Interproton distance measurements in solution for a double-stranded DNA undecamer comprising a portion of the specific target site for the cyclic AMP receptor protein in the *gal* operon

A nuclear Overhauser enhancement study

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Using the proton-proton nuclear Overhauser effect, a large number (150) of interproton distances, both intra- and internucleotide, are determined in solution for a non-self-complementary DNA undecamer comprising a portion of the specific target site for the cAMP receptor protein in the *gal* operon. It is shown that these distances are very similar to those expected for classical B DNA (RMS difference of 0.5 Å) but are significantly different from those expected for classical A DNA (RMS difference 1.1Å). Glycosidic (χ) and C4′-C3′ (δ) bond torsion angles are obtained by model building on the basis of the intranucleotide interproton distances. Whereas the sugar pucker exhibits little base-to-base variation with δ lying in the range $120 \pm 10°$, the glycosidic bond torsion angles of the pyrimidine and purine residues are significantly different, with $\chi_{pyr} = -120 \pm 10°$ and $\chi_{pur} = -90 \pm 10°$.

DNA oligonucleotide *NOE* *Interproton distance* *Solution structure* *CRP specific site*

1. INTRODUCTION

In [1] we presented a 500 MHz ^{1}H-NMR study on the double-stranded non self-complementary DNA undecamer

5′d A_1 A_2 G_3T_4 G_5T_6 $G_7A_8C_9A_{10}T_{11}$ 3′ A strand

3′d $T_{11}T_{10}C_9A_8C_7A_6C_5T_4$ G_3T_2 $A_1$5′ B strand

comprising a portion of the specific target site for the cyclic AMP receptor (also known as CRP or CAP) in the *gal* operon. Using pre-steady state nuclear Overhauser enhancement (NOE) measurements all exchangeable imino, non-exchangeable base, methyl and H1′, H2′ and H2″ sugar proton resonances were assigned in a sequential manner. It is important to bear in mind that this sequential resonance assignment strategy does not require the initial assumption of a particular helix type. This is because the general pattern of NOEs observed for right-handed helices is quite different from that expected for left-handed Z DNA, and because the additional demands and constraints extracted from the *J* connectivities, the known nucleotide sequence, the nature of the terminal residues, and, most of all, the directionality of some of the internucleotide NOEs, makes the assignments based on the NOE data completely unambiguous [1–8]. The NOE data on the undecamer were interpreted on a qualitative basis and shown to be indicative of a right-handed B type conformation [1], in agreement with the CD data [9]. Here, the pre-steady state NOEs are quantified and used to obtain a large number of interproton distances, both intra-

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Table 1

Direct pre-steady state NOEs (irradiation time 0.4 s) and $(\langle r^{-6}\rangle)^{-1/6}$ mean interproton distances for the double-stranded DNA undecamer

(A) Intranucleotide NOEs	H1′–H8/H6[a]		H2′–H8/H6[b]		H1′–H2′[b,d]		H1′–H2″[b]	
	% NOE	r_{ij} (Å)	% NOE	r_{ij} (Å)	% NOE	r_{ij} (Å)	% NOE	r_{ij} (Å)
A strand								
A_{1A}	−11	2.9	−9	2.3				
A_{2A}	−3	3.6						
G_{3A}			−14	2.1				
T_{4A}	−4	3.5	−21	2.0				
G_{5A}	−6	3.2	−14	2.1				
T_{6A}	−3	3.6	−23	1.9				
G_{7A}	−5	3.3	−18	2.0				
A_{8A}	−10	3.0	−37	1.8				
C_{9A}	−2	3.9	−15	2.1	−6	2.4	−10	2.2
A_{10A}	−6	3.2	−34	1.8	−10	2.2	−21	2.0
T_{11A}			−35	1.8				
B strand								
A_{1B}	−13	2.8			−8	2.3	−17	2.0
T_{2B}	−4	3.5	−22	2.0				
G_{3B}	−6	3.2	−19	2.0	−11	2.2	−12	2.2
T_{4B}			−25	1.9	−11	2.2	−15	2.1
C_{5B}	−3	3.6	−18	2.0				
A_{6B}	−6	3.2	−27	1.9	−12	2.2	−28	1.9
C_{7B}	−3	3.6	−15	2.1				
A_{8B}	−6	3.2	−34	1.8				
C_{9B}	−3	3.6	−15	2.1				
T_{10B}	−6	3.2	−15	2.1				
T_{11B}	−4	3.5						
B DNA[c]		3.9/3.7		2.2/1.9		3.0		2.3
A DNA[c]		3.9/3.6		3.9/3.8		2.6		2.3
RMS difference								
NMR − B DNA		0.6		0.2		0.8		0.3
NMR − A DNA		0.6		1.9		0.4		0.3

[a] Base–base and H1′ sugar–base distances are calculated from eqs 1 and 2 using the C(H5)–C(H6) NOE and distance (2.5 Å) as an internal reference [3]. At 23°C, the C(H5)–C(H6) interproton vectors have a cross-relaxation rate of $0.7 \pm 0.1\ s^{-1}$ and an apparent correlation time of 2.8 ± 0.6 ns [14]. (Note that the T(CH_3)–T(H6) vectors which have a $(\langle r^{-6}\rangle)^{-1/6}$ distance of 2.7 Å have a cross-relaxation rate of $0.4 \pm 0.05\ s^{-1}$ which also corresponds to a correlation time of 2.8 ± 0.6 ns [14].)

[b] Sugar–sugar and sugar–base (with the exception of H1′ sugar–base) distances are calculated from eqs 1 and 2 using the H2′–H2″ NOE and distance (1.8 Å) as an internal reference [3]. At 23°C, the H2′–H2″ interproton vectors have a cross-relaxation rate of $0.9 \pm 0.1\ s^{-1}$ and an apparent correlation time of 0.7 ± 0.2 ns [14]

[c] From the fibre diffraction data of [15]

[d] The H1′–H2′ distances represent lower limits as spin diffusion through the H1′↔H2″↔H2′ pathway is inevitable owing to the very short $r_{H2'-H2''}$ distance of 1.8 Å. Also note that $r_{H1'-H2''}$ is usually shorter and can never be longer than $r_{H1'-H2'}$ for all sugar pucker conformations

Table 1 (cont.)

(B) Internucleotide NOEs involving non-exchangeable protons

	5′-residue → 3′-residue															
	H1′→H8/H6[a]		H2′→H8/H6[b]		H2″→H8/H6[b]		H1′→H5/CH$_3$[a]		H2′→H5/CH$_3$[b]		H2″→H5/CH$_3$[b]		H8/H6→H5/CH$_3$[a]		H8/H6→H8/H6[a]	
	% NOE	r_{ij} (Å)	% NOE	r_{ij} (Å)	% NOE	r_{ij} (Å)	% NOE	r_{ij} (Å)	% NOE	r_{ij} (Å)	% NOE	r_{ij} (Å)	% NOE	r_{ij} (Å)	% NOE	r_{ij} (Å)
A strand																
$A_{1A}pA_{2A}$	−1	4.4													−1	4.4
$A_{2A}pG_{3A}$															−2	3.9
$G_{3A}pT_{4A}$	−4	3.5	−20	2.0	−11	2.2	−8	3.1	−14	2.1	−11	2.2	−13	2.8	−1	4.4
$T_{4A}pG_{5A}$	−6	3.2			−10	2.2									−1	4.4
$G_{5A}pT_{6A}$	−6	3.2	−7	2.4	−3	2.7	−3	3.6	−10	2.2	−10	2.2	−13	2.8	−2	3.9
$T_{6A}pG_{7A}$	−2	3.9	−6	2.4	−8	2.3									−2	3.9
$G_{7A}pA_{8A}$	−8	3.1													−1	4.4
$A_{8A}pC_{9A}$	−6	3.2	−5	2.5	−10	2.2							−2	3.9	−3	3.6
$C_{9A}pA_{10A}$	−5	3.3	−1	3.3	−2	2.9										
$A_{10A}pT_{11A}$	−4	3.5	−16	2.1	−10	2.2	−3	3.6	−6	2.4	−6	2.4	−13	2.8	−1	4.4
B strand																
$A_{1B}pT_{2B}$	−3	3.6	−14	2.1	−10	2.2	−4	3.5					−11	2.9	−1	4.4
$T_{2B}pG_{3B}$	−6	3.2													−1	4.4
$G_{3B}pT_{4B}$					−7	2.4	−10	3.0	−10	2.2	−10	2.2	−13	2.8	−1	4.4
$T_{4B}pC_{5B}$	−3	3.6			−9	2.3							−2	3.9	−3	3.6
$C_{5B}pA_{6B}$	−6	3.2			−7	2.4										
$A_{6B}pC_{7B}$	−4	3.5	−7	2.4	−16	2.1	−3	3.6					−2	3.9	−2	3.9
$C_{7B}pA_{8B}$	−3	3.6	−6	2.4	−9	2.3										
$A_{8B}pC_{9B}$	−6	3.2	−7	2.4	−14	2.1					−5	2.5	−4	3.5	−2	3.9
$C_{9B}pT_{10B}$	−3	3.6	−6	2.4	−6	2.4	−2	3.9	−10	2.2			−14	2.8	−1	4.4
$T_{10B}pT_{11B}$	−4	3.5					−2	3.9					−12	2.9		
B DNA[c]		2.8/2.8		3.7/3.9		2.3/2.4		3.6/3.7		3.3/2.6		2.9/2.4		3.9/3.3		5.0
A DNA[c]		4.0/3.4		1.6/1.6		3.3/3.1		3.5/4.7		3.0/2.9		4.7/4.6		3.8/3.1		4.8
RMS difference																
NMR − B DNA		0.7		0.9		0.2		0.3		0.4		0.2		0.4		0.9
NMR − A DNA		0.5		1.5		0.9		1.2		0.7		2.3		0.2		0.7

[a] Base–base and H1′ sugar–base distances are calculated from eqs 1 and 2 using the C(H5)–C(H6) NOE and distance (2.5 Å) as an internal reference [3]. At 23°C, the C(H5)–C(H6) interproton vectors have a cross-relaxation rate of $0.7 \pm 0.1\ s^{-1}$ and an apparent correlation time of 2.8 ± 0.6 ns [14]. (Note that the T(CH$_3$)–T(H6) vectors which have a $(\langle r^{-6} \rangle)^{-1/6}$ distance of 2.7 Å have a cross-relaxation rate of $0.4 \pm 0.05\ s^{-1}$ which also corresponds to a correlation time of 2.8 ± 0.6 ns [14].)

[b] Sugar–sugar and sugar–base (with the exception of H1′ sugar–base) distances are calculated from eqs 1 and 2 using the H2′–H2″ NOE and distance (1.8 Å) as an internal reference [3]. At 23°C, the H2′–H2″ interproton vectors have a cross-relaxation rate of $0.9 \pm 0.1\ s^{-1}$ and an apparent correlation time of 0.7 ± 0.2 ns [14]

[c] From the fibre diffraction data of [15]

[d] The H1′–H2′ distances represent lower limits as spin diffusion through the H1′↔H2″↔H2′ pathway is inevitable owing to the very short $r_{H2'-H2''}$ distance of 1.8 Å. Also note that $r_{H1'-H2''}$ is usually shorter and can never be longer than $r_{H1'-H2'}$ for all sugar pucker conformations

Table 1 continued on next page.

Table 1 (cont.)

(C) Internucleotide NOEs involving exchangeable protons[a]

	NMR		Fibre diffraction[c]	
	% NOE	r_{ij} (Å)	B DNA	A DNA
G_{3A}(H1)–T_{4A}(H3)	−3	3.6	3.8	4.5
G_{3A}(H1)–A_{8B}(H2)	−2	3.9	3.8	4.5
T_{4A}(H3)–A_{8B}(H2)	−12	2.9	2.9	2.9
T_{4A}(H3)–G_{5A}(H1)	−3	3.6	4.1	5.0
A_{8B}(H2)–G_{5A}(H1)	−2	3.9	4.3	5.3
G_{5A}(H1)–T_{6A}(H3)	−3	3.6	3.5	3.4
G_{5A}(H1)–A_{6B}(H2)	−4	3.5	3.8	4.5
T_{6A}(H3)–A_{6B}(H2)	−10	3.0	2.9	2.9
T_{6A}(H3)–G_{7A}(H1)	−4	3.5	4.1	5.0
A_{6B}(H2)–G_{7A}(H1)	−4	3.5	4.3	5.3
T_{4B}(H3)–A_{8A}(H2)	−10	3.0	2.9	2.9

[a] Base–base and H1′ sugar–base distances are calculated from eqs 1 and 2 using the C(H5)–C(H6) NOE and distance (2.5 Å) as an internal reference [3]. At 23°C, the C(H5)–C(H6) interproton vectors have a cross-relaxation rate of $0.7 \pm 0.1\ s^{-1}$ and an apparent correlation time of 2.8 ± 0.6 ns [14]. (Note that the T(CH_3)–T(H6) vectors which have a $(\langle r^{-6} \rangle)^{-1/6}$ distance of 2.7 Å have a cross-relaxation rate of $0.4 \pm 0.05\ s^{-1}$ which also corresponds to a correlation time of 2.8 ± 0.6 ns [14].)

[b] Sugar–sugar and sugar–base (with the exception of H1′ sugar–base) distances are calculated from eqs 1 and 2 using the H2′–H2″ interproton vectors have a cross-relaxation rate of $0.9 \pm 0.1\ s^{-1}$ and an apparent correlation time of 0.7 ± 0.2 ns [14]

[c] From the fibre diffraction data of [15]

[d] The H1′–H2′ distances represent lower limits as spin diffusion through the H1′↔H2″↔H2′ pathway is inevitable owing to the very short $r_{H2'-H2''}$ distance of 1.8 Å. Also note that $r_{H1'-H2''}$ is usually shorter and can never be longer than $r_{H1'-H2'}$ for all sugar pucker conformations

and internucleotide, which are compared to those expected for classical B and A DNA. In addition, glycosidic and C4′–C3′ bond torsion angles are deduced from the intranucleotide interproton distances by means of model building.

2. EXPERIMENTAL

The two strands of the undecamer were synthesized as in [1]. The experimental conditions were as follows: 2.3 mM duplex undecamer in 99.96% D_2O or 90% H_2O/10% D_2O containing 300 mM KCl, 15 mM potassium phosphate pH* 6.8 (meter reading uncorrected for the isotope effect on the glass electrode) and 0.18 mM EDTA. All experiments were carried out at 23°C.

NMR spectra were recorded on a Bruker AM500 spectrometer as described previously [1]. Spectra in H_2O were recorded using a time-share hard 1-1 observation pulse [10]. The NOEs were observed by interleaved difference spectroscopy with a presaturation pulse of 0.4 s, and delays of 2 s between scans to permit relaxation of the system. 1600 transients were averaged for each NOE difference spectrum. The power of the selective irradiation pulse used was sufficient to achieve effective saturation as regards NOE effects (i.e., the high power limit) whilst at the same time maintaining selectivity [11]. The estimated relative error in the NOEs, $\Delta N_{ij}/N_{ij}$, is $\leqslant \pm 0.15$. Assuming an error of ± 0.05 Å in the structure invariant reference interproton distances, the error in the interproton distances measured from the NOE data is $\leqslant \pm 0.2$ Å.

3. RESULTS

For short irradiation times t, the magnitude of the NOE, $N_{ij}(t)$, observed on the resonance of proton i following irradiation of proton j is given by

$$N_{ij}(t) \sim \sigma_{ij} t \qquad (1)$$

as the initial build-up rate of the NOE is equal to the cross-relaxation rate σ_{ij} between the two protons [11,12]. Distance ratios and distances, if one distance is already known, can then be obtained from the equation

$$r_{ij}/r_{kl} = (\sigma_{kl}/\sigma_{ij})^{1/6} \sim [N_{kl}(t)/N_{ij}(t)]^{1/6} \qquad (2)$$

providing the correlation times for the i–j and k–l interproton vectors are the same. (Note that the approximation in eq.2 remains valid up to values of t 3–4-times longer than that in eq.1 [13].)

In the quantitative pre-steady state NOE measurements presented here, the selective irradiation pulse was applied for 0.4 s as time-dependent NOE measurements for the H5–H6, CH_3–H6 and H2′–H2″ interproton vectors of the undecamer under identical experimental conditions indicated that

eqs. 1 and 2 are valid at this irradiation time [14].

To calculate interproton distances, we have used 3 intranucleotide interproton reference distances which are completely independent of the structure of the DNA, namely: $r_{H2'-H2''}$, $r_{C(H5)-C(H6)}$ and $r_{T(CH_3)-T(H6)}$ which have values of 1.8, 2.5 and 2.7 Å, respectively (note the latter distance is a $(\langle r^{-6}\rangle)^{-1/6}$ mean to take account of free rotation of the methyl group). The choice of a particular reference distance is not entirely straightforward as the apparent correlation time of the deoxyribose H2′–H2″ vector (0.7 ± 0.2 ns at 23°C) is shorter than that of the C(H5)–C(H6) and T(CH_3)–T(H6) vectors (2.8 ± 0.6 ns at 23°C) on account of its higher degree of internal mobility [14]. This choice, however, is easily made on the basis of the expected ranges of the various interproton distances and the expected motions of the different protons. This has been extensively discussed by us in [3], where we concluded that sugar–sugar and sugar–base (with the exception of the H1′ sugar–base) interproton distances should be calculated using the H2′–H2″ NOE and distance as a reference, and that base–base and H1′ sugar–base distances should be calculated using the C(H5)–C(H6) or T(CH_3)–T(H6) NOE and distance as a reference.

The complete set of intra- and internucleotide NOEs that could be quantified, together with the $(\langle r^{-6}\rangle)^{-1/6}$ mean interproton distances calculated from them, is given in table 1. A comparison of the NMR distances with those of classical B and A DNA derived from fibre diffraction data [15] is shown in table 2.

4. DISCUSSION

Examination of tables 1 and 2 clearly indicates that the interproton distances determined from the NOE measurements on the double-stranded DNA undecamer are very close to those expected for classical B DNA (RMS difference of 0.5 Å) but significantly different from those expected for classical A DNA (RMS difference of 1.1 Å). Indeed the difference between the NMR distances and those for classical A DNA is as great as that between classical B and classical A DNA (RMS difference of 1.1 Å). Further inspection of table 1 also reveals that the most sensitive distance markers for distinguishing between A and B DNA are the intranucleotide H2′–H8/H6 distance and the internucleotide distances (with the exception of the H8/H6(5′)–H5/CH_3(3′) and H8/H6(5′)–H8/H6(3′) distances and the T(N3H)–A(H2) distance in an AT base pair).

On the assumption of a single conformation, the glycosidic bond torsion angle (χ) and the sugar pucker conformation, defined in terms of the C4′–C3′ bond torsion angle (δ), can be deduced from model building on the basis of two intranucleotide sugar–base distances, namely $r_{H1'-H8/H6}$ and $r_{H2'-H8/H6}$. The *syn* and *anti* ranges for χ are 60 ± 90° and 240 ± 90° respectively. The distance $r_{H1'-H8/H6}$ has a maximum value of 3.7–3.9 Å at $\chi = 240°$ (*anti*) and a minimum value of 2.3–2.5 Å at $\chi = 60°$ (*syn*). Furthermore, each value of $r_{H1'-H8/H6}$ is compatible with two values of χ: $60° < \chi_1 < 240°$ and $\chi_2 = (240° - \chi_1) + 240°$.

Table 2

Root mean square (RMS) differences between the $(\langle r^{-6}\rangle)^{-1/6}$ mean interproton distances determined by NMR and the idealized interproton distances for classical B and A DNA derived from fibre diffraction data[a]

	Number of distances	RMS difference in interproton distances (Å)		
		NMR – B DNA	NMR – A DNA	B DNA – A DNA
Intranucleotide[b]	50	0.5	1.2	1.1
Internucleotide (non-exchangable protons)[c]	89	0.6	1.0	1.2
Internucleotide (exchangeable protons)[d]	11	0.4	1.0	0.8
Overall	150	0.5	1.1	1.1

[a]Fibre diffraction data of [15]. [b]Interproton distances given in table 1A. [c]Interproton distances given in table 1B. [d]Interproton distances given in table 1C

Given the restricted degrees of freedom imposed by the 5-membered deoxyribose ring, the $r_{H2'-H8/H6}$ distance enables one to distinguish between χ_1 and χ_2 unambiguously and to simultaneously determine the C4′–C3′ bond torsion angle (δ). Values of χ and δ obtained in this manner are given in table 3 and compared to average values derived from single crystal X-ray diffraction on short DNA oligonucleotides. It is apparent from table 3 that there is little base-to-base variation in the sugar pucker conformation which lies in the C1′–*exo* range with $\delta_{mean} = 120 \pm 10°$. This value is in good agreement with the crystallographic findings for the B DNA dodecamer 5′d(CGCGAATTCGCG)$_2$ where $\delta_{mean} = 123 \pm 21°$ [16] and with the NMR findings based on NOE distance data for the B DNA hexamer 5′d(CGTACG)$_2$ where $\delta_{mean} = 115 \pm 10°$ [2,3]. In the case of the glycosidic bond torsion angles, however, there is a significant difference between the pyrimidine and purine residues with $\chi_{pyr} = -120 \pm 10°$ and $\chi_{pur} = -90 \pm 10°$. This difference is more marked than in the crystal structure of the dodecamer where $\chi_{pyr} = -124 \pm 8°$ and $\chi_{pur} = -110 \pm 4°$ [16] and in the solution structure of the hexamer where $\chi_{pyr} = -113 \pm 6°$ and $\chi_{pur} = 108 \pm 6°$ [2,3]. This finding is therefore suggestive of a dinucleotide repeating unit in the stretches of alternating pyrimidine-purine residues within the undecamer. However the degree of variation is considerably less than in the solution structure of the DNA octamer 5′d(ACGCGCGT)$_2$ [2,3] which was found to exhibit a distinct dinucleotide repeating unit with alternations both in the glycosidic bond torsion angles ($\chi_{pur} = -70 \pm 10°$, $\chi_{pyr} = -100 \pm 10°$) and the sugar pucker conformations (purines 01′–*endo* with $\delta = 105 \pm 10°$, and pyrimidines C1′–*exo* with $\delta = 125 \pm 10°$).

With χ and δ determined, the internucleotide distances can be used to model the overall structure of the undecamer [2,3,8]. This procedure generates models with a helical rise of 3.4 ± 0.1 Å, a helical twist of $36 \pm 10°$ and a base tilt of $0 \pm 5°$, as expected for B DNA. In addition, the internucleotide distances involving the imino and A(H2) protons (see table 3C) enable one to obtain information on base-pair propellor twisting [2,3,8]. In this respect we find that the distance between the H2 proton of A_{8B} and the imino proton of G_{5A} and between the H2 proton of A_{8B} and the imino proton of G_{7A} is significantly shorter than expected for classical B DNA. Taken together with the other imino–imino and imino–A(H2) interproton distances, these findings are indicative of positive propel-

Table 3

Glycosidic (χ) and C4′–C3′(δ) bond torsion angles for the DNA undecamer deduced from model building on the basis of the intranucleotide interproton distance data and on the assumption of a single conformation

	$\chi(°)$[a]	$\delta(°)$[a]
A strand		
A_{1A}	−50	90
T_{4A}	−130	120
G_{5A}	−90	120
T_{6A}	−120	140
G_{7A}	−100	120
A_{8A}	−90	120
C_{9A}	−120	120
A_{10A}	−90	120
B strand		
T_{2B}	−130	120
G_{3B}	−90	120
C_{5B}	−120	125
A_{6B}	−90	120
C_{7B}	−120	130
A_{8B}	−90	120
C_{9B}	−120	120
T_{10B}	−100	110
Pur(mean)[b]	−90 ± 10	120 ± 10
Pyr(mean)	−120 ± 10	123 ± 10
B DNA crystal[c]		
Pur(mean)	−110 ± 14	129 ± 20
Pyr(mean)	−124 ± 8	117 ± 19
A DNA crystal[d]		
Pur(mean)	−166 ± 9	88 ± 4
Pyr(mean)	−155 ± 3	88 ± 3

[a] χ and δ are defined as δ = C5′–C4′$\overset{\delta}{}$C3′–03′, χ_{pur} = 01′–C1′$\overset{\chi}{}$N9–C4 and χ_{pyr} = 01′–C1′$\overset{\chi}{}$N1–C2, with zero at the *cis* position and positive angles by clockwise rotation of the further pair of atoms. The error in the estimation of the individual χ and δ angles is ~±10°

[b] The mean for the purine residues does not include residue A_{1A} as the values of χ and δ for this residue represent distortions due to end effects

[c] From [16]

[d] From [17]

lor twisting of the $T_{4A}A_{8B}/G_{5A}C_{7B}$ and $T_{6A}A_{6B}/G_{7A}C_{5B}$ base pairs.

5. CONCLUDING REMARKS

Here, we have demonstrated that a large number of interproton distances for a double-stranded DNA undecamer can be obtained reliably in solution by means of pre-steady state NOE measurements. These distances are similar to those expected for classical B DNA, confirming the previous qualitative interpretation of the NOE data [1] as well as the CD results [9]. The interproton distances provide a wealth of structural information with which to refine the solution structure of the undecamer. As a first approach we have used manual model building which allows one to obtain accurate and reliable estimates for the glycosidic and C4′–C3′ bond torsion angles. An alternative and more sophisticated approach is to use a non-linear least squares optimization algorithm in which all covalent bond lengths, fixed bond angles, van der Waals contacts, and hydrogen bond lengths and geometry are constrained within narrow limits, in order to refine an initial idealized B DNA model on the basis of the interproton distance data. Such a procedure is currently under development and it is hoped will yield a solution structure of the undecamer at a resolution comparable to that attainable by single crystal X-ray diffraction.

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