Internal Dynamics in a DNA Triple Helix Probed by ^{1}H-^{15}N-NMR Spectroscopy

Lihong Jiang* and Irina M. Russu†
Departments of *Molecular Biology and Biochemistry and †Chemistry, Molecular Biophysics Program, Wesleyan University, Middletown, Connecticut 06459 USA

ABSTRACT The amino group of adenine plays a key role in maintaining DNA triple helical structures, being the only functional group in DNA that is involved in both Watson-Crick and Hoogsteen hydrogen bonds. In the present work we have probed the internal dynamics of the adenine amino group in the intramolecular YRY triple helix formed by the 31-mer DNA oligonucleotide d(AGAGAGAACCCCTTCTCTCTTTTTCTCTCTT). The DNA triple helix was specifically labeled with ^{15}N at the amino group of the adenine in the fifth position. The rotation rate of the labeled amino group was measured as a function of temperature using ^{1}H-^{15}N heteronuclear NMR spectroscopy. The results indicate that, in the DNA triple helix, the rotation of the adenine amino group is greatly slowed relative to that in a DNA double helix. The temperature dependence of the rotation rate suggests a large entropic contribution to this effect, which may originate from different hydration patterns of the adenine amino group in the two structures.

INTRODUCTION

Triple-helical DNA structures are of special interest due to their potential applications in biotechnology and molecular medicine (Frank-Kamenetskii and Mirkin, 1995; Plum et al., 1995; Radhakrishnan and Patel, 1994a; Soyfer and Potaman, 1995; Vasquez and Wilson, 1998; Wang and Feigon, 1999). Formation of these structures involves binding of a third DNA strand into the major groove of a DNA double helix. The binding is highly sequence-specific. The specificity resides, in part, in the Hoogsteen basepairing between the nucleotides in the third strand and purines in the double-helical part of the structure. Due to these sequence-specific interactions, triplex-forming oligonucleotides can target unique sites in DNA, and thus inhibit binding of proteins involved in transcriptional regulation in vitro and in vivo (Cooney et al., 1988; Duval-Valentin et al., 1992; Maher et al., 1992; Postel et al., 1991; Young et al., 1991).

Triple-helical DNA structures undergo a variety of internal conformational fluctuations that make significant contributions to their stabilities, for example, wobbling about P—O bonds in the phosphodiester backbone, rotation of exocyclic hydrogen bonding groups, and basepair opening (Cain and Glick, 1998; Laughton and Neidle, 1992; Powell et al., 2001; Weerasinghe et al., 1995). Among the groups participating in these fluctuations, the amino group of adenine is especially important because it is the only functional group in DNA triple helices that is engaged in both Watson-Crick and Hoogsteen hydrogen bonds. In T·AT triads (Fig. 1), the group forms two hydrogen bonds, one in the Watson-Crick AT basepair, and the other in the Hoogsteen T·A basepair. Thus, through these two hydrogen bonds, the amino group anchors the two bases in the pyrimidine strands onto the central purine. In the present work we have probed the conformational dynamics of the adenine amino group using selective ^{15}N-labeling and ^{1}H-^{15}N heteronuclear NMR spectroscopy.

The DNA investigated is an intramolecular triple helix formed by the 31-mer DNA oligonucleotide shown in Fig. 1. Previous NMR studies (Macaya et al., 1992) have shown that, in acidic conditions, the homopyrimidine sequence T_{24} through T_{31} binds in the major groove of the hairpin double helix, parallel to the homopurine sequence A_1 through A_8. The resulting structure belongs to the YRY family of triple helices, and contains four canonical T·AT triplets (T_{26}·A_3T_{18}, T_{28}·A_5T_{16}, T_{30}·A_7T_{14}, and T_{31}·A_8T_{13}) and three canonical C^+·GC triplets (C_{25}^+·G_2C_{19}, C_{27}^+·G_4C_{17}, and C_{29}^+·G_6C_{15}). The first thymine in the third strand (T_{24}) does not form a Hoogsteen pair with A_1, possibly due to the constraints imposed by the three-base loop between the two pyrimidine sequences (Macaya et al., 1992). The dynamics of individual basepairs in the same triple helix has been recently characterized by our laboratory using proton exchange (Powell et al., 2001). In the present work we have labeled the DNA triple helix with ^{15}N at the N6 amino group of the adenine in position 5 (Fig. 1). This site-specific labeling allows observation and characterization of the individual adenine amino group by ^{1}H-^{15}N-NMR methods.

MATERIALS AND METHODS

DNA samples

The DNA oligonucleotide was synthesized using solid-support H-phosphonate chemistry on an automated DNA synthesizer (Applied Biosystems, Foster City, CA). ^{15}N-labeled deoxyadenosine H-phosphonate

Submitted October 10, 2001, and accepted for publication February 6, 2002.

Address reprint requests to Irina M. Russu, 203 Hall-Atwater Laboratories, Middletown, CT 06459-0175. Tel.: 860-685-2428; Fax: 860-685-2211; E-mail: irussu@wesleyan.edu.

0006-3495/02/06/3181/05 $2.00

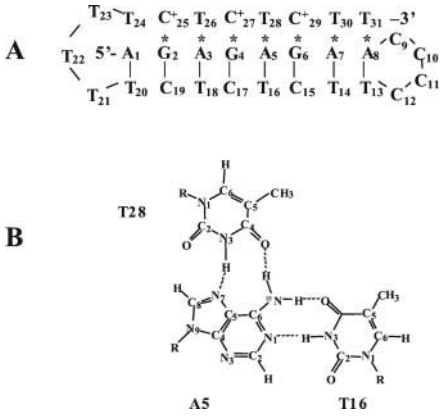

FIGURE 1 (*A*) The DNA triple helix investigated and its folding deduced from NMR data (Macaya et al., 1992). Watson-Crick basepairing is indicated by vertical bars and Hoogsteen hydrogen bonding is indicated by asterisks. (*B*) Canonical T·AT triad. The adenine amino group that was labeled with ^{15}N is indicated.

was synthesized from 6-chloropurine as described previously (Kelly et al., 1995; Michalczyk et al., 1996). The DNA oligonucleotide was purified by reverse-phase HPLC on a PRP-1 column (Hamilton, Reno, NV) in 50 mM triethylamine acetate buffer at pH 7.0 with a gradient of 10 to 20% acetonitrile in 32 min. The counterions were replaced with Na^+ ions by repeated centrifugation through Centricon YM-3 tubes (Amicon, Inc., Bedford, MA). The final samples were in 100 mM NaCl, 5 mM $MgCl_2$ in 90% H_2O/10% D_2O. The pH of the samples before and after the NMR measurements was 5.30 ± 0.05 (at 20°C). The samples contained ~300 $O.D._{260}$ units of DNA.

NMR methods

The NMR experiments were carried out on a Varian INOVA 500 spectrometer operating at 11.75 T and on a Varian VXR 400 spectrometer operating at 9.4 T. Regular, unedited ^1H spectra were obtained using the jump-and-return pulse sequence (Plateau and Gueron, 1982). ^{15}N-edited spectra were obtained using the 1D version of the HSQC with water flip-back pulse sequence (Grzesiek and Bax, 1993). The rates of rotation of the adenine amino group were measured in transfer of magnetization experiments using the pulse sequence that we have previously described (Michalczyk and Russu, 1997). In this pulse sequence, each amino proton resonance is selectively inverted using a rectangular soft pulse (4.6 ms) and, following the delay for transfer of magnetization, the observation is with the 1D version of the HSQC with water flip-back pulse sequence (Grzesiek and Bax, 1993). Thirty-two values of the transfer of magnetization delay, in the range from 0.001 to 4 s, were used in each experiment.

In the transfer of magnetization experiments used, the magnetizations of the two amino protons (labeled A and B) depend on time as (Michalczyk and Russu, 1999):

$$M_{A,B}(t) = M^0_{A,B} + C_{A,B} \cdot \exp(\lambda_1 t) + F_{A,B} \cdot \exp(\lambda_2 t) \quad (1)$$

where $M^0_{A,B}$ are the equilibrium magnetizations, and the constant coefficients $C_{A,B}$ and $F_{A,B}$ depend on the relaxation rates of the two protons and on the initial conditions of the experiment. In the DNA triple helix investigated the longitudinal relaxation rates of the two protons, R_{1A} and R_{1B}, have similar values (e.g., 8.4 and 8.1 s^{-1}, respectively, at 30°C; see below). In this case, the rates λ_1 and λ_2 are (Michalczyk and Russu, 1999):

$$\lambda_1 = -(R_{1A} + R_{1B})/2 - \sigma \quad (2)$$

and

$$\lambda_2 = -(R_{1A} + R_{1B})/2 - 2k_r + \sigma$$

where σ is the rate of cross-relaxation between the two protons and k_r is the rate of rotation of the amino group. For each determination of the rotation rate four sets of experimental data for the magnetization as a function of time were used: one for each inverted proton and the other for the proton receiving the transfer of magnetization. The rates λ_1 and λ_2 were obtained by fitting the four sets of data simultaneously to Eq. 1 with appropriate initial conditions (Michalczyk and Russu, 1999).

The rate of rotation was obtained from the rates λ_1 and λ_2 based on Eqs. 2. The longitudinal relaxation rates of the two amino protons, R_{1A} and R_{1B}, were calculated using inter-proton distances derived from the canonical structure of a YRY triple helix (Protein Data Bank 1AT4). The cross-relaxation rate σ was calculated using an inter-proton distance of 1.75 Å. The overall correlation time of the DNA triple helix was determined from a ^1H-^1H NOESY experiment at 25°C and a magnetic field of 9.4 T. Five mixing times in the range from 0.03 to 0.07 s were used. The initial rates of the NOESY build-up curves for cytosine H5-H6 proton pairs were measured from the intensities of the corresponding crosspeaks normalized to the intensity of diagonal peaks at a mixing time of zero. The obtained cross-relaxation rate (-0.74 ± 0.02 s^{-1}) corresponds to a correlation time of 3.3 ± 0.1 ns at 25°C. The correlation time at other temperatures was calculated from the Stokes-Einstein equation, assuming that the viscosity of the DNA solution depends on temperature, as does that of water (Weast, 1987).

The time-dependence of the magnetizations of the two adenine amino protons in transfer of magnetization experiments can be influenced by the exchange of these protons with solvent. We have measured the exchange rates of the two protons using hydrogen/deuterium exchange (from 1 to 10°C) and ^{15}N-edited experiments of transfer of magnetization from water (at 45°C). The results indicated that, at these temperatures, the exchange rates of the adenine amino protons in the DNA triple helix range from 1×10^{-4} to 0.6 s^{-1}. These values are at least one order of magnitude smaller than the rotation rates (see Results). Therefore, the effect of solvent exchange in rotation rate measurements can be neglected.

RESULTS AND DISCUSSION

The ^1H-NMR spectra of the ^{15}N-labeled DNA triple helix are shown in Fig. 2. In the unedited spectrum, the resonances of the two adenine amino protons overlap resonances from aromatic protons and from other amino protons. In the ^{15}N-edited spectrum two resonances are observed, corresponding to the two amino protons of the adenine in position 5. The chemical shift separation between these two resonances is only 0.39 ppm, as expected from the fact that both protons are involved in hydrogen bonds (Fig. 1).

The rotation rate of the amino group in adenine A5 was measured as a function of temperature, in the temperature range from 30 to 45°C. Selection of this temperature range was dictated by two facts. First, for temperatures lower than 30°C, the rotation rate is too small to be measured accurately in transfer of magnetization exper-

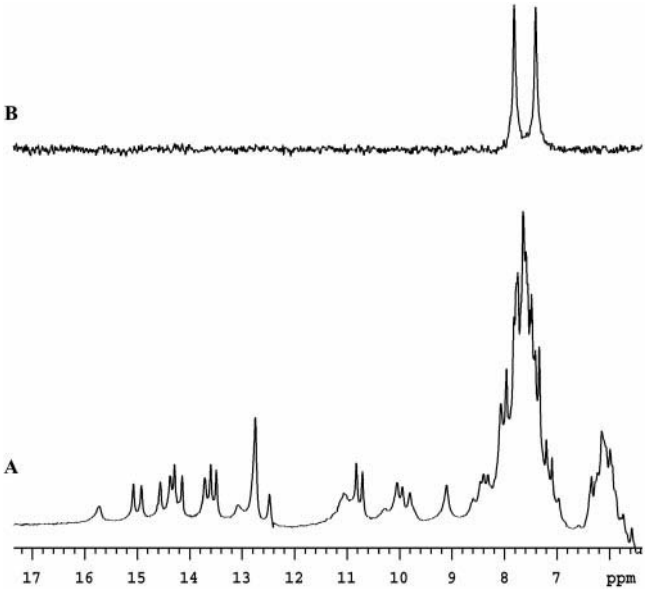

FIGURE 2 (*A*) Expanded region of the 1D ^{1}H-NMR spectrum of the ^{15}N-labeled DNA triple helix. (*B*) Same spectral region in the ^{15}N-edited 1D ^{1}H-NMR spectrum. The vertical scale in (*B*) is ~20 times higher than in (*A*). Solution conditions: 100 mM NaCl, 5 mM $MgCl_2$ in 90% H_2O/10% D_2O at pH 5.3 and at 30°C.

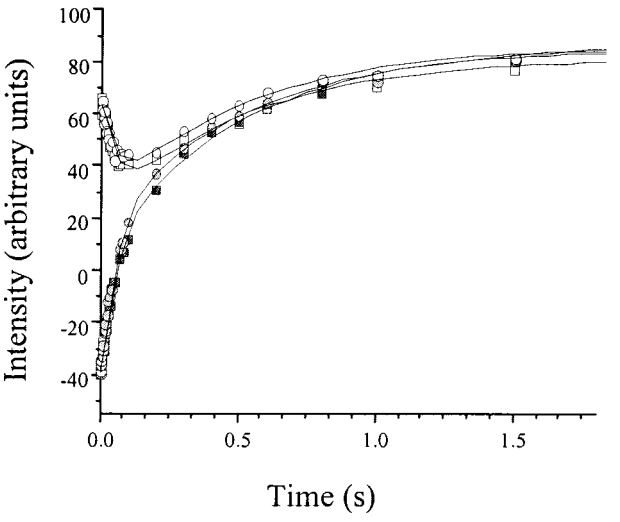

FIGURE 3 Time dependence of the intensities of the two adenine amino proton resonances in transfer of magnetization experiments. For clarity, only the data in the time range from 0.001 and 1.5 s are shown. Filled symbols correspond to the inverted proton, open symbols correspond to the proton receiving the transfer of magnetization. Circles represent the intensity of the upfield resonance, rectangles represent the intensity of the downfield resonance. The curves represent nonlinear least-squares fits to Eq. 1.

iments (i.e., $k_r < \sim 2\ s^{-1}$). Second, the ^{1}H- and ^{15}N-NMR spectra indicated that, for temperatures up to 45°C, the DNA molecule maintains a triple-helical structure, and no other conformations are present in solution. The results from measurements of rotation rates are illustrated in Fig. 3.

The rotation rates were fitted as a function of temperature to the Arrhenius equation:

$$\ln k_r = \ln A - \frac{E_a}{R} \cdot \left(\frac{1}{T}\right) \quad (3)$$

where E_a is the activation energy for rotation and A is the frequency factor (Fig. 4). The activation parameters obtained from this fit are $E_a = (16 \pm 2)$ kcal/mol and $\ln A = 28 \pm 3$.

The rotation of the adenine amino groups in DNA double helices has been previously characterized by our laboratory (Michalczyk and Russu, 1997, 1999). One of the DNA molecules investigated is the double helix formed by the self-complementary 12-mer oligonucleotide 5′-d(CGCGAGCTCGCG)-3′. The molecule contains two structurally equivalent adenines, flanked on both 5′- and 3′-sides by guanines. This is the same sequence context as that of the ^{15}N-labeled adenine in the DNA triple helix investigated (Fig. 1). The rotation rate of the adenine amino groups and its temperature dependence in this DNA double helix are also shown in Fig. 4 (see Note 1 at end of text). Comparison between the two sets of results reveals that, in the DNA triple helix, the rotation of the adenine amino group is greatly slowed relative to that in the DNA double helix. For example, at 45°C, the rotation rate in the triplex is $(6.3 \pm 0.4)\ s^{-1}$ and that in the duplex is $3800\ s^{-1}$. One may have anticipated this result because, in the DNA triple helix, the adenine amino group is involved in the additional Hoogsteen hydrogen bond (Fig. 1). Nevertheless, the activation parameters suggest that this Hoogsteen hydrogen bond does

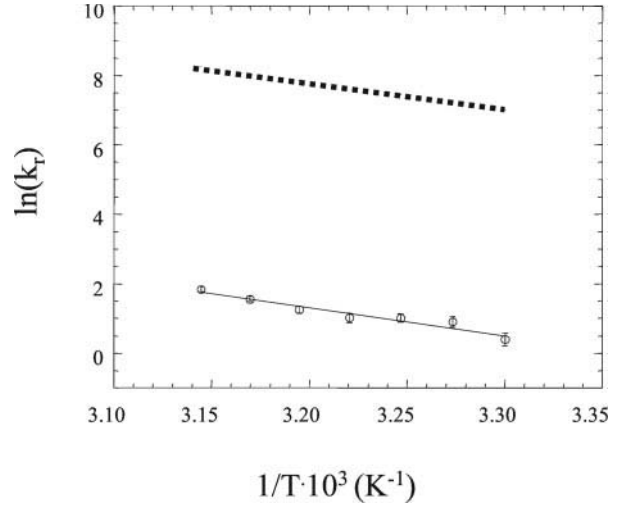

FIGURE 4 Temperature dependence of the rotation rate of the A5 amino group in the DNA triple helix. The full line represents the fit of the data to Eq. 3. The dotted line represents the temperature dependence of the rotation rate of the adenine amino groups in the DNA double helix $[d(CGCGAGCTCGCG)]_2$ (Michalczyk and Russu, 1999).

not make a large enthalpic contribution to the observed effect. In the DNA triple helix, the activation energy for rotation (16 ± 2 kcal/mol) is, within experimental errors, the same as that in the DNA double helix, namely 15.9 ± 0.2 kcal/mol (Michalczyk and Russu, 1999). The difference in rotation rate between the two structures results, in large part, from a difference in the frequency factor A: $\ln A = 28 \pm 3$ in the DNA triple helix, and $\ln A = 33.5 \pm 0.3$ in the DNA double helix. Therefore, a main source for the decrease in the rotation rate in the triple helix is entropic: in this structure, formation of the activated state during rotation involves a smaller change in entropy than that in the double helix. The structural origin of this difference cannot be rigorously described because the nature of the activated state during rotation is not known. However, a qualitative explanation is suggested by hydration patterns of the adenine amino groups in DNA triple and double helices. Analysis of 14 crystallographic structures of DNA double helices in canonical B-form has revealed that the amino group of adenine is one of the main hydration sites (Schneider and Berman, 1995). In the most common hydration pattern, the amino group donates its free hydrogen to a hydrogen bond with water. For DNA triple helices, single-crystal x-ray structures are not available. However, hydration sites have been defined by Patel and co-workers (Radhakrishnan and Patel, 1994b) using homonuclear 2D NMR methods. For YRY triple helices, no hydration sites (i.e., sites with residence times longer than 1 ns) have been detected in the vicinity of adenine amino groups. This result suggests that, in DNA triple helices, the hydration at or near adenine amino groups could be lower or the bound water molecules could be short-lived. In a DNA double helix, rotation of the adenine amino group should break the Watson-Crick hydrogen bond and the hydrogen bond(s) between the amino nitrogen and water molecule(s). This disturbance of the hydration shell should increase the enthalpy and entropy changes for formation of the activated state during rotation. In a DNA triple helix, both Watson-Crick and Hoogsteen hydrogen bonds should break during rotation, thus making the activation energy comparable to that in the double helix. However, for lower hydration, the favorable contribution to the activation entropy from bound water would be less, and the activation entropy would therefore decrease.

In summary, in the present work we have shown that the internal dynamics of the adenine amino group in DNA structures is not a simple function of the number of interbase hydrogen bonds the amino group participates in. In DNA triple helices, formation of the Hoogsteen hydrogen bond to the third strand also affects the rotational dynamics of the adenine amino group through an entropic effect. Similar hydrogen bonds from the adenine amino groups are also involved in the binding of proteins into the major groove of DNA double helices. It will be of interest to determine whether the local energetic changes induced at the adenine amino group by binding of a protein follow a similar pattern to that observed here for binding of a third DNA strand.

NOTES

1. We have attempted to measure the rotation rate of the A5 amino group under experimental conditions in which the DNA 31-mer folds into a duplex hairpin (e.g., pH 8.0 and temperature higher than 20°C). We have found that, as in the other DNA duplexes previously investigated, the fast rotation of the adenine amino group at these temperatures broadens the amino proton resonances beyond detection.

We thank Dr. Ryszard Michalczyk for the synthesis of the ^{15}N-labeled deoxyadenosine H-phosphonate.

This work was supported by a grant from the National Science Foundation.

REFERENCES

Cain, R. J., and G. D. Glick. 1998. Use of cross-links to study the conformational dynamics of triplex DNA. *Biochemistry.* 37:1456–1464.

Cooney, M., G. Czernuszewicz, E. H. Postel, S. J. Flint, and M. E. Hogan. 1988. Site-specific oligonucleotide binding represses transcription of the human c-*myc* gene in vitro. *Science.* 241:456–459.

Duval-Valentin, G., N. T. Thuong, and C. Helene. 1992. Specific inhibition of transcription by triple helix-forming oligonucleotides. *Proc. Natl. Acad. Sci. U.S.A.* 89:504–508.

Frank-Kamenetskii, M. D., and S. M. Mirkin. 1995. Triplex DNA structures. *Annu. Rev. Biochem.* 64:65–95.

Grzesiek, S., and A. Bax. 1993. The importance of not saturating H_2O in protein NMR. Application to selectivity enhancement and NOE measurements. *J. Am. Chem. Soc.* 115:12593–12594.

Kelly, J., D. A. Ashburn, R. Michalczyk, and L. A. Silks. 1995. An improved synthesis of [amino-^{15}N] adenine useful in the large scale synthesis of 2′-deoxy [amino-^{15}N] adenosine. *J. Labelled Compd. Radiopharm.* 36:631–635.

Laughton, C. A., and S. Neidle. 1992. Molecular dynamics simulation of the DNA triplex $d(TC)_5 \cdot d(GA)_5 \cdot d(C^+T)_5$. *J. Mol. Biol.* 223:519–529.

Macaya, R., E. Wang, P. Schultze, V. Sklenar, and J. Feigon. 1992. Proton nuclear magnetic resonance assignments and structural characterization of an intramolecular DNA triplex. *J. Mol. Biol.* 225:755–773.

Maher, L. J., P. B. Dervan, and B. Wold. 1992. Analysis of promoter-specific repression by triple-helical DNA complexes in a eukaryotic cell-free transcription system. *Biochemistry.* 31:70–81.

Michalczyk, R., and I. M. Russu. 1997. Studies of the dynamics of adenine amino protons in DNA by ^{15}N-labeling and heteronuclear NMR spectroscopy. *In* Structure, Motion, Interaction and Expression of Biological Macromolecules. R. Sarma and M. Sarma, editors. Adenine Press, Albany, NY. 181–189.

Michalczyk, R., and I. M. Russu. 1999. Rotational dynamics of adenine amino groups in a DNA double helix. *Biophys. J.* 76:2679–2686.

Michalczyk, R., L. A. Silks, and I. M. Russu. 1996. Heteronuclear ^{15}N and ^{1}H magnetic resonance study of a DNA dodecamer containing an A_3T_3 tract. *Magn. Res. Chem.* 34:S97–S104.

Plateau, P., and M. Gueron. 1982. Exchangeable proton NMR without base-line distortion, using new strong-pulse sequences. *J. Am. Chem. Soc.* 104:7310–7311.

Plum, G. E., D. S. Pilch, S. F. Singleton, and K. J. Breslauer. 1995. Nucleic acid hybridization: triplex stability and energetics. *Annu. Rev. Biophys. Biomol. Struct.* 24:319–350.

Postel, E. H., S. J. Flint, D. J. Kessler, and M. E. Hogan. 1991. Evidence that a triplex-forming oligodeoxyribonucleotide binds to the c-*myc* promoter in HeLa cells, thereby reducing c-*myc* mRNA levels. *Proc. Natl. Acad. Sci. U.S.A.* 88:8227–8231.

Powell, S. W., L. Jiang, and I. M. Russu. 2001. Proton exchange and base-pair opening in a DNA triple helix. *Biochemistry.* 40:11065–11072.

Radhakrishnan, I., and D. J. Patel. 1994a. DNA triplexes: solution structure, hydration sites, energetics, interactions, and function. *Biochemistry.* 33:11405–11416.

Radhakrishnan, I., and D. J. Patel. 1994b. Hydration sites in purine·purine·pyrimidine and pyrimidine·purine·pyrimidine DNA triplexes in aqueous solution. *Structure.* 2:395–405.

Schneider, B., and H. M. Berman. 1995. Hydration of the DNA bases is local. *Biophys. J.* 69:2661–2669.

Soyfer, V. N., and V. N. Potaman. 1995. Triple-Helical Nucleic Acids. Springer, New York.

Vasquez, K. M., and J. H. Wilson. 1998. Triplex-directed modification of genes and gene activities. *Trends Biochem. Sci.* 23:4–9.

Wang, E., and J. Feigon. 1999. Structures of nucleic acid triplexes. *In* Oxford Handbook of Nucleic Acid Structure. S. Neidle, editor. Oxford University Press, New York. 355–388.

Weast, R. C., editor. 1987. CRC Handbook of Chemistry and Physics, 67th Ed. CRC Press, Boca Raton, FL.

Weerasinghe, S., P. E. Smith, V. Mohan, Y.-K. Cheng, and B. M. Pettitt. 1995. Nanosecond dynamics and structure of a model DNA triple helix in saltwater solution. *J. Am. Chem. Soc.* 117:2147–2158.

Young, S. L., S. H. Krawczyk, M. D. Matteucci, and J. J. Toole. 1991. Triple helix formation inhibits transcription elongation in vitro. *Proc. Natl. Acad. Sci. U.S.A.* 88:10023–10026.