DNA with 2′−5′ Phosphodiester Bonds Forms a Duplex Structure in the A-Type Conformation

Howard Robinson,† Kyeong-Eun Jung,‡
Christopher Switzer,*,‡ and Andrew H.-J. Wang*,†

Biophysics Division and
Department of Cell & Structural Biology
University of Illinois at Urbana−Champaign
Urbana, Illinois 61801
Department of Chemistry
University of California at Riverside
Riverside, California 92521

Received September 9, 1994

Present-day genetic material contains a phosphodiester linkage between the O3′ and O5′ atoms. The reason why nature selects the 3′−5′ linkage is not obvious. Template-directed chemical synthesis of RNA has shown that either the 2′−5′ or the 3′−5′ bond may form predominantly, depending on the conditions used.[1,2] Selection of the 3′,5′-linkage may be related to the stability of the molecules. Theoretical studies proposed that 2′,5′-linked RNA does not support duplex structures due to the unfavorable backbone geometry.[3] NMR studies of small dinucleotides seemed to verify this conclusion.[4] More recently, the crystal structure of r(C-2′,5′-A) revealed an unusual parallel-stranded miniduplex, incorporating C^+:C and A:A base pairs.[5] The issue of whether an extended fragment of 2′,5′-linked nucleotides can form a duplex was addressed by the successful synthesis of defined-sequence 2′,5′-linked oligonucleotides.[6−8] The spectroscopic studies of those molecules clearly established that they have well-defined cooperative melting profiles, but lower T_m values than their 3′−5′ counterparts. To gain further insight into the properties of 2′,5′-linked DNA, we undertook a structural analysis of the self-complementary octamer 5′-CGGCGCCG-2′ by NMR refinement.

The H_2O exchangeable spectrum revealed three clear imino proton resonances at 12.53 (G2), 12.95 (G3), and 13.11 (G5) ppm, plus a rapidly exchanging peak at 12.15 (G8) ppm, suggesting Watson−Crick base pairs (Figure 1A).[9] There are several unusual NOE crosspeaks associated with the terminal base pair, including those between G8H2′ and C7NH4 and interstrand G2NH1. The 2D-NOESY and TOCSY spectra in D_2O were used to assign the resonances of all nonexchangeable protons. Figure 1B shows the aromatic-H1′ crosspeak regions of the 2D-NOESY spectra. The uninterrupted sequential crosspeak connectivity of the H6/H8 protons to the H1' protons suggested a helical structure, and their intensities indicated that all nucleotides are in the *anti* conformation, except for the surprising *syn* conformation of the C1 residue.[15]

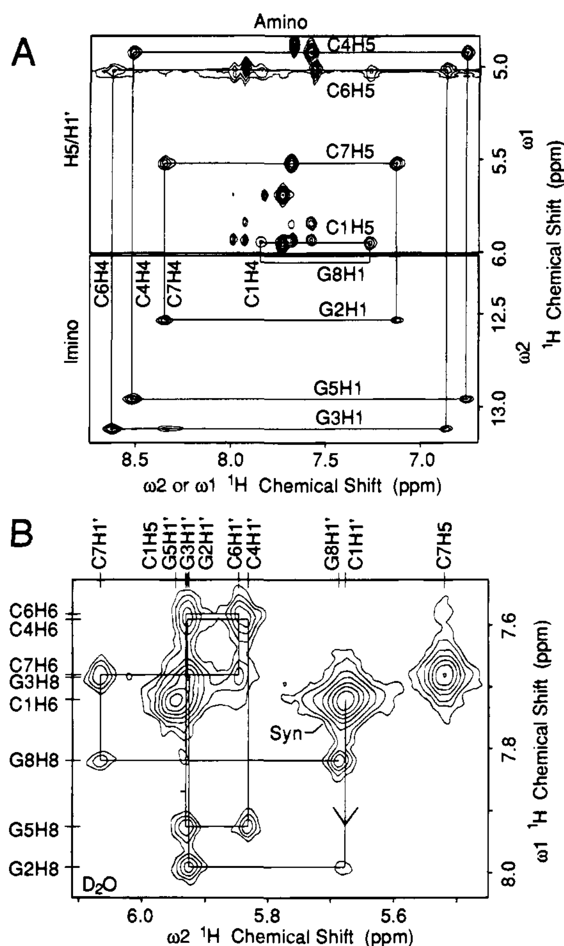

Figure 1. (A) Imino to amino and amino to H1′/H5 regions in the exchangeable spectra. These spectra provide evidence for the Watson−Crick base pairs and the assignment of the exchangeable proton resonances. (B). The expanded aromatic−H1′ region of the nonexchangeable proton 2D-NOESY spectra of the 5′-CGGCGCCG-2′, showing the internucleotide connectivity. The unusually strong C1H1′−C1H6 crosspeak suggests a *syn*-glycosyl conformation for the C1 nucleotide.

† University of Illinois at Urbana−Champaign.
‡ University of California at Riverside.

(1) Abbreviations used: NMR, nuclear magnetic resonance; 2D-NOESY, two-dimensional nuclear Overhauser effect spectroscopy; TOCSY, total-correlated spectroscopy.

(2) For reviews of template-directed RNA synthesis, see: (a) Joyce, G. F. *Cold Spring Harbor Symposia on Quantitative Biology*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1987; Vol. LII, pp 41−52. (b) Orgel, L. E. *Nature* **1992**, *358*, 203−209.
(3) Srinivasan, A. R.; Olson, W. K. *Nucleic Acids Res.* **1986**, *14*, 5461−5479.
(4) Dhingra, M. M.; Sarma, R. H. *Nature* **1978**, *272*, 798−801.
(5) Krishnan, R.; Seshadri, T. P.; Viswamitra, M. A. *Nucleic Acids Res.* **1991**, *19*, 379−384.
(6) Hashimoto, H.; Switzer, C. *J. Am. Chem. Soc.* **1992**, *114*, 6255−6256. Jung, K.-E.; Switzer, C. *J. Am. Chem. Soc.* **1994**, *116*, 6059−6061.
(7) Dougherty, J. P.; Rizzo, C. J.; Breslow, R. *J. Am. Chem. Soc.* **1992**, *114*, 6254−6255. Jin, R. Z.; et al. *Proc. Natl. Acad. Sci. U.S.A* **1993**, *90*, 10568−10572.
(8) Kierzek, R.; He, L.; Turner, D. H. *Nucleic Acids Res.* **1992**, *20*, 1685−1690. Note that the assignment of the imino proton resonances in this paper differs from ours.

(9) Solutions (~0.45 mM duplex with 0.15 M NaCl and 0.05 M phosphate buffer at pH 7.0) of the 2′,5′-linked DNA oligomers were prepared in 0.55 mL of D_2O as described earlier.[10] NMR spectra were collected on a Varian VXR500 500 MHz spectrometer, and the data were processed with FELIX v1.1 (Hare Research, Woodinville, WA). The nonexchangeable 2D-NOE spectra were collected at 2 °C at a mixing time of 150 ms and a total recycle delay of 5.4 s, where the average T_1 relaxation was 2.7 s. The data were collected by the States/TPPI technique with 512 t_1 increments and 2048 t_2 complex points, each the average of 32 transients. Refinement of the starting model was carried out by the sequence of procedures comprising the SPEDREF package.[12] This includes a full-matrix relaxation calculation of the NOEs[13] for the model with comparison of the experimental and simulated spectra to deconvolute overlapped areas of the spectra. Minimization of the residual errors within the program X-PLOR[14] is then performed by low-temperature simulated annealing and conjugate gradient minimization of the NOE derived force springs together with the chemical force field. TOCSY spectra were used together with the NOE spectra to derive the assignment. 2D-NOESY spectra in 90% H_2O were collected with the 1-not-1 pulse sequence as the read pulse of the NOESY. In all, 24 transients were averaged with a recycle delay of 2.9 s and a mixing time of 0.15 s. The excitation offset was set to one-quarter of the spectral bandwidth, which was set to 10 000 Hz so that the imino resonances around 13 ppm were nearly maximally excited.

(10) Robinson, H.; van der Marel, G. A.; van Boom, J. H.; Wang, A. H.-J. *Biochemistry* **1992**, *31*, 10510−10517. Robinson, H.; Wang, A. H.-J. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 5224−5228.
(11) States, D. J.; Haberkorn, R. A.; Ruben, D. J. *J. Magn. Reson.* **1982**, *48*, 286−292.
(12) Robinson, H.; Wang, A. H.-J. *Biochemistry* **1992**, *31*, 3524−3533.

0002-7863/95/1517-0837$09.00/0 © 1995 American Chemical Society

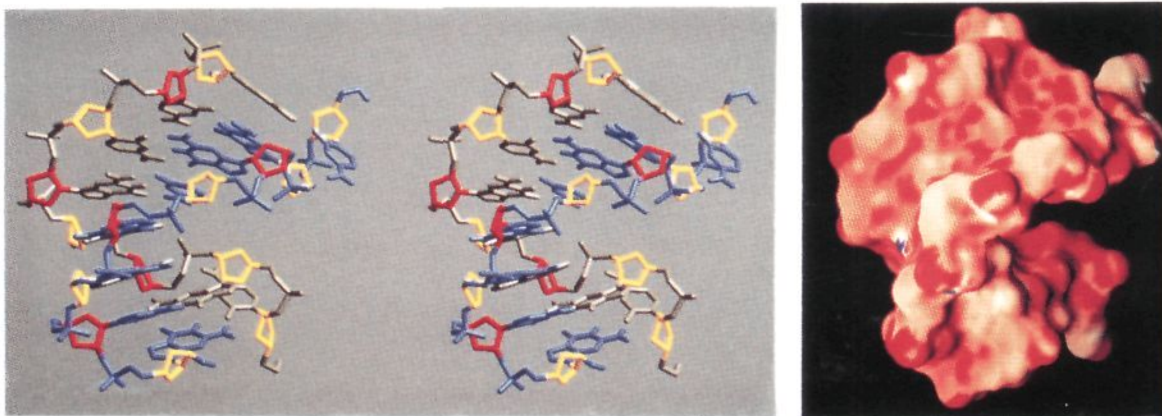

Figure 2. Molecular model of the 5′-CGGCGCCG-2′-refined structure as drawn by the program GRASP.[16] This model represents a consensus of an ensemble of related conformers which fit the NOE data to a similar degree. (A, left) Skeletal model. The sugars are colored according to their puckers (yellow for the N-type and red for the S-type). (B, right) Surface drawing with its electrostatic potential distribution. The large patch of red color indicates that the duplex has a negative surface charge distribution, similar to that of the A-DNA.

The 3D structure was obtained by a combined SPEDREF[12] and NOE-constrained molecular dynamics refinement.[14] Two grossly different starting models were constructed beginning with B-DNA and A-DNA incorporating 2′−5′ linkages and were subsequently energy-minimized. Both refined models converged to an A-like structure (Figure 2A), in which the base pairs have an average *x*-displacement of −4.2 Å (excluding the terminal base pairs). The base pairs are tilted with respect to the helix axis by ~21°. The major groove is narrow and deep, as in the natural A-DNA, but the minor groove is not nearly as broad due to the 2′−5′ linkages, which bring the two anti-parallel backbones closer together (Figure 2B).

The backbone of the central six nucleotides has alternating sugar puckers with the G2, C4, and C6 nucleotides of the N-type (pseudorotation angle $P = 20°$, 48°, and 28°) and the G3, G5, and C7 nucleotides of the S-type ($P = 228°$, 174°, and 101°). This is consistent with the TOCSY data, in which strong H1′−H2′ crosspeaks were observed only for the G2, C4, and C6 nucleotides, but weak crosspeaks were observed for the remaining nucleotides. The helical twist angles for the inner G2pG3, G3pC4, and C4pG5 steps are 38°, 43°, and 40°, respectively. Extensive *inter*strand G5:G5* stacking, but no intrastrand stacking, of the CpG step exists. Interestingly, G2-NH2 forms an *intra*strand hydrogen bond (2.83 Å) to the O4′ of residue G3. The conformation of the terminal base pair (C1:G8*) is dynamic with the *syn*-C paired with the *anti*-G in an ill-defined manner. The highly unusual *syn* conformation of the terminal C1 may relieve strain associated with the stretched CpG step.

The NOE-refined structure of the 2′,5′-linked DNA suggests that 2′,5′-linked RNA is likely to form an A-type duplex as well, since the insertion of O3′ atoms into our model does not cause any steric clashes. Turner and colleagues have studied a 2′,5′-linked RNA of the same sequence, 5′-r(CGGCGCCG)-2′, and their data support a duplex, although no structure was proposed.[8] Our results here point out that the presence of O2′ atoms in nucleic acid, whether they exist as free hydroxyl groups in normal RNA or as part of the backbone as in 2′,5′-linked DNA or RNA, causes the nucleic acid to adopt the A-genus of conformations. Such information may be useful in understanding the role of RNA in the origins of life.[2,6] We observe that 2′,5′-linked DNA inversion recovery T_1 relaxation times of about 3 s are much longer than those of normal DNA (for the nonterminal nucleotides, data not shown). We suppose that these longer T_1 values are due to restriction of ring inversion for the sugar rings caused by the 2′,5′-linkages. This restriction in ring motion may possibly not be suitable for certain vital biological DNA characteristics.

Acknowledgment. This work was supported by a grant from the NIH to A.H.-J.W. (GM-41612) and C.S. (GM-47375) and also by a grant from the National Aeronautics and Space Administration to C.S.

Supplementary Material Available: Three figures showing the experimental and simulated 2D NMR spectra, superposition of two refined models, base pair stacking diagrams, and two tables listing the chemical shifts and torsional angles (7 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JA9429879

(13) Macura, S.; Ernst, E. E. *Mol. Phys.* **1980**, *41*, 95−117. Keepers, J. W.; James, T. L. *J. Magn. Reson.* **1984**, *57*, 404−426.

(14) Brünger, A. *X-plor,* version 3.1; The Howard Hughes Medical Institute and Yale University: New Haven, CT, 1993.

(15) The rare *syn*-pyrimidine conformation has been observed previously only in the Z-DNA with out-of-alternating pyrimidine−purine sequence, see: Wang, A. H.-J.; Gessner, R.; van Boom, J. H.; van der Marel, G. A.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 3611−3615. Feigon, J.; Wang, A. H.-J.; van der Marel, G. A.; van Boom, J. H.; Rich, A. *Science* **1985**, *230*, 82−84.

(16) Nicholls, A. *GRASP,* version 1.1; Columbia University, New York, NY, 1993.