Volume 306, number 2,3, 223–228 FEBS 11309 July 1992


# NMR study of self-paired parallel duplex of d(AAAAACCCCC) in solution

Jia Luo, Mukti H. Sarma, Ren-da Yuan and Ramaswamy H. Sarma

*Institute of Biomolecular Stereodynamics, State University of New York at Albany, NY 12222, USA*

Received 12 May 1992

The oligonucleotide $d(A_5C_5)$ in solution forms a parallel self-duplex at neutral and low pH values. $H_2O$ NMR spectra at pH 5.1 indicate the presence of five imino resonances at lower temperatures; and the structure is stable up to 60°C. These signals can arise only from the hemiprotonated $C^+ \cdot C$ pairs [Westhof, E. and Sundaralingham, M. (1980) Biochemistry 77, 1852–1856; Westhof, E. and Sundaralingham, M. (1980) J. Mol. Biol. 142, 331–361] and constitute the first direct observation of $C^+ \cdot C$ hemiprotonated pairs in solution. The cross peaks from H1's and more than five distinct AH8's in 500 MHz $^1H$ 2D-NOESY spectra indicate that there are two conformationally different and energetically similar A-tracts. There is good qualitative agreement between NOESY data and two theoretically derived structures in which A-tracts are reverse Watson–Crick and reverse Hoogsteen base-paired, respectively.

Oligonucleotide $d(A_5C_5)$; Self-parallel duplex; Hemiprotonated; 2D NMR; Computer modeling

## 1. INTRODUCTION

X-Ray diffraction studies on polyadenylic acid [3] and polycytidylic acid [4] had indicated that, at acidic pH values, they form parallel double helices. X-Ray crystallographic studies on cytidylyl-3′, 5′-adenosine(CpA)-proflavine complex [1,2] and cytidylyl- 2′, 5′-adenosine (C2′p5′A) [5] had indicated that both these dimers, as repeat units, form right-handed parallel double helices with hemiprotonated $C^+ \cdot C$ self-pairs and neutral A–A self-pairs. In addition, other identical $C^+ \cdot C$ base pairings have been reported from X-ray crystallographic studies for d(C3′p5′G) NH4 [6] and d(C3′p5′G) Na [7]. From gel electrophoresis studies a similar $C^+ \cdot C$ base pairing for the (C,A)-hairpin structure in the telomeric sequence $G_4T_2 \cdot A_2C_4$ had been proposed [8]. Adenines forming self base pairs through N6–N7 atoms have also been observed in the X-ray diffraction studies of ApApA [9]. We report here our NMR experimental data and computer modeling study to demonstrate that the decamer, $d(A_5C_5)$, in solution forms a right- handed parallel self-paired duplex in near neutral and low pH (5.1). The cytosines and adenines form self-pairs with three and two hydrogen bonds, respectively. Our results also indicate a possible N1–N6 hydrogen bonding to form a reverse Watson–Crick pair for the A- tracts.

*Correspondence address:* R.H. Sarma, Institute of Biomolecular Stereodynamics, State University of New York at Albany, NY 12222, USA. Fax: (1) (518) 452-4955.

## 2. MATERIALS AND METHODS

The decamer $d(A_5C_5)$ was synthesized on a DNA synthesizer (Applied Biosystems Model 380A) following the method of Matteuci and Caruthers [10]. The products were purified on a 1.1 × 50 cm column of Q-Sepharose (Pharmacia) with a linear gradient of 0.2–0.8 M NaCl in 10 mM NaOH (pH 12.0) and further purified by several ethanol precipitations.

### 2.1. *NMR spectroscopy*

For both $H_2O$ and $D_2O$ samples, the DNA concentration was 200 $A_{260}$ units. The solutions were prepared in 20 mM sodium phosphate buffer (pH 7.0) with 1 mM EDTA. One-dimensional NMR spectra of $d(A_5C_5)$ in $H_2O$ and $D_2O$ were recorded for a temperature range of 1–80°C at pH 5.1, by use of a time-shared long pulse sequence at 500 MHz. The temperature profiles of the imino/amino protons and base protons for $d(A_5C_5)$ in $H_2O$ and $D_2O$, respectively, were measured by equilibrating the sample at each temperature for at least 30 min. The pH titration was performed at 1 and 10°C for a pH range of 2.1–8.0. NOE difference spectra of $d(A_5C_5)$ at 1, 10 and 30°C in $H_2O$ at pH 5.1 and 6.7 were recorded for presaturation times ($\tau_m$) of 250 ms with a relaxation delay (RD) of 2.5 s and 5,000 transients (NS). The NOESY spectra of $d(A_5C_5)$ at 10°C in $D_2O$ were collected using the pulse sequence $[RD\text{-}90°\text{-}t_1\text{-}90°\text{-}\tau_m\text{-}90°\text{-}A_{acq}]_{NS}$ with $\tau_m$ of 250 and 150 ms in the pure absorption mode [11] with RD of 1.8 s. The data matrix (2,048 × 256) for 64 scans was processed to a size of 2K×2K. Two MINSY [12] spectra in $D_2O$ were collected at 10°C under the same conditions with $\tau_m$ of 250 ms, and decoupler irradiating at 2.1 and 2.5 ppm, respectively. The HDO signal was presaturated in MINSY and NOESY experiments.

### 2.2. *Energy minimization and computer modeling*

Using the parallel self-paired, two-fold symmetry, we constructed two $d(A_5C_5)_2$ double helical structures by rotating the strand $d(A_5C_5)$ of the duplex $d(A_5C_5) \cdot d(G_5T_5)$ produced by AMBER [13–15]. The A-tract self-pair bases were adjusted after rotation, based on the X-ray data of CpA-proflavine complex [1,2]. While keeping the $C^+ \cdot C$ hemiprotonated hydrogen bonding pattern unchanged, resembling the X-ray structures [1,2,4,5], the glycosyl torsional angles of the A–A motif were then set to anti (molecule A) and syn (molecule B), respectively,

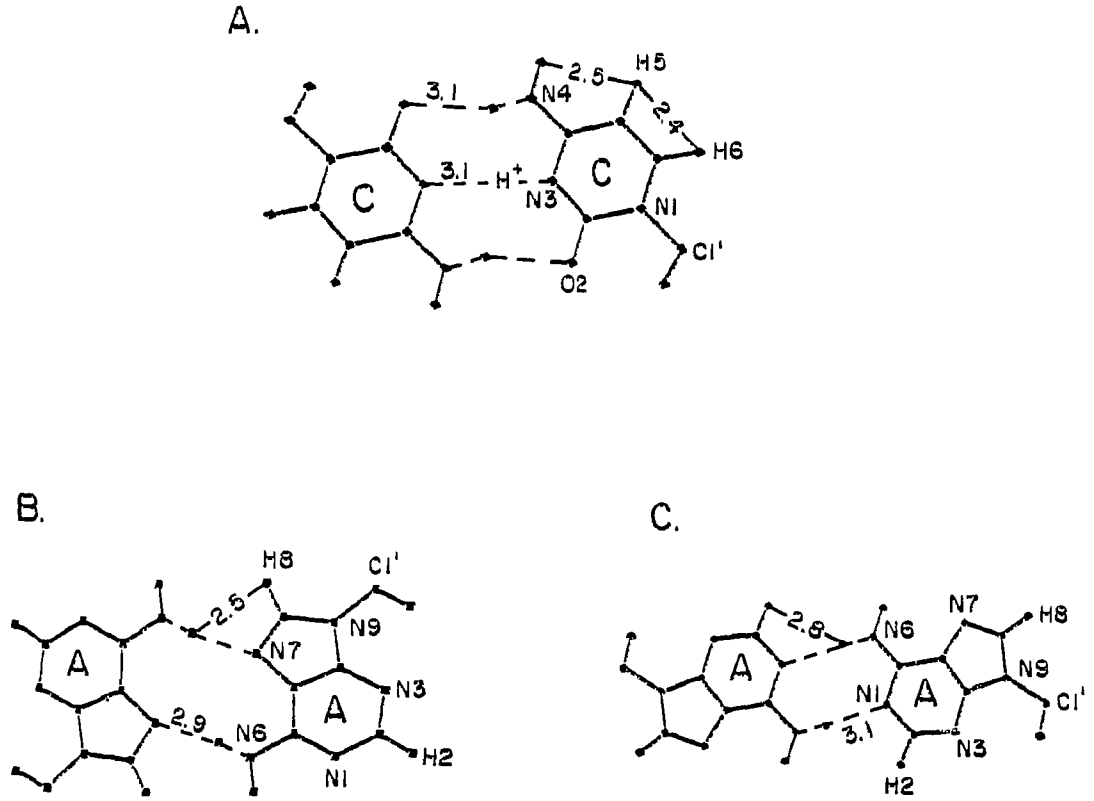


Fig. 1. (A) The scheme of C-tract. (B and C). The possible schemes of A-tract.

based on the indication by the experimental data that there are two conformationally different and energetically similar favored structures co-existing in the solution. The glycosyl torsional angles are anti for the cytosine residues for both molecule A and B. The all-atom structures were energy minimized using AMBER to arrive at two different refined structures, one parallel reverse Hoogsteen self-paired duplex and one parallel reverse Watson–Crick self-paired duplex.

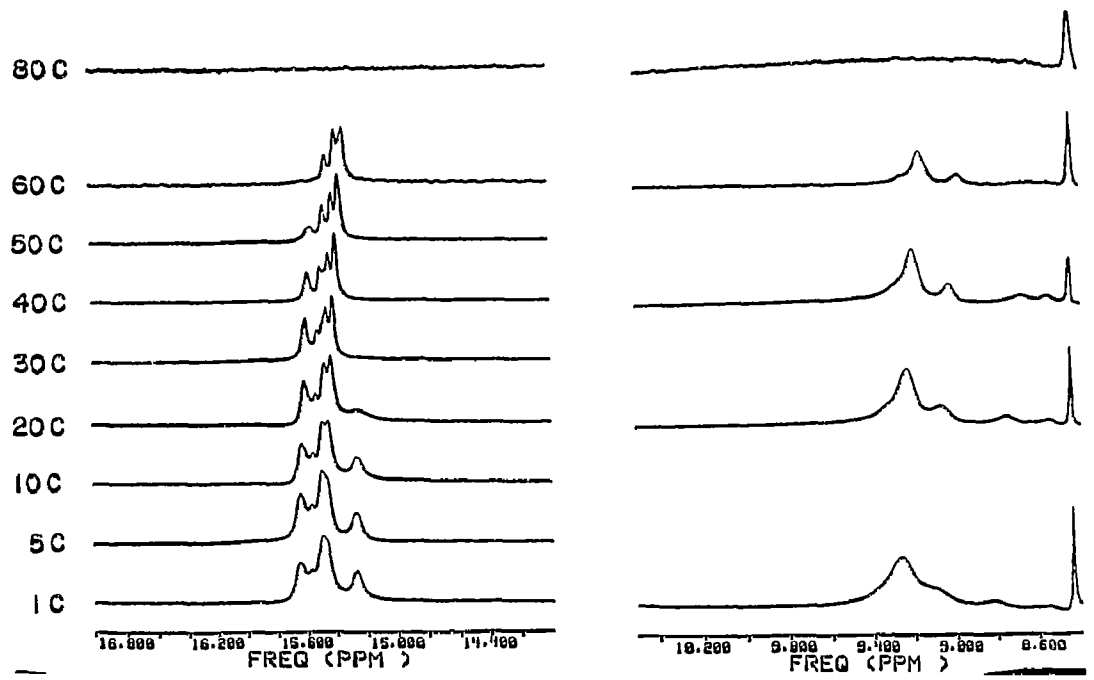


Fig. 2. 500 MHz 1H NMR spectra of $d(A_5C_5)$ in $H_2O$ at pH 5.1. In the temperature range of 1–80°C. The left panel indicates the imino protons from $C^+$·C hemiprotonated pairs, and the right panel from the hydrogen bonded amino protons of C and A (Fig. 1).

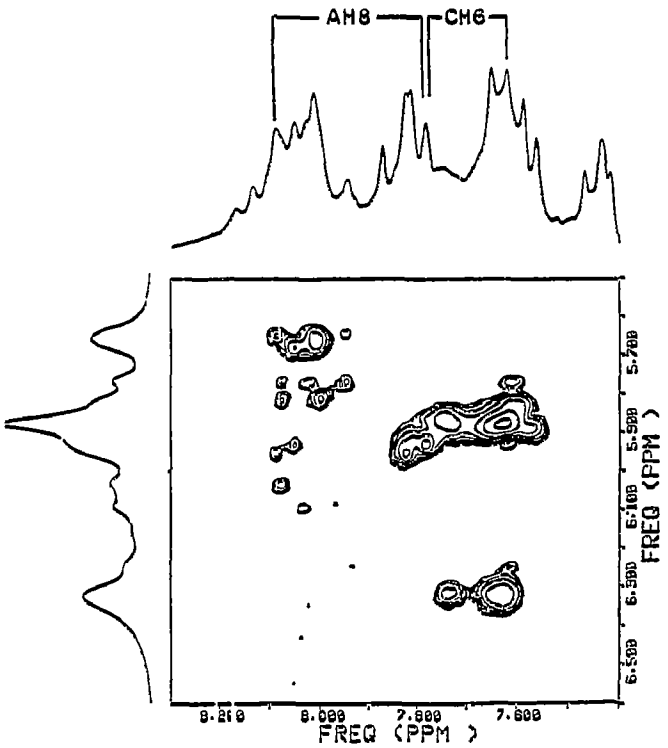


Fig. 3. 500 MHz 1H 2D-NOESY contour plot showing cross peaks between H1', H5 and base proton AH8/CH6s.

## 3. RESULTS AND DISCUSSION

The possible schemes of hydrogen bonding in the parallel double helix of $d(A_5C_5)_2$ are shown in Fig. 1. The parallel self-paired C–C, A–A structures in Fig. 1A and B have been familiar since they were reported by the first X-ray diffraction studies of polyadenylic acid and polycytidylic acid respectively [3,4]. Fig. 1C shows a possible parallel reverse Watson–Crick pair pattern for A-tract. The structures in Fig. 1 are the results of the energy minimizations. The A-tract structure in Fig. 1B represents a reverse Hoogsteen pair (molecule A) and that in Fig. 1C represents a reverse Warson–Crick N1–N6 pair (molecule B).

### 1.1. *Temperature and pH titration effects*

Fig. 2 shows the 500 MHz $^1H$ NMR spectra of $d(A_5C_5)$ in $H_2O$ at acidic pH 5.1 in the temperature range of 1–80°C. Five imino resonances are present between 15–15.7 ppm at 1–10°C. These signals can arise only from the hemiprotonated $C^+ \cdot C$ pairs since there is no imino proton in both A and C bases. These are the first reported direct experimental observation of hemiprotonated $C^+ \cdot C$ pairs under solution conditions. The temperature profile of the imino protons of $d(A_5C_5)$ at pH 5.1 indicates that the duplex is stable up to 60°C with three prominent imino signals between 15.4–15.6

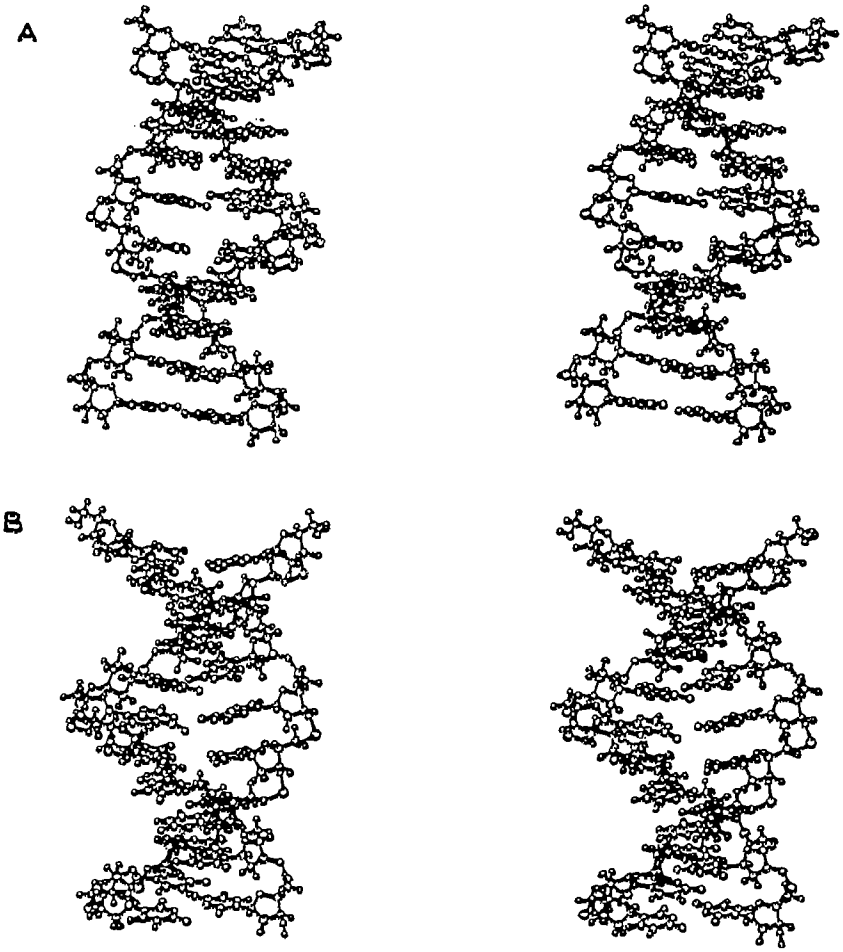


Fig. 4. Stereo views of molecule A and B.

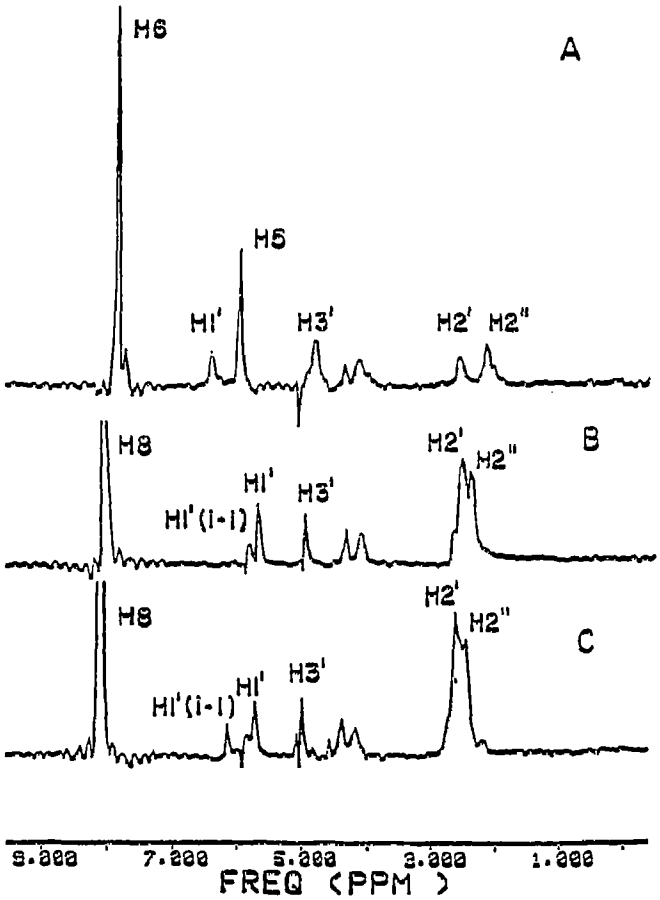


Fig. 5. (A) 1D-NOE slices through the base region for representitive cytosine, (B) adenine (molecule A) and (C) adenine (molecule B).

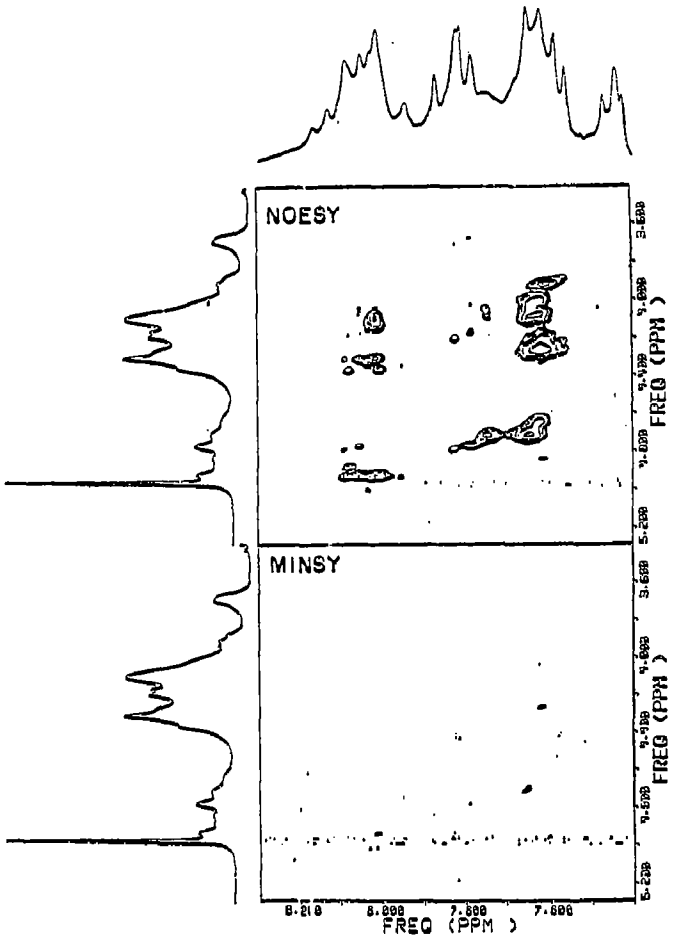


Fig. 6. H8/H6–H3′ crosspeaks in NOESY and MINSY contour plots observed for a mixing time of 250 ms at 10°C.

ppm. The amino signals between 9–9.5 ppm that arise from H-bonded amino protons of adenine and cytosine are also present at 60°C. All amino protons, however, disappear at 80°C. Increasing pH to 8.0 at 1°C and decreasing pH to 2.5 at 10°C abolishes all imino signals. This one expects because at pH 8.0 the cytosine is no longer hemiprotonated; at pH 2.5, the bases become protonated and they can no longer form hydrogen-bonded duplexes.

### 3.2. *2D NMR spectra and two stereochemically sound structures*

The complexity of the spectra, along with resonance doublings (see later), does not enable us to present a quantitative story, but several interesting and reliable qualitative conclusions can be made. From Fig. 3 one can easily isolate the chemical shifts of the AH8 and CH6 since the C-region contains very strong cross peaks between H6 and its neighbouring H5, in addition to the cross peaks between H6 and H1′. This enables the latter to be distinguished. The chemical shift of AH8's are confined to 7.76–8.10 ppm and that of CH6's to 7.60–7.76 ppm. For the A-region in Fig. 3 there are nine chemical shifts with distinctive cross-peaks with H1′. This clearly indicates that there are two possible A–A pairing structures, because if only one symmetrical structure is present one would expect only five such cross peaks. Non-symmetrical pairings were ruled out by energy and stereochemical studies.

The two starting structures (molecule A, molecule B) constructed (see section 2) have anti glycosyl torsional angles for C-tracts and anti for molecule A and syn for molecule B for A-tracts. After energy minimization using AMBER [13–15], the cytosine tracts remained unchanged at the anti glycosyl condition for both structures (molecule A, molecule B); while the anti glycosyl adenines (molecule A) remained unchanged, the syn glycosyl adenines (molecule B) flipped back to anti and the base pairing scheme changed from N6–N7 to N1–N6. The stereo view of molecules A and B are shown in Fig. 4A and B, respectively. A surprising result from the energy minimization of molecule B is that the A–A base-pairing pattern changed from the reverse Hoogsteen N6–N7 amino hydrogen bonding type to the reverse Watson-Crick N1–N6 amino hydrogen bonding type while the C1′–C1′ distance expanded from 10.9 to 13.3 Å. This is the second possible structure to explain

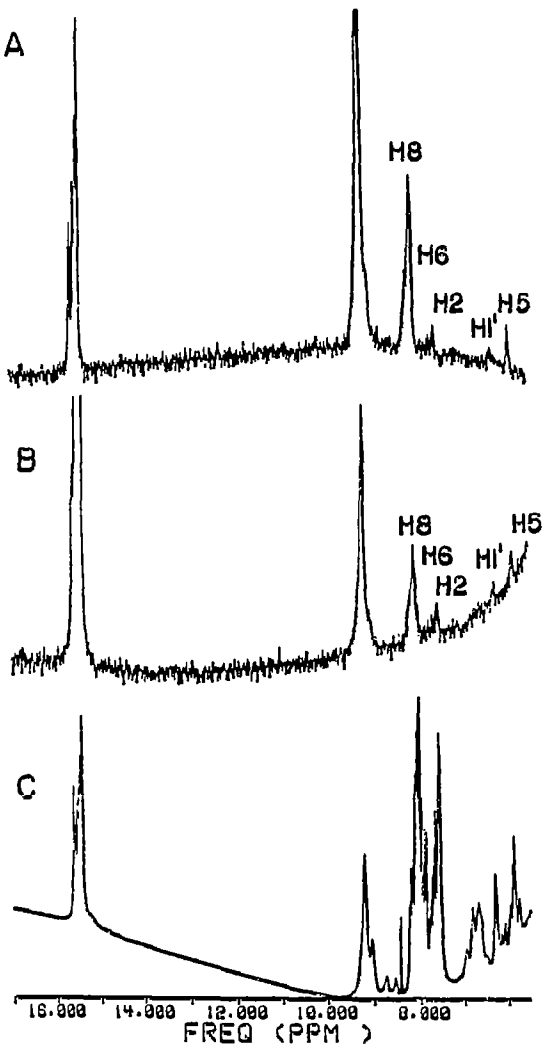


Fig. 7. 1D NOE difference spectra of d($A_5C_5$) in $H_2O$ at 30°C. (A) The amino resonance at 9.26 ppm, saturated. (B) The imino resonance at 15.52 ppm saturated. (C) The control spectrum.

the resonance doublings observed for AH8 in the experimental data. In the two energy-minimized structures, both A–A and C–C tracts have slightly larger hydrogen bond lengths near the A–C junction. This indicates that both molecules bend in the junction. The two structures are right handed, with a rise of 3.5 Å and twist angle of 31°.

According to the structures that are presented in Fig. 4, the intra-residue proton distances between H8/H6(i) and H1′(i) are in the range of 3.7–3.9 Å, the inter-residue H8(i) to H1′(i-1) is in the range of 3.6–3.8 Å for molecule B and beyond 4.7 Å for molecule A. The H6(i) to H1′(i-1) is in the range of 4.3 Å for both molecule B and A. The CH6(6) to AH1′(5) distances are 4.4 and 4.6 Å for molecule B and A, respectively, at the A–C junction. The structures would predict intra-residue H8/H6(i)–H1′(i) cross peaks for both molecules A and B, with inter-residue H8(i)–H1′(i-1) cross peaks and weak H6(i)–H1′(i-1) cross peaks for molecule B and very weak inter-residue H8(i)–H1′(i-1) cross peaks and weak H6(i)–H1′(i-1) cross peaks for molecule A. The base-H1′ cross peaks for the A–C junction for molecule A and B are both weak, with the B slightly stronger: these are what were observed.

In Fig. 5A, B and C we show 1D NOE slices through the base region for representitive cytosine, adenine (molecule A) and adenine (molecule B), respectively, and the data show strong NOE at H5 for cytosine (Fig. 5A), average NOE's at H1′ and different H1′ NOE features for molecule A and B (Fig. 5B and C). In Fig. 5 the observed NOE's at the H2′, H2″ region are entirely expected of these two structures, but the observed relatively strong NOE's at the H3′ region (H4′ also) cannot be rationalized on the basis of the structures because in these structures the distances between H8(i) and H3′(i) are 4.5–4.8 Å, and H6(i) and H3′(i), 3.5–3.9 Å. We believe that this originates via spin diffusion through H2′ and H2″. In order to verify this we preformed a MINSY experiment [12]. The decoupler was placed at 2.5 ppm and the decoupling power was such that the entire H2′, H2″ signals were irradiated. Fig. 6 shows H8/H6–H3′ cross peaks in NOESY and MINSY contour plots observed for a mixing time of 250 ms at 10°C. The MINSY spectrum shows no H3′ cross peak. This establishes that H8/H6 would transfer magnetization to the H3′ through spin diffusion mediated via the H2′/H2″ and to the H4′ through H3′.

### 3.3. *NOE from the exchangeable imino and amino protons to the base protons*

In Fig. 7 the amino resonance at 9.26 ppm and the imino resonance at 15.52 ppm are saturated, there are magnetization transfers at the CH5 region at 5.9 ppm, CH6 region at 7.6 and 7.7 ppm, AH2 region at 7.58 ppm, AH8 region at 8.0 ppm and H1′ region at 6.3 ppm in both difference spectra. This indicates that magnetization transfers exist between imino and amino, as well as base, protons mediated through H-bonded N4H in the case of C-tracts and through N6H in the case of both A-tracts.

(i) In the case of the A-tracts the distance between H-bonded N6H and the H8 across the strand is 2.5 Å for molecule A, while the distance between H-bonded N6H and the H2 across the strand is 2.7 Å for molecule B (Fig. 1) so the magnetization from N6H can be transferred to the H8 and H2 for molecule A and B, respectively.

(ii) In the C-tracts of the duplex structure the fixed distance between the H-bonded $N3H^+$ and the hydrogen bonded N4H protons is ~2.4 Å; that between the non-bonded N4H and CH5 is 2.5 Å, and CH5 and CH6 2.4 Å (Fig. 1), so if $CN3H^+$ is presaturated it would transfer magnetization to the CH5 through spin diffusion and chemical exchange mediated via the H-bonded NH4 protons and to the CH6 through CH5.

(iii) The structures of the duplex are such that the grooves in the C-tracts are aligned by the H-bonded N4H's separated from each other by 3.5 Å. In the A-tracts section the N6H are aligned along the same groove separated from each other by 3.5 Å for both molecule A and molecule B. At the ApC junctions the distances between the N6H of A and the N4H of C is 3.3 Å for molecule B and 4.5 Å for molecule A. The grooves of the duplex structures present pathways for the transfers of magnetization between the exchangeable imino and amino protons to the base protons.

*Acknowledgements:* This research was supported by a grant from the National Institutes of Health (GM 29787) and a contract from the National Foundation for Cancer Research. The high-field NMR experiments were performed at the NMR Facility for Biomolecular Research located at the F. Bitter National Magnet Laboratory, MIT. The NMR facility is supported by Grant RR00995 from the Division of Research Resources of the NIH and by the National Science Foundation under contract C-67.

## REFERENCES

[1] Westhof, E. and Sundaralingam, M. (1980) Biochemistry 77, 1852–1856.
[2] Westhof, E. and Sundaralingam, M. (1980) J. Mol. Biol. 142, 331–361.
[3] Rich, A., Davis, D.R., Crick, F.H.C. and Watson, J.D. (1961) J. Mol. Biol. 3, 71–86.
[4] Langridge, R. and Rich, A. (1963) Nature 198, 725–728.
[5] Krishnan, R., Seshadri, T.P. and Viswamitra (1991) Nucleic Acids Res. 19, 379–384.
[6] Cruse, W.B.T., Egert, E., Kennard, O., Sala, G.B., Salisbury, S.A. and Viswamitra, M.A. (1983) Biochemistry 22, 1833–1839.
[7] Coll, M., Solans, X., Font-Altaba, M. and Subirana, J.A. (1987) J. Biomol. Str. Dyn. 4, 797–811.
[8] Lyamichev, V.I., Mirkin, S.M., Danilevskaya, O.N., Voloshin, O.N., Balatskaya, S.V., Dobrynin, V.N., Filippov, S.A. and Frank-Kamenetskii, M.D. (1989) Nature 339, 634–637.
[9] Suck, D., Manor, P.C. and Saenger, W. (1976) Acta Cryst. B32, 1727–1737.
[10] Matteuci, M.D. and Caruthers, M.H. (1981) J. Am. Chem. Soc. 103, 3185–3191.
[11] States, D.J., Haberkorn, R.H. and Ruben, D.J. (1982) J. Magn. Reson. 48, 286–292.
[12] Massefski Jr., W. and Redfield, A.G. (1988) J. Magn. Reson. 78, 150–155.
[13] Creighton, S., Rudolph, B., Lybrand, T., Singh, U.C., Shafer, R., Brown, R., Kollman, P., Case, D.A. and Andrea, T. (1989) J. Biomol. Str. Dyn. 6, 929–969.
[14] Weiner, S.J., Kollman, P.A., Case, D.A., Singh, U.C., Ghio, C., Alagona, G., Profeta Jr., S. and Weiner, P. (1984) J. Am. Chem. Soc. 106, 765–784.
[15] Weiner, S.J., Kollman, P.A., Nguyen, D.R. and Case, D.A. (1986) J. Comp. Chem. 7, 230–252.