Probing the Role of Hydrogen Bonds in the Stability of Base Pairs in Double-Helical DNA

Alicia E. Every, Irina M. Russu
Department of Chemistry and Molecular Biophysics Program, Wesleyan University, Middletown, CT 06459

Received 30 May 2007; revised 11 July 2007; accepted 11 July 2007
Published online 17 July 2007 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/bip.20811

ABSTRACT:

Aromatic stacking and hydrogen bonding between nucleobases are two of the key interactions responsible for stabilization of DNA double-helical structures. The present work aims at defining the specific contributions of these interactions to the stability of individual base pairs in DNA. The two DNA double helices investigated are formed, respectively, by the palindromic base sequences 5′-dCCAACGTTGG-3′ and 5′-dCGCAGATCTGCG-3′. The strength of the N—H · · · N inter-base hydrogen bond in each base pair is characterized from the measurement of the protium–deuterium fractionation factor of the corresponding imino proton using NMR spectroscopy. The structural stability of each base pair is evaluated from the exchange rate of the imino proton, measured by NMR. The results reveal that the fractionation factors of the imino protons in the two DNA double helices investigated fall within a narrow range of values, between 0.92 and 1.0. In contrast, the free energies of structural stabilization for individual base pairs span 3.5 kcal/mol, from 5.2 to 8.7 kcal/mol (at 15°C). These findings indicate that, in the two DNA double helices investigated, the strength of N—H · · · N inter-base hydrogen bonds does not change significantly depending on the nature or the sequence context of the base pair. Hence, the variations in structural stability detected by proton exchange do not involve changes in the strength of inter-base hydrogen bonds. Instead, the results suggest that the energetic identity of a base pair is determined by the number of inter-base hydrogen bonds, and by the stacking interactions with neighboring base pairs. © *2007 Wiley Periodicals, Inc. Biopolymers 87: 165–173, 2007.*

Keywords: *proton exchange; stacking interactions; protium-deuterium fractionation factors; structural energetics; base-pair opening*

This article was originally published online as an accepted preprint. The "Published Online" date corresponds to the preprint version. You can request a copy of the preprint by emailing the Biopolymers editorial office at biopolymers@wiley.com

Correspondence to: Irina M. Russu; e-mail: irussu@wesleyan.edu

INTRODUCTION

The double-helical structure of the DNA molecule is maintained by a finely tuned array of intra- and inter-molecular interactions. Among these interactions the Watson–Crick hydrogen bonding between complementary bases and the stacking of bases play dominant roles.[1] Early studies have established that the DNA stability is strongly affected by base composition. For example, the melting temperature of DNA increases linearly with the G-C content of the molecule.[2] This relationship has been attributed to the hydrogen bonding between bases, namely, G-C base pairs should be more stable than A-T base pairs because the former contain three inter-base hydrogen bonds whereas the latter contain only two. Subsequent investigations have augmented this simple model by demonstrating that the sequence of bases also plays an important role in DNA stability.[3–5] The role of base sequence has been rational-

A.

10-mer DNA

```
          1 2 3 4 5
5' - d ( C C A A C G T T G G ) - 3'
3' - d ( G G T T G C A A C C ) - 5'
                    5 4 3 2 1
```

12-mer DNA

```
          1 2 3 4 5 6
5' - d ( C G C A G A T C T G C G ) - 3'
3' - d ( G C G T C T A G A C G C ) - 5'
                      6 5 4 3 2 1
```

B.

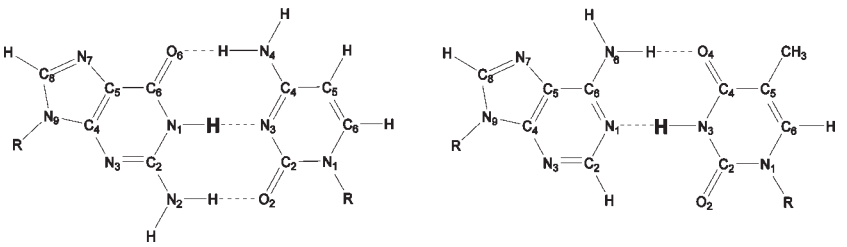

FIGURE 1 (A) Base sequences and numbering of base pairs in the DNA molecules investigated. (B) The structures of AT base pair (*right*) and GC base pair (*left*). The imino protons investigated in the present work are highlighted.

ized by postulating that the DNA structure is, thermodynamically, a sum of nearest-neighbor interactions. Accordingly, each base pair contributes to the overall stability of the DNA molecule a stabilization free energy that is specifically determined by its nature and by its neighbors.

The energetics of nearest-neighbor interactions in DNA are generally determined from thermal melting transitions monitored, for example, by differential scanning calorimetry or UV-spectroscopy.[3,4,6–9] The energetic parameters of the transition (e.g., free energy and enthalpy changes) are analyzed to obtain the thermodynamics of nearest-neighbor interactions at each of the 10 distinct dinucleotide steps present in double-helical DNA. An important advantage of this approach is its predictive power, namely, the energetic parameters for the 10 dinucleotide steps can be used to predict the stability of DNA duplexes of any base sequence. However, like in any thermodynamic approach, the intramolecular interactions that contribute to the obtained energetic parameters of dinucleotide steps are not known. Therefore, new methodologies have been sought to partition the near-neighbor energies into contributions from base stacking and base-pairing hydrogen bonds.[10–12] Furthermore, theoretical studies have suggested that base stacking interactions can affect the hydrogen bonding ability of bases.[13] Experimental evidence for the existence of this interplay between stacking and hydrogen bonding is not yet available. Our laboratory has begun to address these questions using nuclear magnetic resonance (NMR) spectroscopy. In our approach, the stability of individual DNA base pairs is measured by imino proton exchange. In agreement with DNA melting experiments, the measured stability is strongly dependent on the sequence context of the base pair.[14–17] To evaluate the contribution of base-pairing interactions to these sequence-induced changes in stability we also measured the strength of Watson–Crick hydrogen bonds using protium–deuterium (H-D) fractionation factors. In this article, we report our first series of results using this approach.

The DNA molecules investigated are shown in Figure 1. One molecule contains 10 base pairs, and is henceforth abbreviated 10-mer DNA. The structure of this DNA duplex has been solved at high-resolution (1 Å) by X-ray crystallography.[18] The other DNA molecule contains 12 base pairs, and is henceforth abbreviated 12-mer DNA. The processes of base-pair opening in the 12-mer DNA and the binding of Ca^{2+} and Mg^{2+} ions to it have been previously characterized by our laboratory using NMR spectroscopy.[19–21]

MATERIALS

The DNA oligonucleotides were synthesized on an automated DNA synthesizer (Applied Biosystems, Foster City, CA) using phosphoramidite chemistry. They were purified by reverse-phase HPLC in formate buffer at pH 7 with a gradient of 5–32% acetonitrile in 39 min. Counterions were exchanged with Na^+ ions by repeated (five times) centrifugation in Centricon YM-3 tubes (Amicon, Bedford, MA) against 0.5*M* NaCl. The samples were desalted by repeated centrifugation (eight times) against water. Each sample was dried down, and the final buffer used in each experiment was added to a volume of 0.6 ml. In the measurements of fractio-

nation factors the DNA samples were in 50 m*M* Tris buffer at pH 8.58. The DNA concentration was 0.6 m*M* (duplex) for the 10-mer and 1.3 m*M* (duplex) for the 12-mer. In the imino proton exchange measurements increasing concentrations of Tris were obtained by adding to each sample appropriate amounts of a Tris stock solution (3*M*). The pH of the samples varied between 8.49 and 8.58. The DNA concentration was 1.6 m*M* (duplex) for the 10-mer and 1.3 m*M* (duplex) for the 12-mer. All DNA samples used in this work contained 1.3 m*M* triethanolamine. The methylene resonances from triethanolamine were used to determine the pH directly in the NMR tube as we described.[15]

METHODS

NMR Experiments

The NMR experiments were carried out at 15°C, on a Varian INOVA 500 spectrometer operating at 11.75 T. The 1D NMR spectra were obtained with the Jump-and-Return pulse sequence,[22] with the maximum excitation at 13 ppm and a relaxation delay of 10 s. This delay ensures complete longitudinal relaxation of the magnetization of imino protons to equilibrium (for example, for the imino proton in the AT_6 base pair of the 12-mer DNA, the longitudinal relaxation times are 0.30 ± 0.03 s in 90% H_2O/10% D_2O and 0.44 ± 0.03 s in 50% H_2O/50% D_2O). The ^1H-^1H NOESY experiments in H_2O were carried out at a mixing time of 100 ms using a WATERGATE pulse sequence.[23]

The exchange rates of imino protons were measured by transfer of magnetization from water. The water proton resonance was selectively inverted using a Gaussian 180° pulse (5.8 ms) followed by a variable delay for the exchange of magnetization between water and imino protons. A gradient of 0.21 G/cm was applied during the exchange delay to prevent the effects of radiation damping upon the recovery of water magnetization to equilibrium. At the end of the relaxation delay, a second Gaussian pulse on water was applied to bring the water magnetization back on the *z* axis. The observation was with the Jump-and-Return pulse sequence.[22] Twenty-four values of the exchange delay in the range from 2 to 600 ms were used in each experiment. The exchange rates were calculated from the dependence of the intensity of the imino proton resonance (I) on the exchange delay (t) using the following equation:[14]

$$I(t) = I^0 + [I(0) - I^0 - A] \times e^{-(R_1 + k_{ex}) \times t} + A \times e^{-R_{1w} \times t} \quad (1)$$

where I^0 is the equilibrium intensity, $I(0)$ is the intensity immediately after the first selective pulse on water, k_{ex} is the exchange rate, and R_1 is the longitudinal relaxation rate of the observed proton. The factor A is defined as

$$A = \left[\frac{I_w(0)}{I_w^0} - 1\right] \times \frac{k_{ex}}{R_1 + k_{ex} - R_{1w}} \times I^0 \quad (2)$$

where $I_w(0)$ and I_w^0 are the intensities of the water proton resonance after the inversion pulse and at equilibrium, respectively, and R_{1w} is the longitudinal relaxation rate of water protons. $I_w(0)$, I_w^0, and R_{1w} were measured in separate experiments. The exchange rate of each imino proton was obtained by fitting the intensity of the corresponding resonance to Eq. (1) using a nonlinear least-squares program.

Determination of H-D Fractionation Factors

For each molecule investigated two samples of equal DNA concentrations were used. One sample was in H_2O and the other was in D_2O. Various [D]/[H] ratios in the range from 0 to 1 were obtained by adding appropriate volumes of the D_2O sample to the sample in H_2O. For the range of [D]/[H] ratios from 1 to 4, appropriate volumes of the H_2O sample were added to the sample in D_2O. The [D]/[H] ratios were calculated from the corresponding volumes. The ratios were corrected for the amount of residual water in the D_2O sample, which was measured by NMR. No correction was made for the density difference between H_2O and D_2O because this difference affects the calculated [D]/[H] ratios by only 0.4–0.5%.

The intensity of each imino proton resonance was measured from the 1D NMR spectra at each [D]/[H] ratio by integration using Varian software. The intensity was normalized to the intensity of a thymine methyl resonance in each spectrum. To calculate the fractionation factor (Φ) of each imino proton the resonance intensity (I) was fitted as a function of the [D]/[H] ratio to the following equations:[24]

$$\frac{1}{I} = \frac{1}{I_0} + \frac{\Phi}{I_0} \times \frac{[D]}{[H]} \quad (3)$$

$$I = \frac{I_0}{1 + \Phi \times \frac{[D]}{[H]}} \quad (4)$$

where I_0 is the intensity of the resonance in 100% H_2O. The Φ values obtained from the fits to Eq. (3) were, within experimental errors, the same as those obtained from the fit to Eq. (4).

Analysis of Imino Proton Exchange Data

The general kinetic equation for exchange of imino protons in DNA is[14,25,26]:

$$k_{ex} = \frac{k_{op} \times k_{ex,open}}{k_{cl} + k_{ex,open}} \quad (5)$$

where k_{ex} is the measured exchange rate, k_{op} and k_{cl} are the rates of opening and closing, respectively, of the base containing the imino proton, and $k_{ex,open}$ is the rate of exchange from the open state. The rate of exchange from the open state depends on the concentration of Tris base B, which is the acceptor of the imino proton in our experiments. This dependence is:

$$k_{ex,open} = \alpha \times k_B \times [B] + k_{aac} \quad (6)$$

where k_B is the rate constant for the transfer of the imino proton to Tris base in isolated nucleotides, α is a factor that accounts for differences in proton transfer rate between isolated nucleotides and

open base pairs, and k_{aac} is the rate of exchange from the open state in the absence of added catalyst. The rate constant for proton transfer to Tris was calculated according to the analysis of Benight et al.[27] as 9.8×10^6 M^{-1} s^{-1} for guanine and 3.1×10^7 $M^{-1}s^{-1}$ for thymine at 15°C. The factor α was assumed to have a value of 1.[28] The concentration of Tris base was calculated from the total Tris concentration C_0 and the pH as:

$$[B] = C_0 \times \frac{10^{-pK}}{10^{-pH} + 10^{-pK}} \quad (7)$$

The pH was measured at each Tris concentration, directly in the NMR tube, using the proton resonances of triethanolamine.[15] The pK value of Tris at 15°C was measured by NMR and a value of 8.55 was found.

The exchange rates measured experimentally were fitted to Eq. (5) with $k_{ex,open}$ expressed as in Eq. (6). The fits provided the rates of opening and closing, k_{op} and k_{cl}, of each base pair in the two DNA molecules investigated. The equilibrium constant for opening of the base pair was calculated as:

$$K_{op} = \frac{k_{op}}{k_{cl}} \quad (8)$$

The equilibrium constants K_{op} can also be directly calculated from the exchange rates at low concentrations of Tris base. Under these conditions, $k_{ex,open} \ll k_{cl}$, and Eq. (5) can be approximated as:

$$k_{ex} = K_{op} \times k_{ex,open} = K_{op} \times (\alpha \times k_B \times [B] + k_{aac}) \quad (9)$$

Hence, at low Tris base concentrations, the exchange rate depends linearly on the concentration of Tris base, and the slope of the line provides the equilibrium constant K_{op}.

RESULTS

The NMR resonances of imino protons in the two DNA molecules investigated (namely, N_1—H from guanines and N_3—H from thymines) are shown in Figure 2. Since the base sequences are palindromic, five resonances are expected for the 10-mer DNA and six resonances are expected for the 12-mer DNA. However, the resonances from the terminal base pairs are not observed because, at 15°C, they are broadened by fraying at the ends of the helix. The resonances of the 10-mer DNA were assigned to specific imino protons using ^{1}H-^{1}H NOESY spectra (not shown). The assignments of the imino proton resonances in the 12-mer DNA were previously obtained by this laboratory.[19]

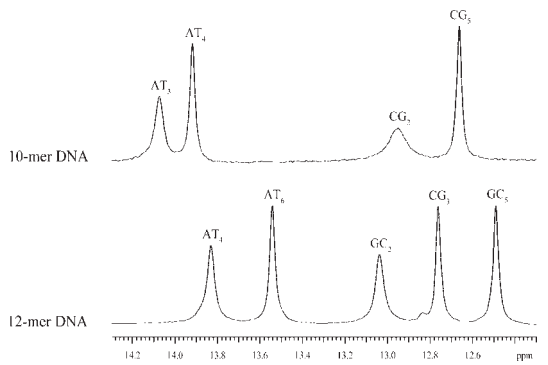

FIGURE 2 NMR resonances of imino protons in the DNA molecules investigated in 50 m*M* Tris buffer at 15°C.

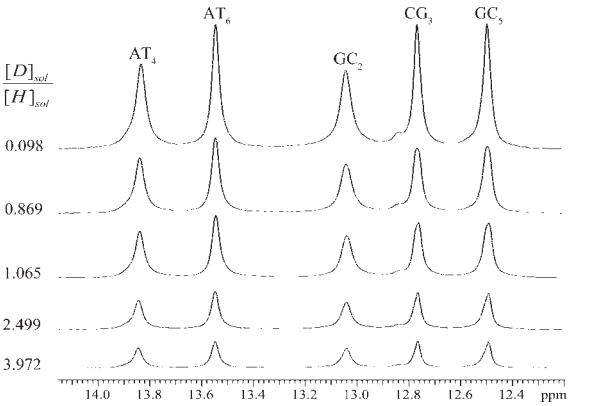

FIGURE 3 The imino proton resonances of the 12-mer DNA at various [D]/[H] ratios in the solvent, in 50 m*M* Tris buffer at pH 8.58 and at 15°C.

The measurements of H-D fractionation factors are illustrated in Figures 3 and 4. Figure 3 shows the dependence of the imino proton resonances of the 12-mer DNA on the [D]/[H] ratio in the solvent. The calculations of fractionation factors are illustrated in Figure 4 for the CG_5 imino proton in the 10-mer DNA. The fit of the resonance intensity to either Eqs. (3) or (4) gave a fractionation factor of 0.95 ± 0.07 for this proton. The fractionation factors for the other imino protons are reported in Table I.

The results of the imino proton exchange measurements are summarized in Figures 5 and 6. For the penultimate base pairs (i.e., CG_2), the exchange rates could not be measured because fraying at the ends of the duplex increases them above ~100 s^{-1}. This is the upper limit for the exchange rates that can be measured by the method of transfer of magnetization from water.[14] For two other imino protons, namely, AT_3 in the 10-mer DNA and AT_4 in the 12-mer DNA, the exchange rates reach a value of ~100 s^{-1} at Tris base concentrations of 0.2 m*M* or less (Figures 5 and 6). Therefore, for these two imino protons, the equilibrium constant of the opening reaction K_{op} was obtained by fitting the data to Eq. (9). The K_{op} values for the remaining base pairs were calculated from Eq. (8), using k_{op} and k_{cl} obtained from fits to Eq. (5) [with $k_{ex,open}$ expressed as in Eq. (6)]. The results are summarized in Table I.

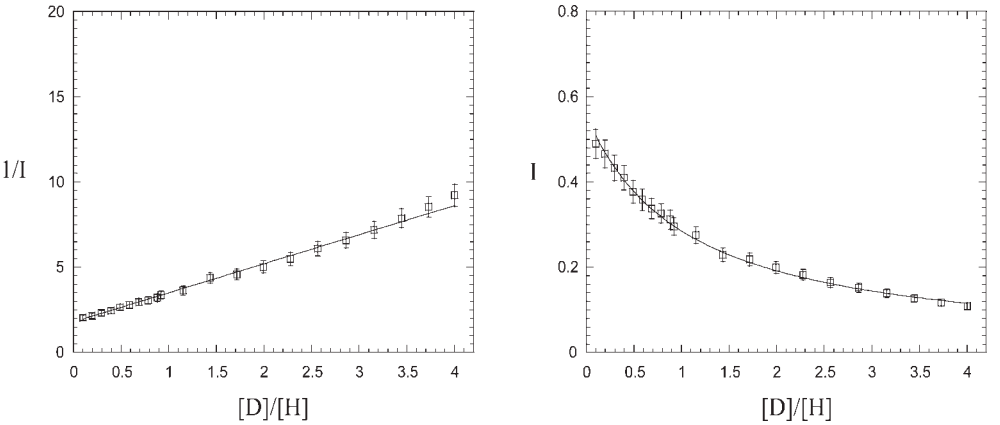

FIGURE 4 Dependence of the intensity of imino proton resonance from CG_5 base pair in the 10-mer DNA on the [D]/[H] ratio. In the left panel, the data are fitted to Eq. (3). In the right panel the data are fitted to Eq. (4).

DISCUSSION

H-D Fractionation Factors and Strength of Base-Pairing Hydrogen Bonds

The fractionation factor of a N-H site in DNA (Φ) represents the equilibrium constant of the following isotope exchange reaction:

$$N—H + D \rightleftarrows N—D + H$$

where D and H are the bulk deuterium and protium in the solvent. A Φ value less than 1 reflects a preference of the site for protium over deuterium. This is generally observed when the hydrogen bond involving the N—H group is stronger than hydrogen bonds in the solvent.[29–31] When the Φ value is 1, there is no preference for protium over deuterium since the hydrogen bond at the N—H site is as strong as those in the solvent.

The fractionation factors determined in the present work provide a measure of the strength of the hydrogen bonds formed by imino groups on the nucleobases, namely, the $N_1—H\cdots N_3$ hydrogen bond in GC/CG base pairs, and the $N_3—H\cdots N_1$ hydrogen bond in AT/TA base pairs (Figure 1). The measured Φ values span a narrow range from 0.92 to 1 (Table I), indicating that the strength of the N—H···N hydrogen bonds in these duplexes is comparable to that of hydrogen bonds in water. No significant differences in the Φ values between different base pairs are observed. Hence, these results suggest that the strength of N—H···N hydrogen bonds does not depend on the nature of the base pair or on sequence context.

A dependence of the strength of the N—H···N hydrogen bond on the nature of the base pair has been recently proposed by a theoretical study using compliance constraints.[32] The results of this study predicted that the N—H···N hydrogen bond in a GC base pair should be twice as strong as the

Table I Fractionation Factors for Imino Protons and Base-Pair Opening Parameters in the Two DNA Duplexes Investigated at 15°C

DNA	Base Pair	Φ	K_{op}	ΔG_{op}[a]	k_{op} (s^{-1})
10-mer	CG_2	0.92 ± 0.07	[b]	[b]	[b]
10-mer	AT_3	0.99 ± 0.07	$(1.171 \pm 0.009) \times 10^{-4}$	5.165 ± 0.004	[c]
10-mer	AT_4	0.99 ± 0.07	$(3.86 \pm 0.08) \times 10^{-5}$	5.80 ± 0.01	68.0 ± 0.8
10-mer	CG_5	0.95 ± 0.07	$(3.0 \pm 0.3) \times 10^{-7}$	8.60 ± 0.06	7.0 ± 0.3
12-mer	GC_2	0.98 ± 0.07	[b]	[b]	[b]
12-mer	CG_3	1.00 ± 0.07	$(7.5 \pm 0.8) \times 10^{-7}$	8.05 ± 0.06	16.4 ± 0.8
12-mer	AT_4	0.96 ± 0.07	$(6.34 \pm 0.03) \times 10^{-5}$	5.515 ± 0.003	[c]
12-mer	GC_5	0.98 ± 0.07	$(2.3 \pm 0.2) \times 10^{-7}$	8.70 ± 0.05	3.9 ± 0.2
12-mer	AT_6	0.93 ± 0.07	$(1.54 \pm 0.04) \times 10^{-5}$	6.30 ± 0.02	77 ± 1

[a] In kcal/mol at 15°C.
[b] The exchange of the imino proton is too fast to be measurable by NMR.
[c] The exchange rate at high concentration of Tris base is too fast to be measurable by NMR (Figures 5 and 6).

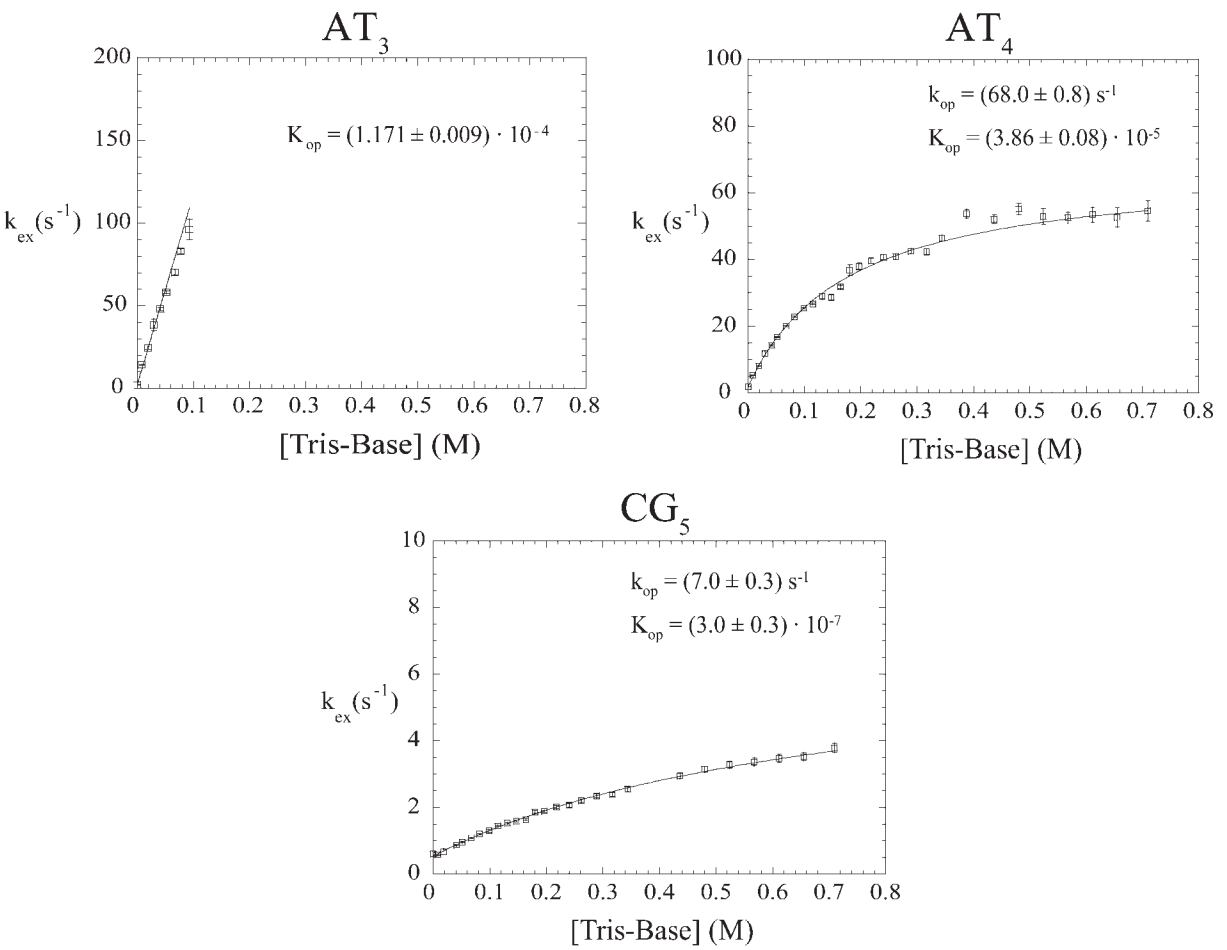

FIGURE 5 Dependence of imino proton exchange rates on the concentration of Tris base in the 10-mer DNA at 15°C. The curves represent nonlinear least-squares fits to Eq. (5) [with $k_{ex,open}$ expressed as in Eq. (6)]. For AT_3, the data were fitted to Eq. (9). The k_{op} and K_{op} values were obtained as described in *Methods*.

N—H · · · N hydrogen bond in a AT base pair. Our investigation does not support this theoretical prediction since the Φ values for GC/CG base pairs do not differ significantly from those for AT/TA base pairs (Table I). Variations in the strength of N—H · · · N hydrogen bonds induced by the base sequence have been observed previously from isotopic shifts of adenine $^{13}C_2$ NMR resonance and from one-bond $^{15}N—^{1}H$ J-coupling constants.[33] According to this previous study, the N—H · · · N hydrogen bonds may be the strongest in polypyrimidine-polypurine tracts of at least four base pairs. The DNA duplexes investigated in the present work contain only short polypyrimidine-polypurine tracts, for example, 5′-AA-3′/3′-TT-5′ in the 10-mer DNA, and 5′-AGA-3′/3′-TCT-5′ in the 12-mer DNA. The length of these tracts may not be sufficient to induce changes in the base-pairing hydrogen bonds that can be detected by H-D fractionation factors.

Fractionation factors of N—H · · · N hydrogen bonds have been previously measured by this laboratory for the intramolecular Y · RY triple helix formed by the DNA oligonucleotide 5′-AGAGAGAACCCCTTCTCTCTTTTTCTCTCTT-3′.[34] The fractionation factors in the triple helix are lower than those measured in the present work for DNA duplexes, namely, they range from 0.6 to 0.8 for most Watson–Crick and Hoogsteen imino protons. These values suggest that the strength of N—H · · · N hydrogen bonds in this triple helical structure is enhanced relative to that in the two DNA duplexes. This enhancement could result from the binding of the third "strand" in the major groove of the DNA double-helical structure. The enhancement may also be due to the presence of long polypyrimidine and polypurine tracts in this Y · RY triple helix, as previously suggested for DNA duplexes containing such tracts.[33]

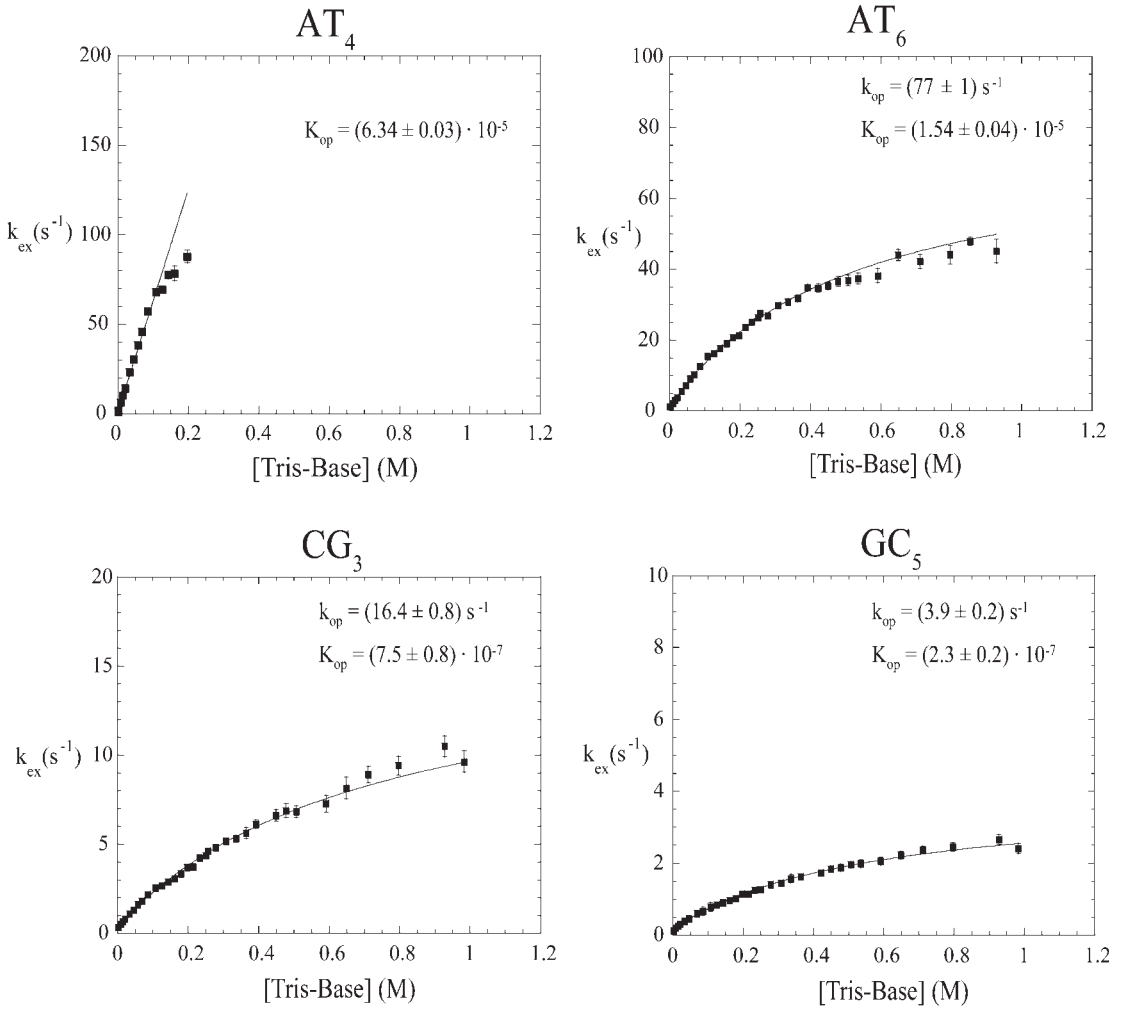

FIGURE 6 Dependence of imino proton exchange rates on the concentration of Tris base in the 12-mer DNA at 15°C. The curves represent nonlinear least-squares fits to Eq. (5) [with $k_{ex,open}$ expressed as in Eq. (6)]. For AT_4, the data were fitted to Eq. (9). The k_{op} and K_{op} values were obtained as described in *Methods*.

Imino Proton Exchange and Stabilities of Individual Base Pairs

The exchange of imino protons in DNA occurs by a conformational transition of each base pair from its native, closed state to an open state. In this transition, the base-pairing hydrogen bonds, including that at the imino group, break. In the open state of the base pair the imino proton is accessible to the acceptor present in solution (i.e., Tris base in the present experiments), and thus can exchange. The equilibrium constant of the opening reaction, K_{op} [Eq. (8)] is related to the free energy change in the reaction as:

$$\Delta G_{op} = -R \times T \times \ln K_{op} \qquad (10)$$

where T is the absolute temperature and R is the gas constant. The free energy ΔG_{op} provides a measure of the structural stability of the base pair in its native conformation: the larger ΔG_{op} is, the higher the stability of the base pair.

Our present results show that the equilibrium constants K_{op} in the DNA duplexes investigated vary from 2.3×10^{-7} to 1.2×10^{-4} (Table I). Accordingly, the corresponding opening free energies range from 5.2 to 8.7 kcal/mol (at 15°C). These variations result from both, the nature and the sequence context of the corresponding base pair. Overall, the ΔG_{op} values for GC/CG base pairs are all higher than 8 kcal/mol while, for AT/TA base pairs, they range from 5.2 to 6.3 kcal/mol. Hence, the structural stabilities of GC/CG base pairs are higher than those of AT/TA base pairs. Similar trends have been observed by this laboratory in other DNA duplexes, such as, DNA unwinding elements,[16] and TATA-box DNA.[15] For the same type of base pair, ΔG_{op} values vary depending on neighboring base pairs. For example, among

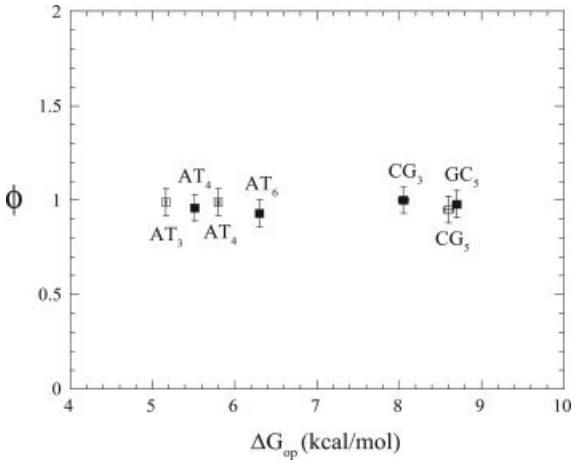

FIGURE 7 A plot of the fractionation factor (Φ) *vs.* the opening free energy for each base pair in the DNA duplexes investigated. Open symbols are for the 10-mer DNA and filled symbols are for the 12-mer DNA.

AT/TA base pairs in the 12-mer DNA, the stability of the AT_4 base pair is ~0.8 kcal/mol lower than that of the AT_6 base pair. This difference is most likely due to the stacking energies at the 5′-CA-3′/3′-GT-5′ and 5′-AG-3′/3′-TC-5′ steps being lower than those at the 5′-GA-3′/3′-CT-5′ and 5′-AT-3′/3′-TA-5′ steps.[35]

Correlation Between the Strength of Inter-Base Hydrogen Bonds and the Stabilities of Individual Base Pairs

A summary of our results on H-D fractionation factors and base-pair opening free energies in the two DNA duplexes investigated is presented in a graphical form in Figure 7. While the fractionation factors, and thus the strength of the N—H · · · N hydrogen bonds, do not change significantly among different base pairs, the ΔG_{op} values vary by as much as 3.5 kcal/mol (Table I). The implication of these results is that the observed variations in structural stabilities among different base pairs are not accompanied by changes in the strength of N—H · · · N hydrogen bonds. This lack of correlation between hydrogen bond strength and base-pair stability does not support theoretical predictions that stacking interactions affect the hydrogen bonding capacity of DNA base pairs.[13] Instead, our results suggest that the variations in stability within the same type of base pairs, i.e., AT/TA or GC/CG, are due to different stacking interactions between the observed base pair and its neighbors. The N—H · · · N hydrogen bonds do contribute, however, to the overall difference in stability between GC/CG and AT/TA base pairs. As our results show, GC/CG base pairs are ~2.4–2.8 kcal/mol more stable than AT/TA base pairs (Table I and Figure 7). This increase is most likely due to the additional inter-base hydrogen bond present in GC/CG base pairs. The general validity of these suggestions awaits similar investigations of other DNA duplexes of different lengths and base sequences.

In the present work we have measured the fractionation factors for the central N—H · · · N hydrogen bonds in each base pair of the two DNA duplexes. These hydrogen bonds were selected because their proton resonances are well separated in the NMR spectra (Figure 2). Observation of the other hydrogen bonds in each base pair, i.e., N—H · · · O, is difficult due to the overlap of their resonances with aromatic proton resonances of each duplex (results not shown). In addition, these N—H · · · O resonances are also broadened by the rotation of the amino group around the exocyclic N—C bond.[36] Because of these experimental limitations we were not able to measure fractionation factors for amino-group N—H · · · O hydrogen bonds. Therefore, at the present time, a dependence of the strength of these hydrogen bonds on the nature and the sequence context of the base pair cannot be excluded.

The DNA duplexes studied in the present work are representative for the B-form family of DNA structures. This fact is indicated by the high-resolution crystallographic structure of the 10-mer DNA[18] and by our NMR structural characterization of the 12-mer DNA.[19,20] For other base sequences, the DNA structure can deviate significantly from the canonical B-form structure. It is possible that such changes in structure may affect the strength of the N—H · · · N hydrogen bonds and its correlation with base-pair stabilities. Investigation of some of these DNA duplexes is currently in progress in our laboratory.

We thank Dr. Daniel Coman for his help in the measurements of fractionation factors.

REFERENCES

1. Saenger, W. Principles of Nucleic Acid Structure; Springer-Verlag: New York, 1983.
2. Marmur, M. N.; Doty, P. J Mol Biol 1962, 5, 109–118.
3. Breslauer, K. J.; Frank, R.; Blocker, H.; Marky, L. A. Proc Natl Acad Sci USA 1986, 83, 3746–3750.
4. SantaLucia, J. J.; Allawi, H. T.; Seneviratne, P. A. Biochemistry 1996, 35, 3555–3562.
5. Sugimoto, N.; Nakano, S.; Yoneyama, M.; Honda, K. Nucleic Acids Res 1996, 24, 4501–4505.
6. Doktycz, M. J.; Goldstein, R. F.; Paner, T. M.; Gallo, F. J.; Benight, A. S. Biopolymers 1992, 32, 849–864.
7. Doktycz, M. J.; Morris, M. D.; Dormady, S. J.; Beattie, K. L.; Jacobson, K. B. J Biol Chem 1995, 270, 8439–8445.
8. SantaLucia, J. J. Proc Natl Acad Sci USA 1998, 95, 1460–1465.
9. Chalikian, T. V.; Volker, J.; Plum, G. E.; Breslauer, K. J. Proc Natl Acad Sci USA 1999, 96, 7853–7858.

10. Kool, E. T. Chem Rev 1997, 97, 1473–1487.
11. Holbrook, J. A.; Capp, M. W.; Saecker, R. M.; Record, M. T. J. Biochemistry 1999, 38, 8409–8422.
12. Yakovchuk, P.; Protozanova, E.; Frank-Kamenetskii, M. D. Nucleic Acids Res 2006, 34, 564–574.
13. Mignon, P.; Loverix, S.; Steyaert, J.; Geerlings, P. Nucleic Acids Res 2005, 33, 1779–1789.
14. Russu, I. M. Methods Enzymol 2004, 379, 152–175.
15. Chen, C.; Russu, I. M. Biophys J 2004, 87, 2545–2551.
16. Coman, D.; Russu, I. M. J Biol Chem 2005, 280, 20216–20221.
17. Coman, D.; Russu, I. M. Biophys J 2005, 89, 3285–3292.
18. Chiu, T. K.; Dickerson, R. E. J Mol Biol 2000, 301, 915–945.
19. Folta-Stogniew, E. J.; Russu, I. M. Biochemistry 1994, 33, 11016–11024.
20. Folta-Stogniew, E.; Russu, I. M. Biochemistry 1996, 35, 8439–8449.
21. Griffiths, K. K.; Russu, I. M. J Biomol Struct Dyn 2006, 23, 667–676.
22. Plateau, P.; Gueron, M. J Am Chem Soc 1982, 104, 7310–7311.
23. Lippens, G.; Dhalluin, C.; Wieruszeski, J. M. J Biomol NMR 1995, 5, 327–331.
24. Loh, S. N.; Markley, J. L. In Techniques in Protein Chemistry; Angeletti, R., Ed.; Academic Press: San Diego, CA, 1993; pp 517–524.
25. Englander, S. W.; Kallenbach, N. R. Q Rev Biophys 1984, 16, 521–655.
26. Gueron, M.; Leroy, J.-L. Methods Enzymol 1995, 261, 383–413.
27. Benight, A. S.; Schurr, J. M.; Flynn, P. F.; Reid, B. R.; Wemmer, D. E. J Mol Biol 1988, 200, 377–399.
28. Gueron, M.; Charretier, E.; Hagerhorst, J.; Kochoyan, M.; Leroy, J. L.; Moraillon, A. In Structure and Methods: DNA and RNA, Vol. 3; Sarma, R. H.; Sarma, M. H., Eds; Adenine Press: New York, NY, 1990; pp 113–137.
29. Edison, A. S.; Weinhold, F.; Markley, J. L. J Am Chem Soc 1995, 117, 9619–9624.
30. Harris, T. K.; Mildvan, A. S. Proteins: Struct Funct Genet 1999, 35, 275–282.
31. Loh, S. N.; Markley, J. L. Biochemistry 1994, 33, 1029–1036.
32. Grunenberg, J. J Am Chem Soc 2004, 126, 16310–16311.
33. Manalo, M. N.; Kong, X.; LiWang, A. J Am Chem Soc 2005, 127, 17974–17975.
34. Coman, D.; Russu, I. M. J Am Chem Soc 2003, 125, 6626–6627.
35. SantaLucia, J. J.; Hicks, D. Annu Rev Biophys Biomol Struct 2004, 33, 415–440.
36. Michalczyk, R.; Russu, I. M. Biophys J 1999, 76, 2679–2686.

Reviewing Editor: David Wemmer