Structure and conformation in solution of the parallel-stranded hybrid α-d(CGCAATTCGC)·β-d(GCGTTAAGCG) by high-resolution 2D NMR

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SUMMARY

The unnatural duplex oligonucleotide α-d(CGCAATTCGC)·β-d(GCGTTAAGCG) was analyzed by high-resolution NMR methods. All of the exchangeable imino and nonexchangeable protons of the duplex were assigned. Detection of all 10 of the exchangeable imino protons confirms that a parallel, unsymmetrical duplex is formed. The thermal stability of the parallel duplex is similar to the analogous antiparallel β–β duplex. The right handedness of the helix is confirmed by inter-residue [H8/H6-H1'] and [H8/H6-H2"] NOEs to the 5'-neighbor in the β-strand and [H8/H6-H1'] NOEs to the 3'-neighbor in the α-strand. Intra-residue and inter-residue distances between base protons and deoxyribose protons in both strands were determined using the isolated spin-pair approximation for NOESY cross peaks acquired with mixing times 50 ms or less. The NOE data are consistent with a B-form geometry adopted by the α/β hybrid decamer.

INTRODUCTION

Sequin using Dreiding stereomodels considered the possibility of oligonucleotides, consisting of α-deoxynucleotide units, to exhibit a secondary structure similar to that of the natural nucleic acids, featuring base pairing, base stacking and helix formation (Sequin, 1973). Although some approximations relating to exact parameters (angles and dimensions of the bases, conformation of the deoxyribose ring and the phosphate backbone) were made with respect to the geometry of the B-DNA structure, this study predicted that an α-strand may form a duplex with the comple-

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mentary β- or α-strand by base pairing and that the two strands should exhibit parallel and anti-parallel polarity, respectively. Several α-oligonucleotides have been synthesized and their properties examined (Morvan et al., 1986a, 1987a,b,c; Gagnor et al., 1987; Gautier et al., 1987; Bertrand et al., 1989; Paoletti et al., 1989; Gmeiner et al., 1990). Biophysical evidence was obtained, principally by 2D ^{1}H NMR, for stable antiparallel self-recognition of short six-base-pair α-sequences which display some self-complementarity and of stable parallel duplex formation between α-oligomers and their β-complements, of either DNA (Morvan et al., 1986, 1987a; Lancelot et al., 1989) or RNA (Gmeiner et al., 1990). In view of recent advances in the application of α- and β-oligonucleotides (Morvan et al., 1986; Praseuth et al., 1988; Gagnor et al., 1989; Gmeiner et al., 1990), especially the implication of triplex formation (Griffin and Dervan, 1987; Francois et al., 1989; Maher et al., 1989) with double-stranded DNA cellular targets, it was evident that detailed structural and conformational information on α-DNA-containing duplexes of at least one helical turn should be made available. Accordingly, we report a detailed examination of the structure and conformation in solution of one complete turn of the parallel-stranded hybrid duplex α-d(CGCAATTCGC)·β-d(GCGTTAAGCG) by high-resolution 2D NMR.

MATERIALS AND METHODS

Chemicals

The β-oligonucleotides were synthesized on an Applied Biosystems 381A DNA synthesizer using phosphoramidite chemistry. Reverse-phase HPLC analysis with detection at 254 nm indicated purity greater than 97%. The α-oligonucleotides were synthesized as described by Morvan et al. (1986). Purification was performed by preparative reverse-phase HPLC using 6% acetonitrile in ammonium bicarbonate (0.05 M, pH 7.2) as eluent. Analytical HPLC indicated purity greater than 99%. The nucleoside composition of the α- and β-oligonucleotides was checked by degradation with snake venom phosphodiesterase and alkaline phosphatase and yielded dA, dC, dG and dT in the proper molar rations.

NMR spectroscopy

All NMR spectra were acquired on a Varian Unity spectrometer at 499.84 MHz. Data processing was done on a SUN 4/470 computer using the VNMR software. All experiments were temperature-regulated to within 1 and were run without sample spinning. Phase-sensitive spectra were acquired using the hypercomplex method (States et al., 1982). All correlation spectroscopy experiments including COSY (Jeener et al., 1979), DQCOSY, TOCSY (Braunschweiler and Ernst, 1983), and PECOSY (Mueller, 1987) were acquired with acquisition windows of 4200 Hz in F1 and F2 with 4096 points in T2 and 800 points in T1. The T1 dimension was zero-filled to 2K for a final digital resolution of 1.02 Hz/pt in F2 and 2.04 Hz/pt in F1. Sixteen scans per T1 increment were acquired with a 2.4-s delay between scans. Shifted sine bells were used to apodize the data in both dimensions. The baseline was corrected according to the procedure of Otting (Otting et al., 1986). COSY spectra with delays set to emphasize small- or long-range couplings (Bax and Freeman, 1982) were acquired with identical parameters but with a fixed delay during the evolution period and prior to acquisition. The delay was optimized for correlations involving a 4-Hz coupling constant. TOCSY spectra were acquired with spin-lock times of 60 and 100 ms. All nonselective pulses were π/2 pulses except the second pulse of the PECOSY which was a π/4 pulse.

NOESY spectra were acquired with similar acquisition parameters to the correlation experiments except with 512 points in T1 and 32 scans per T1 increment. Recycle delays were either 2.4 or 5 s. Longitudinal relaxation times are less than 1.6 s (except A(H2) = 2.1 s) as measured by the inversion/recovery method with recovery delays between 0.125 and 8 s and 30 s between scans. Data were apodized with Gaussian functions in T1 and T2 prior to Fourier transformation. NOESY spectra with mixing times of 0, 30, 40, 50, 60, 70, 80, 100, 150, 200 and 300 ms were acquired. 2D NOESY spectra acquired in 90% H_2O/10% D_2O utilized a 1–1 echo selective excitation pulse (Sklenar and Bax, 1987). Mixing times of 150 and 300 ms were used. A 12 000 Hz spectral window was used in both dimensions. A homospoil pulse of duration half the mixing time was employed in the pulse sequence. A NOESY spectrum was also acquired with presaturation of the H_2O resonance. 1D spectra in H_2O were acquired with a 1–2–1 selective excitation pulse. 1D NOEs used a 300 ms pre-irradiation of a single frequency at a power level sufficient to saturate the resonance for 90%. Distances were determined from the magnitude of cross peaks in NOESY spectra acquired with mixing times less than 50 ms and recycle times at least 5 s using the relation:

$$r_{ij} = (a_{ref}/a_{ij})^{1.6} r_{ref}$$

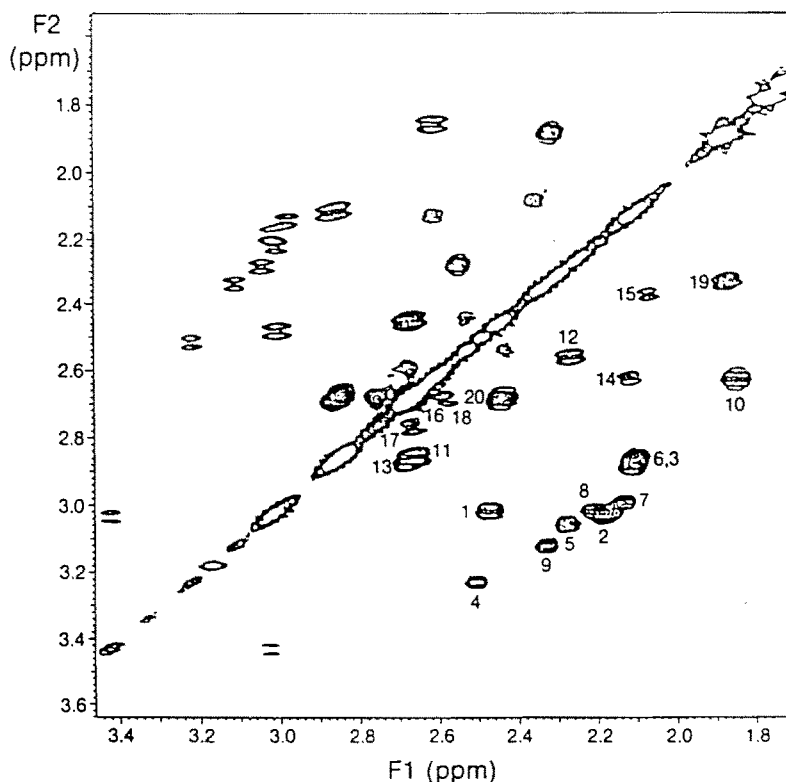Volume integrals were evaluated for the individual cross peaks by evaluating the volume of a rec-

Fig. 1. Expansion of the COSY spectrum about the 2'-2'' region. The correlations are indicated by residue. The cross peaks from the α-nucleotides are further removed from the diagonal than the β-nucleotides.

tangle which circumscribed the peak and algebraically adding the average volume for a series of equally sized rectangles around the peak to correct for baseline fluctuation. Corrections were always less than 10% of the peak volume. The H5-H6 interproton vector served as a reference distance of 2.46 Å (Gronenborn et al., 1984). This evaluation procedure was accurate to within a few percent for other fixed distances in the molecule.

RESULTS

The spin systems for the deoxyribose sugars were assigned using COSY and phase-sensitive TOCSY experiments (Hare et al., 1983). The twenty H2'-H2" cross peaks (Fig. 1) of the absolute-value COSY spectrum provided a starting point for the assignment process. Interestingly, the α-nucleotides all have a larger difference between the chemical shift of H2' and H2" than the β-nucleotides. The TOCSY spectra confirmed the assignments determined from the COSY spectra and allowed assignment of correlations in crowded regions of the COSY spectrum. In order to resolve overlapping cross peaks spectra were acquired at 18°, 24° and 30°C. Correlations for all twenty spin systems were evident and assigned in the spectra except the 5', 5" protons which were not resolved and left unassigned. An analysis of the magnitude of the J-couplings for the deoxyribose
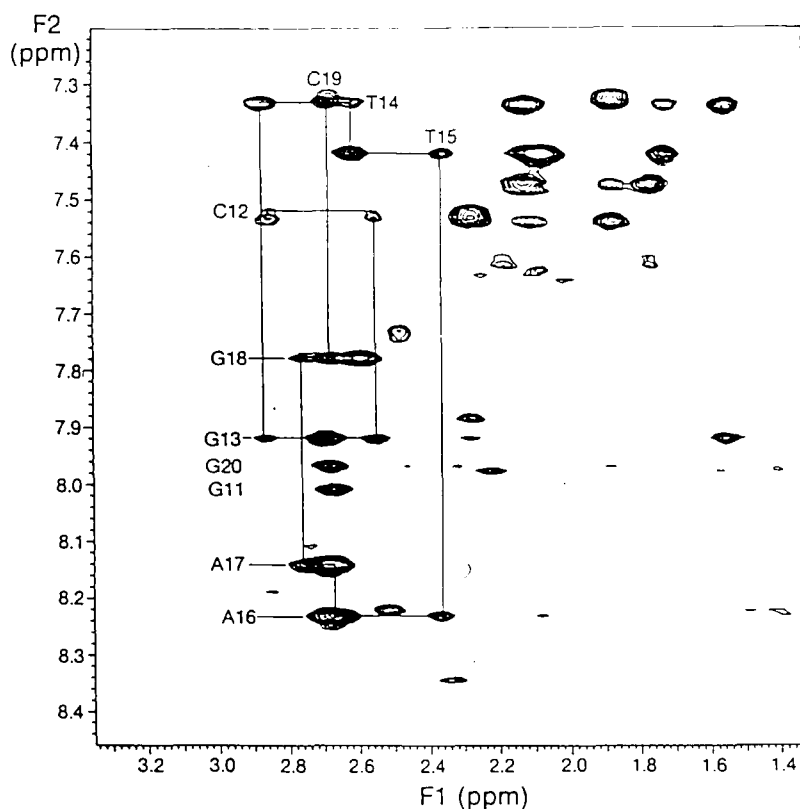

Fig. 2. Expansion of the phase-sensitive NOESY spectrum acquired with a mixing time of 80 ms. The H8/H6-H2', H2" NOEs are indicated for all the β-nucleotides and the pathway of sequential connectivity via interresidue H8/H6-H2', H2" NOEs is outlined.

TABLE I
'H NMR ASSIGNMENTS FOR THE HYBRID DECAMER

Residue	H8/H6	H1'	H2'	H2"	H3'	H4'	H5/CH₃/H2
α-chain 5'							
C1	7.74	6.38	2.50	3.02	4.90	4.66	5.52
G2	7.88	6.06	2.28	3.06	4.94	4.20	
C3	7.48	5.90	2.18	3.00	4.89	4.18	5.12
A4	8.22	6.20	2.56	3.28	5.10	4.20	7.42
A5	8.35	6.04	2.33	3.12	4.99	4.25	6.51
T6	7.54	5.55	2.12	2.88	4.94	4.22	1.89
T7	7.49	5.94	2.17	2.89	4.99	4.08	1.78
C8	7.61	6.06	2.18	3.02	5.08	4.24	5.44
G9	7.98	6.08	2.24	3.02	4.95	4.02	
C10	7.68	5.51	1.87	2.62	4.42	4.25	5.92
β-chain 5'							
G11	8.01	6.12	2.88	2.66	5.06	4.17	
C12	7.52	5.87	2.56	2.27	4.98	4.29	5.54
G13	7.92	6.05	2.88	2.70	5.05	4.17	
T14	7.33	6.06	2.62	2.12	5.02	4.33	1.56
T15	7.42	5.78	2.36	2.10	4.93	4.16	1.74
A16	8.22	5.61	2.71	2.65	5.04	4.38	6.91
A17	8.14	5.76	2.78	2.69	5.08	4.16	1.74
G18	7.78	5.83	2.68	2.60	5.03	4.15	
C19	7.32	5.85	2.32	1.90	4.82	4.20	5.48
G20	7.97	6.21	2.68	2.45	4.74	4.28	

sugars from the PECOSY experiment (Griesinger et al., 1987), along with the structural interpretation, is given below. Two cross peaks in addition to the twenty expected from H2'-H2" correlations were apparent at reduced intensity in the same region of the COSY spectrum but they did not correlate with any other resonances and are attributed to an impurity rather than to a distinct oligonucleotide species. Integration of the methyl resonances at elevated temperatures indicated the ratio of α- to β-oligonucleotide was unity.

Connectivity of spin systems was done using phase-sensitive NOESY spectra (Feigon et al., 1983). The α-nucleotides are typified by moderate $d_i(8/6;2')$ and $d_i(8/6;2'')$ based on observation of the corresponding cross peaks at 100 ms mixing time. Sequential connectivity was established based on $d_s(1',8/6)$ which resulted in observable cross peaks in the NOESY spectrum acquired with 50 ms mixing time for all such distances in the α-chain. It should be noted that the connectivity is from the base proton to the 3'-neighboring H1' as has been previously observed in α-oligonucleotides of a right-handed helix (Lancelot et al., 1987). The short $d_s(6,M)$ distance resulted in a prominent cross peak useful in the assignment process. Again, the polarity of the NOE is reversed from that of a β-oligonucleotide. No independent pathway of sequential connectivity could be traced based on $d_s(2';8/6)$ or $d_s(2'';8/6)$ as is commonly done for β-oligonucleotides in B-form geometry (Scheek et al., 1984) because these NOEs were not detected, not even in NOESY spectra acquired with long mixing times. The structural basis for this observation is given below. The gly-

cosidic bond adopts an anti configuration for all the α-nucleotides based on the moderate $d_i(8/6;4')$.

The base protons of the β-nucleotides were connected to their sugar spin systems on the basis of the prominent cross peaks from the short $d_i(8/6;2')$ and moderate cross peaks from $d_i(8/6;1')$ (Fig. 2). Interresidue connectivity was established by detection of the prominent cross peaks resulting from the short $d_s(2';8/6)$ but a second independent pathway of connectivity based on $d_s(1';8/6)$ was not apparent (Clore and Gronenborn, 1983). The structural implications of this are discussed below. An interresidue NOE from H8 of G13 to the methyl group of T14 aided the assignment process (Weiss et al., 1984). The chemical shift assignments for both strands are given in Table 1.

The pucker of the deoxyribose sugar for both the α- and β-nucleotides was determined by an analysis of the J-couplings from the PECOSY (Fig. 3) spectrum and of the interhydrogen distances measured from the 50 ms NOESY spectrum. The observed sugar conformations are weighted averages of pure C2'-endo and C3'-endo in rapid equilibrium on the NMR time scale (Porschke, 1978). The three-bond couplings involving H1' are diagnostic of sugar conformation. C2'-endo or S-type sugar puckers are characterized by the sum of the couplings of H1' with H2' and H2'' exceeding 13.3 Hz with $J1'2' > J1'2''$ (Rinkel and Altona, 1987). This type of sugar pucker is found in B-DNA (Rinkel et al., 1987). These two criteria are met for 8 of the 10 β-deoxy-
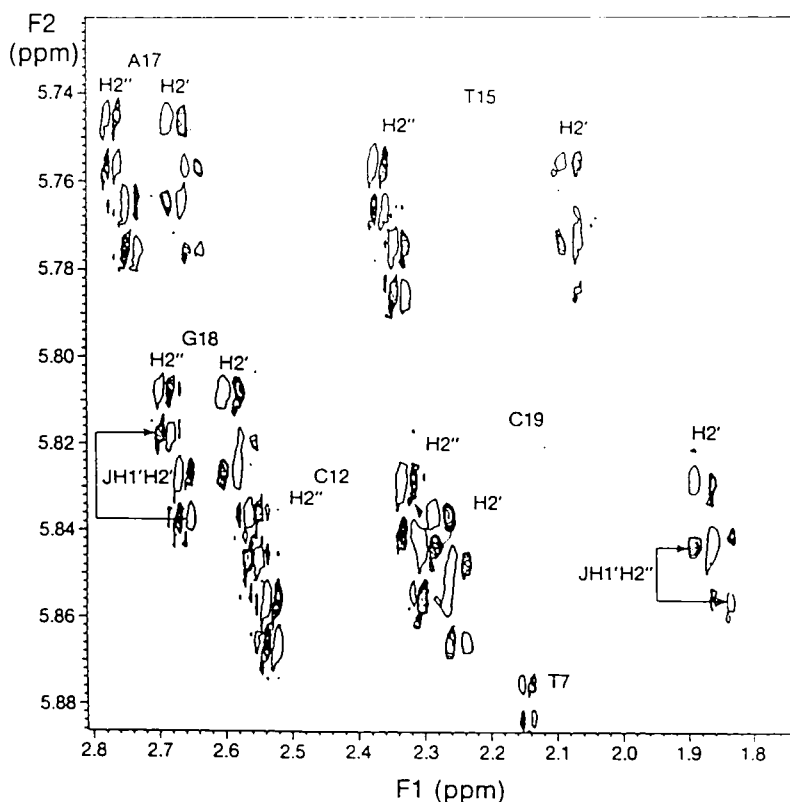

Fig. 3. Expansion of the PECOSY spectrum showing the H1'-H2' and H1'-H2'' cross peaks for several β-nucleotides. The J values for the passive couplings are extracted from the cross peaks as indicated.
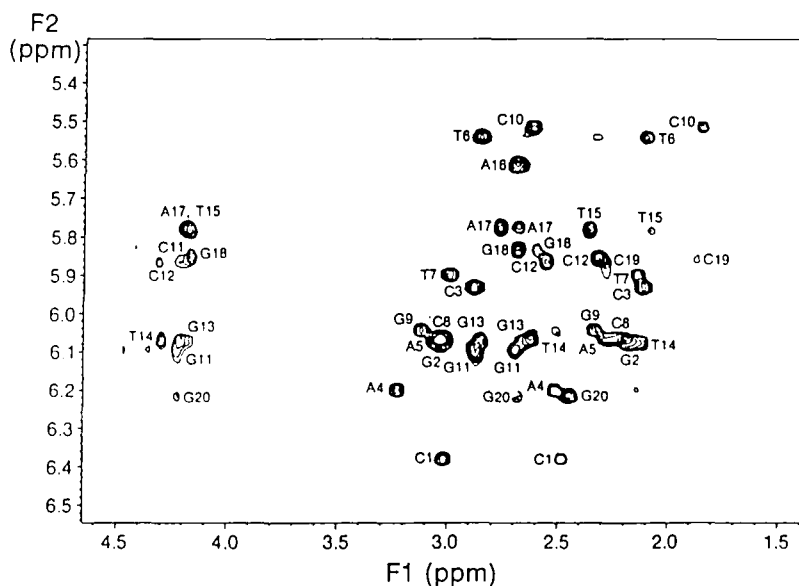
Fig. 4. Expansion about the H1'-H2', H2", H4' region of the 50 ms mixing time phase-sensitive NOESY spectrum. The H1'-H4' NOEs for the α-nucleotides result in NOEs too small to be detected.

ribose sugars. The two nucleotides at the 3' terminus of the β-strand have J1'2':J1'2" ratios near unity indicating a significant contribution from the N-type conformation.

The intraresidue distances as determined by NOE intensities (Wüthrich, 1986) in the 50-ms mixing time NOESY are also consistent with a C2'-endo/S-type sugar pucker. The $d_i(1',4')$ is less than 3 Å for the β-sugars while the $d_i(2'',4')$ is at least 3.5 Å. None of the cross peaks due to 2"-4' interactions were observed in the NOESY spectra with short mixing times while all 10 of the 1'-4' cross peaks were apparent (Fig. 4). A 1'-4' distance of 2.9 Å and a 2"-4' distance of 3.9 Å are typical of C2'-endo/S-type sugar puckers (Wüthrich, 1986). The NOE-derived distance data are in agreement with the coupling constant data with both indicating β-nucleotides in the hybrid duplex have sugar geometry similar to that found in B-DNA. The J-coupling- and NOE-derived distance information is summarized in Table 3.

Evaluation of the J-couplings for the α-sugars shows a trend similar to the β-sugars with a J1'2':J1'2" ratio exceeding unity but with the sum of both couplings less than 13.3 Hz for each α-nucleotide. Both J-values are less than for the corresponding β-sugar indicating that rather than a change in the equilibrium between pure S and pure N conformations occurring, a reduction in the amplitude of the sugar pucker occurs. The relative value of the J-couplings indicates the sugar pucker is predominately C2'-endo. The distances between H1' and the remaining hydrogens of the α-sugar are different from the β-nucleotide and $d_i(1',4')$ is too long to be a useful indicator of sugar pucker for α-nucleotides (Fig. 4). The 2"-4' distances remain greater than 3.5 Å and were not observed in NOESY spectra with a short mixing time. The J-coupling data for α-nucleotides are also included in Table 2.

Distances between base protons and H1', H2' and H2", both intra- and interresidue, are diagnostic of DNA helix type (Wüthrich, 1986). The 10 $d_i(H6/H8;H2')$ and the 9 $d_s(H6/H8;H2'')$ dis-

TABLE 2
SUMMARY OF ^{3}J INVOLVING H1'

Residue	$J_{H1'-H2'}$	$J_{H1'-H2''}$	Σ (J)	Residue	$J_{H1'-H2'}$	$J_{H1'-H2''}$	Σ (J)
C1	4.3	8.3	12.6	G11	4.4	8.8	13.2
G2	3.1	7.5	10.6	C12	4.7	8.8	13.5
C3	3.1	7.9	11.0	G13	5.5	9.0	14.5
A4	3.3	7.9	11.2	T14	6.2	7.7	13.9
A5	3.1	7.3	10.4	T15	5.2	9.8	15.0
T6	2.7	8.2	10.9	A16	5.6	9.4	15.0
T7	4.2	8.1	12.3	A17	5.3	9.5	14.8
C8	3.8	7.9	11.7	G18	4.8	9.2	14.0
G9	3.7	8.5	12.2	C19	5.6	6.7	13.3
C10	3.0	7.0	10.0	G20	7.7	6.6	14.3

tances for the β-strand of the hybrid duplex were measured from the cross peaks in the 50 ms NOESY. NOEs were observed from base protons to 5'-neighboring nucleotides in accordance with the right-handedness of the helix. Standard B-form geometry had values of 2.25 Å and 2 Å for d_i(H6/H8;H2') of β-purine and β-pyrimidine nucleotides respectively while d_s(H6/H8;H2") were 2.1–2.2 Å for either. The corresponding distances in A-form geometry are all considerably longer. The distances measured for the β-nucleotides of the hybrid duplex are typical of B-form geometry although d_s(8/6;2") were considerably more variable. The distances are tabulated in Table 3. The distances from base protons to 3'-neighboring thymidine methyl groups were also in the range expected for a right-handed B-form duplex.

Base to sugar proton distances in the α-oligonucleotide strand are also diagnostic of helix geometry. The d_i(H6/H8;H2") for α-nucleotides are 2.9–3.3 Å while d_i(H6/H8;H2') is longer than 3.5 Å. The interresidue distances d_s(H6/H8;H2') and d_s(H6/H8;H2") are longer than 3 Å and could not be measured from the 50-ms NOESY. Base protons give moderate NOEs to H1' and H4' al-

TABLE 3
SELECTED DISTANCES FROM THE β-CHAIN[a]

	d_i(8/6; 2')	d_s(8/6; 2")	d_i(1',4')	d_i(2",4')
G11	2.7		2.9	< 3.5
C12	3.0	3.7	3.2	< 3.5
G13	3.0	2.9	2.9	< 3.5
T14	2.2	2.6	2.7	< 3.5
T15	2.0	2.6	2.7	< 3.5
A16	2.2	3.1	3.4	< 3.5
A17	2.0		2.7	< 3.5
G18	2.3	3.4	2.5	< 3.5
C19	2.3	2.7	2.5	~ 3.5
G20	3.3		2.9	~ 3.5

[a] Baseline distortions were accounted for in evaluating the volume integrals. Spin diffusion was not explicitly accounted for but should be slight at 50-ms mixing times. Estimated error limits are ± 10%.
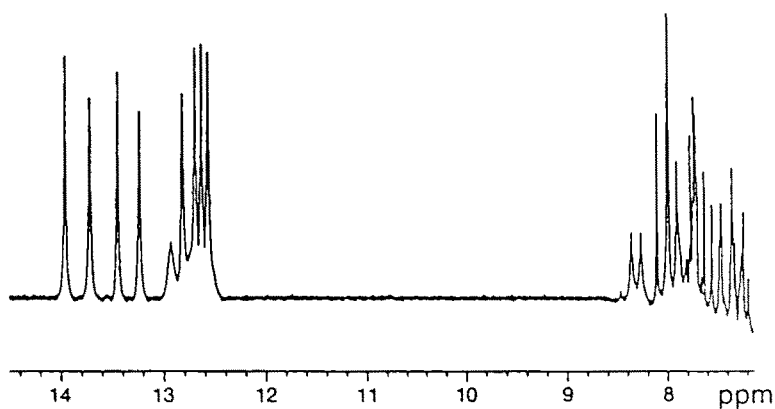
Fig. 5. 1D ¹H NMR spectrum in 90% H₂O of the α/β hybrid decamer obtained with 1 3 3 1 solvent suppression. The imino hydrogens from 8 base pairs are evident in the spectrum with the two guanosine imino protons from the terminal base pairs broadened due to fraying.

though the interresidue NOE d_s(H6/H8;H1′) to the 3′-neighbor is larger than the intraresidue NOE particularly for A4 and A5. This pattern of NOEs is similar to that found for other α/β DNA hybrids (Lancelot et al., 1989).
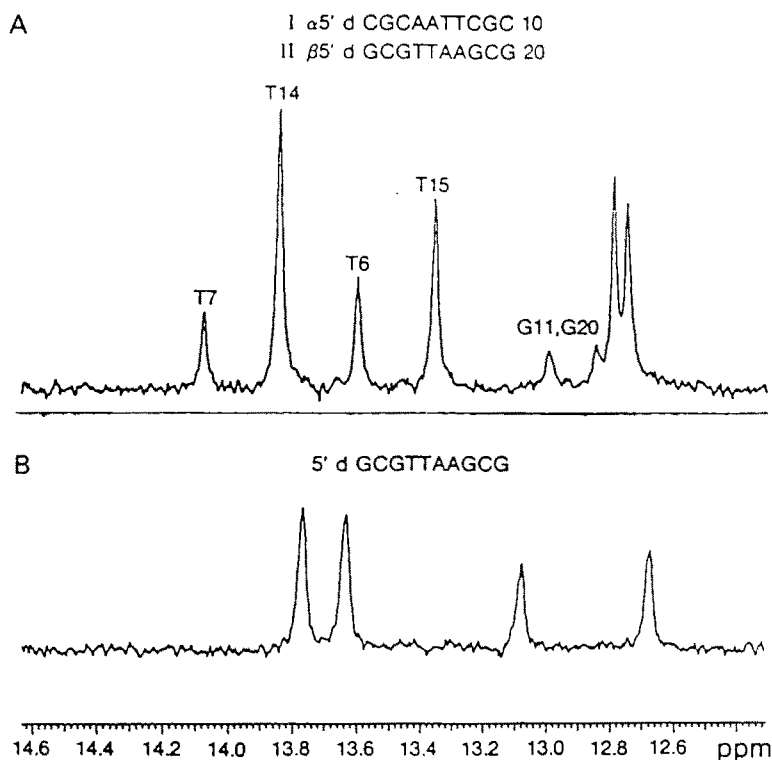

Fig. 6. In (A) is the 1D spectrum of the α/β hybrid decamer obtained with presaturation of solvent while in (B) is the 1D spectrum of a self-complementary β-DNA decamer of identical base pair composition under identical conditions.

Analysis of the exchangeable protons

The hybrid decamer is an unsymmetrical molecule and the imino protons of 10 base pairs are evident in the downfield region of the ^{1}H NMR spectrum in 90% H_2O (Fig. 5). Compared with an analogous sequence of self-complementary β-DNA there is considerably more dispersion among the thymidine imino proton resonances (Fig. 6). The sequential order of the imino protons was established by 1D NOE difference experiments (Fig. 7) and by 2D NOESY spectra acquired in 90% H_2O with a 1–1 echo selective excitation pulse (Fig. 8). The sequential assignment procedure for the imino protons was complicated by the palindromic nature of the base-pair order with information based only on imino–imino NOEs insufficient to distinguish 1–10 from 10–1 ordering. The NOEs from imino hydrogens to H2 in A-T base pairs were readily apparent but the H2-H1' NOEs often observed in B-form DNA, particularly in A-T-rich sequences (Nadeau and Crothers, 1989), were not apparent and could not be used to determine the polarity of the molecule. The discriminating features serving to elucidate the polarity were an NOE from H2 of A17 to the amino group of G18 and an NOE from the T7 imino to the G18 amino (Fig. 8).

Presaturation experiments revealed unusual exchange behavior for the thymidine imino hydrogens (Leroy et al., 1988). In contrast to self-complementary β-DNA for which all imino resonances are substantially reduced in intensity by a 2-s presaturation of the H_2O resonance the two β-thymidine imino hydrogens are not substantially altered in intensity by presaturation of H_2O. The two imino hydrogens from the α-thymidines are substantially reduced in intensity by the presaturation. This disparity in exchange rate parallels the differences in chemical shift apparent between the α- and β-thymidines. There is a 0.4-ppm difference between the exterior (near 1 G-C base pair) and interior thymidine imino chemical shifts for both α- and β-thymidines with the α-imino 0.2 ppm downfield from the β in each case.

Both 1D and 2D experiments indicate the short distances indicative of Watson–Crick base pairing between the imino protons of thymidine and H2 of adenosine occurring in the hybrid duplex. Other distances typically short enough for NOEs to be observed are between adjacent adenine residues $d_s(2;2)$ (Boelens et al., 1988), and intra- and interstrand NOEs of the type d(2;1'). Interstrand NOEs $d_i(2;1')$ are particularly interesting because they provide a measure of the minor groove width (Nadeau and Crothers, 1989). NOEs between adjacent adenosines are apparent in the NOESY spectra, however, all attempts to detect H2-H1' NOEs by either 1D or 2D methods were unsuccessful. The H2 resonances are well-resolved in the 1D spectrum but no NOEs to sugar protons were observed for any H2 resonances at any mixing time. This indicates a considerably wider minor groove in the hybrid duplex which has more structural similarities to B-DNA than occurring in a native B-DNA duplex.

DISCUSSION

The structural details of this α/β hybrid decamer are similar to those found for similar hybrids (Guesnet et al., 1990). The helix is right-handed and comprised of Watson–Crick base pairs. The results are comparable to what has been found for similar hybrids of shorter length with a right-handed helix and comprised of Watson–Crick base pairs (Guesnet et al., 1990). The overall geometry is similar to B-DNA. Several features evident in the analysis of this hybrid decamer are unique and are relevant to the future usefulness of α-DNA in antisense and antigene therapy (Helene, 1990). The geometry of the sugars of the β-chain is predominantly C2'-endo, in agreement
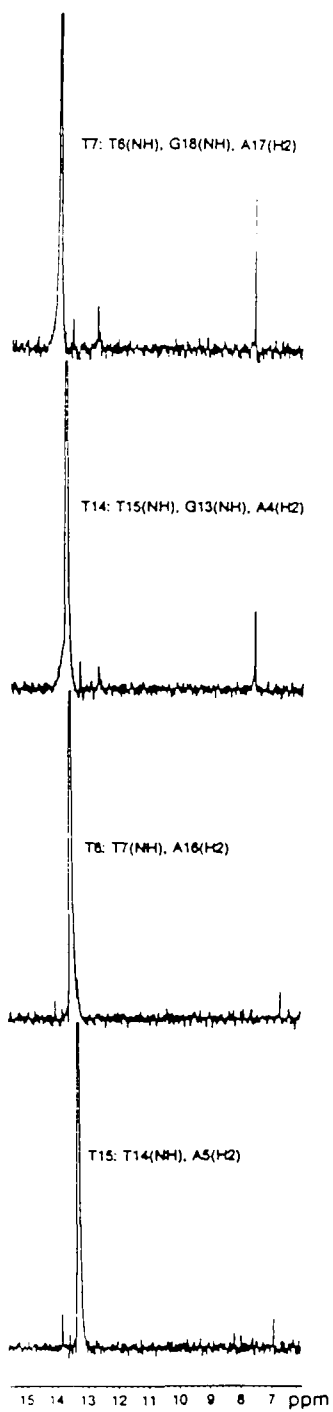
Fig. 7. The 1D NOE difference spectra of the α/β hybrid decamer obtained with selective irradiation of the thymidine imino hydrogens.
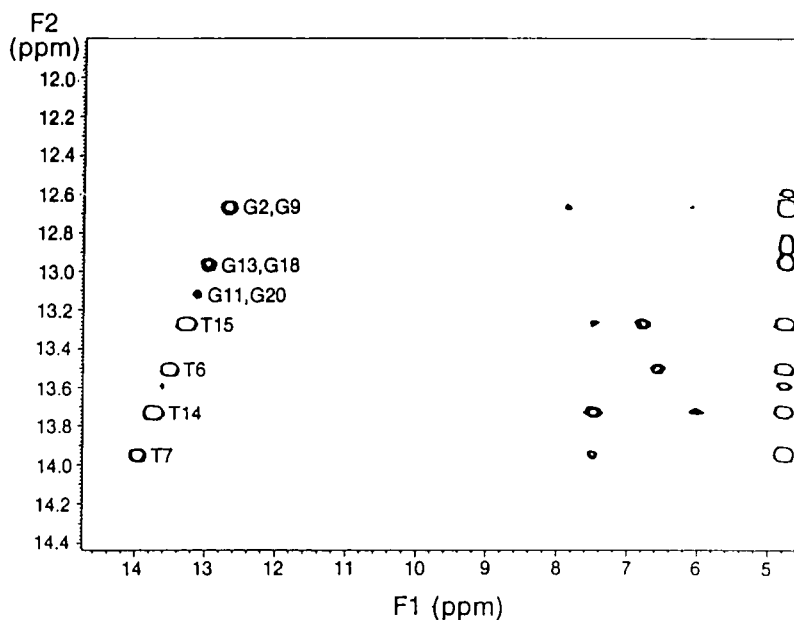
Fig. 8. Expansion of the 2D NOESY obtained in 90% H$_2$O using the 1 1 echo sequence. The imino hydrogens for the 10 base pairs are indicated. Each thymidine imino shows an NOE to the H2 of complementary adenosine, only T14 shows an NOE to a guanosine amino proton. Interimino NOEs were not observed in the experiment.

with previous analyses, with the two β-sugars at the 3' terminus having a significant C3'-endo component in their equilibrium values for coupling constants and intrasugar distances. This suggests there may be an upper limit for the length of α/β duplexes which adopt an overall B-form geometry. No deviation from the C2'-endo sugar pucker has been reported near the termini of shorter hybrid duplexes (Lancelot et al., 1989). The sugar pucker of the α-nucleotides is similar in pseudorotation angle to that previously reported but the amplitude of puckering is reduced compared to the β-nucleotides as evidenced by reduced values for $J_{H1'-H2'}$ and $J_{H1'-H2''}$ but with a near-constant value for the ratio of these two coupling constants. There is no variation in pseudorotation phase for the α-sugars near the termini as for the β-nucleotides. Study of the sugar geometry for α-nucleotides is hampered by a lack of meaningful intrasugar NOEs. In particular d_i(H1';H4'), which is diagnostic of sugar geometry in β-nucleotides (Wüthrich, 1986), is not detectable as a direct NOE in α-sugars.

The dynamics of ^{1}H exchange in the hybrid decamer differ from a self-complementary sequence of identical base-pair composition comprised only of β-nucleotides which was used as a control. Exchange rates were estimated from changes in peak intensity due to presaturation of solvent. The two β-thymidine imino protons of the hybrid decamer exchange slower and the two α-thymidine imino protons exchange faster than the thymidine imino protons of the control. The stability of hybrid A-T base pairs in the observed sequence is αT:βA < βT:αA < βT:βA. The stability of G-C base pairs, apart from the frayed terminal base pairs, goes in the opposite direction. These details are not apparent in the τ_m measurements from hyperchromicity studies which yield similar τ_m values for the hybrid and control decamers with the hybrid being slightly more stable. The rela-

tive stability of hybrid duplexes is likely to be sequence dependent and future studies on the stability of hybrid base pairs are in order to determine if a predictive relationship exists between sequence composition and duplex stability. The chemical shift dispersion of the thymidine imino protons is larger in the hybrid duplex than in the control sequence. This does not result from changes in base stacking as the T-T and A-A distances and torsions are similar for both the α- and β-chains. Studies of the hybrid base pairs would elucidate inherent chemical shift differences and correlate them to intraresidue torsional parameters.

The minor and major grooves of α/β DNA hybrid duplexes are potential targets of drugs (Lown, 1990). The structural data indicate that the binding behavior of the hybrid duplex is likely to be different from analogous sequences of β-DNA. Netropsin and distamycin bind tightly to A-T regions of DNA with one or two drug molecules (in the case of distamycin) occupying the minor groove (Pelton and Wemmer, 1990). The occupancy of the minor groove is dependent on groove width which is compressed in A-T regions due to propeller twisting of the nucleotide bases (Katahira et al., 1988). Propeller twisting is favorable because of the improved stacking that it imparts to consecutive bases in the same strand (Calladine, 1982). The degree of propeller twisting may be determined from the ratio of the distances between the thymidine imino proton and H2 of its complementary adenosine and H2 of the adenosine 3' to the complementary adenosine, sequence permitting (Katahira et al., 1990).

In classical B-DNA the distance to the neighboring adenosine is 5.1 Å (Arnott and Hukins, 1972), too large for detection by NOE. In A-T tracts of β-DNA this distance can be much shorter due to propeller twisting by the bases and the ratio of the NOEs to the complementary H2 and the neighboring H2 approach unity (Nadeau and Crothers, 1989). In the hybrid decamer the NOE to H2 of the complementary A is pronounced, as expected in a Watson–Crick base pair, while the NOE to the neighboring H2 is not observed. The signal-to-noise ratio was sufficient to detect NOEs with 5% of the intensity of the observed imino-to-AH2 NOE. Propeller twisting is not observable directly from NOE data in the hybrid duplex.

The slight base-pair tilt in the hybrid duplex results in a wide minor groove. The $d_s(H2;H1')$ is a measure of minor groove width. While $d_i(H2;H1')$ is larger than 4.6 Å for all DNA conformations and is larger than 5.2 Å for both A- and B-DNA (Wüthrich, 1986), $d_s(H2;H1')$ shortens considerably from its 5.1-Å value in canonical B-DNA in A-T regions as a consequence of propeller twisting. In accordance with the lack of propeller twist derived from T(H3) to A(H2) NOEs the $d_s(H2;H1')$ is too long for the corresponding NOEs to be observed in the hybrid duplex. The minor groove width is not significantly narrower in the hybrid duplex than the 5.1 Å value in B-DNA. Formation of 2:1 complexes of distamycin should be possible with the hybrid duplex and the hybrid may also be bound by thiazole compounds which are AT-avoiding drugs in all β-DNA sequences due to steric repulsions (Lown, 1990).

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