for the U imino proton in the heterocombes is fully consistent with the electrostatic potential-derived negative charge on the acceptor atom as obtained from ab initio calculations at the HF/6-31G(d) level of the 1-methylated purine base: N-1 (7-deaza-A) > N-1 (A) > N-7 (A).

Recently, two-bond internucleotide scalar couplings $^2J_{NN}$ across a hydrogen bond in Watson–Crick base pairs of RNA and DNA have been observed\textsuperscript{23a,b} and extended to the Hoogsteen base pairs of a DNA triple helix.\textsuperscript{23b} In addition to a strong correlation between the size of $^2J_{NN}$ and the $^1H$ chemical shift of the imino proton, a similar but inverse dependency was also found for the covalent $^J_{NN}$ scalar coupling of the imino group donor.\textsuperscript{24} Thus, a more downfield chemical shift observed for the Watson–Crick bound imino protons is generally associated with a stronger $^2J_{NN}$ but a weaker $^1J_{HH}$ coupling in Watson–Crick when compared to Hoogsteen AT base pairs. These scalar coupling constants can provide additional information on the proton localization in a hydrogen bond and a decrease in the $^1J_{HH}$ coupling indicates a gradual shift of the proton from the donor to the acceptor atom with a concomitant lengthening of the covalent N–H bond and a shortening of the donor and acceptor heavy atom distance.\textsuperscript{24,25a–c}

By using selectively $^{15}$N labeled uridine nucleosides, we have measured $^1J_{HH}$ scalar couplings for the individual uridine homodimers and for the heterodimers with adenosine in the slow exchange regime. The results are summarized in Table 1 together with the data obtained from AT base pairs in the DNA triplex.\textsuperscript{24} Clearly, a decrease in the $^1J_{HH}$ coupling constant correlates with a downfield shift of the imino proton signal. Especially the Watson–Crick AU base pair exhibits a significantly reduced $^1H$–$^{15}$N coupling of 80 Hz as compared to 91 Hz in the uridine homodimers. In agreement with the chemical shift data, NHN hydrogen bonds of the Hoogsteen type appear to be weaker with a $^1J_{HH}$ coupling of 84 Hz but are

nevertheless noticeably stronger than hydrogen bonds formed in the self-associates. Interestingly, there is a good qualitative agreement between the data on the isolated AU base pairs and the AT base pairs of the DNA triplex in aqueous solution. However, in addition to the more downfield imino proton chemical shifts and the smaller $^{1}J_{\text{NH}}$ scalar couplings there is also a more pronounced difference in both NMR parameters between the Watson–Crick and Hoogsteen geometry of the free AU base pairs. Clearly, the nature (T vs U) and environment of the hydrogen bonded bases are different in the oligonucleotide and additional contributions from stacking and steric effects of the sugar–phosphate backbone may further influence pairing geometries. Moreover, in contrast to the slowly exchanging low-temperature complexes, NMR parameters of the oligonucleotide strictly reflect averages of fast exchanging hydrogen bonded and small amounts of non-hydrogen bonded bases which may result from local base pair opening.

**Conclusion**

By employing low-temperature NMR techniques even weak intermolecular interactions can be characterized under solution conditions in detail. Thus, studies on the association of nucleobases through specific hydrogen bonds have provided new insight into the geometry and relative strength of hydrogen bonding. The results obtained for the free base pairs of adenosine and uridine nucleosides in solution demonstrate that a Watson–Crick geometry as found in genomic DNA is favored with respect to its energy of hydrogen bond formation. It may be argued that interactions in aprotic solvents are quite different from water, the biologically relevant solvent. However, base pairs in a double helix are efficiently screened from the aqueous environment by the sugar–phosphate backbone and may only experience a moderately polar microenvironment. It is expected that such low-temperature NMR experiments will yield not only a more detailed knowledge of the interactions in biological macromolecules but also a wide variety of different host–guest complexes which rely on the specificity and affinity of hydrogen bond interactions.

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