Comparative NMR analysis of the decadeoxynucleotide d-(GCATTAATGC)₂ and an analogue containing 2-aminoadenine

Walter J.Chazin*, Mark Rance, André Chollet¹ and Werner Leupin⁺

Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037, USA and

¹Glaxo Institute for Molecular Biology, Chemin des Aulx, CH-1288, Plan-des-Ouates, Geneva, Switzerland

Received August 22, 1991; Revised and Accepted September 19, 1991

ABSTRACT

The dodecadeoxynucleotide duplex d-(GCATTAATG-C)₂ has been prepared with all adenine bases replaced by 2-NH₂-adenine. This modified duplex has been characterized by nuclear magnetic resonance (NMR) spectroscopy. Complete sequence-specific ¹H resonance assignments have been obtained by using a variety of 2D NMR methods. Multiple quantum-filtered and multiple quantum experiments have been used to completely assign all sugar ring protons, including 5'H and 5'H resonances. The assignments form the basis for a detailed comparative analysis of the ¹H NMR parameters of the modified and parent duplex. The structural features of both decamer duplexes in solution are characteristic of the B-DNA family. The spin-spin coupling constants in the sugar rings and the relative spatial proximities of protons in the bases and sugars (as determined from the comparison of corresponding nuclear Overhauser effects) are virtually identical in the parent and modified duplexes. Thus, substitution by this adenine analogue in oligonucleotides appears not to disturb the global or local conformation of the DNA duplex.

INTRODUCTION

The introduction of base analogues into regular DNA is a common method of investigating the role and contribution of the standard bases in determining both the three-dimensional structure and the biological activity of DNA. The adenine analogue 2-NH₂-adenine (A'), a 2,6-diamino-purine, is found in S-2L cyanophage DNA, replacing all adenine bases (1,2). Recently, several studies of dA' substitution for dA have been reported. It has been observed that the substitution of dA by dA' leads to inhibition of cleavage for several restriction enzymes (3) and can influence the kinetic parameters of DNA replication, transcription and degradation by DNA and RNA polymerases and nucleases (4,5). Studies of poly (dA'-dT) by CD (6,7) and

two-dimensional ¹H NMR (8) have shown that a salt dependent transition from the standard B-form to the unusual X-form conformation occurs under certain solvent conditions. It has also been shown that small oligodeoxynucleotide duplexes containing GC basepairs with a single dA'-dT basepair crystallize more easily in a Z-conformation than the corresponding dA-dT analogs (9). The latter property of dA'-dT basepairs is not surprising, considering the strong resemblance between dA'-dT and dG-d(5-methyl-C) basepairs (both have an amino group at the C2 position of the purine base and a methyl group at the C5 position of the pyrimidine base) and the known propensity for dG-d(5-methyl-C) basepairs to form Z-DNA duplexes. Analysis of the results from the X-ray crystallographic studies lead to the proposal that in crystals of DNA duplexes containing dA'-dT basepairs, the dA-dT spine of hydration in the minor groove is perturbed due to the presence of the bulky amino group (9). This may explain differences in the biological activity of DNA in which dA' is substituted for dA.

To understand the changes in physical and biochemical properties upon substitution of an amino group for the C2 proton in an adenine base, it is important to determine if there are structural changes created by this derivatization. This requires comparison of structural parameters of DNA duplexes containing dA to those observed for duplexes substituted with dA'. Two-dimensional ¹H NMR is the method of choice for such a detailed structural comparison in solution. However, the only detailed analysis reported to date for a specific duplex is a one-dimensional ¹H NMR study of d-(TATA)₂ (10). In this report, we characterize the decanucleotide d-(GCA'TTA'A'TGC)₂ by ¹H NMR-spectroscopy and compare it to the parent decanucleotide d-(GCATTAATGC)₂ which has been analyzed previously by 2D ¹H NMR spectroscopy (11). The duplex has been designed to contain a core of six dA-dT basepairs flanked by two dG-dC basepairs. The flanking dG-dC basepairs reduce fraying of the ends of the duplex, thereby ensuring that conclusions drawn about the dA-dT core are not heavily influenced by end effects (12).

* To whom correspondence should be addressed

⁺ Present address: Pharma Division, Preclinical Research, F.Hoffmann-LaRoche Ltd, CH-4002 Basel, Switzerland

MATERIALS AND METHODS

Synthesis of Oligonucleotides and Sample Preparation

The 2-amino-2'-deoxyadenine was synthesized according to a previously published procedure (13). Synthesis of the decadeoxy nucleoside nonaphosphate d-(GCA'TTA'A'TGC) was carried out by solid-phase phosphoramidite methodology on an Applied Biosystems 380B automated synthesizer on the 10 μ mol scale. After deblocking, the DNA fragment was purified by HPLC on C₁₈ reverse phase using an acetonitrile gradient in aqueous triethylammonium acetate buffer, followed by cation exchange on a Dowex AG JDW-8X resin (Na⁺ form). The purified decadeoxynucleotide was desalted on a G-25 sephadex column and final traces of triethylammonium counter ions were removed by extensive dialysis against saturated NaCl, then against H₂O. The self-complementary duplex was annealed by heating to 95°C. After lyophilization, the NMR sample was prepared by dissolution in a 50 mM phosphate buffer containing 100 mM NaCl and 0.1% (w/v) NaN₃ at pH 7.0. This solution contained 5% ²H₂O for the spectrometer locking system. For spectra acquired in ²H₂O, the sample was lyophilized repeatedly from ²H₂O (99.996%). The parent oligonucleotide d-(GCATTAATGC) used in the UV-melting study was synthesized on an Applied Biosystems 380B synthesizer on a 1 μ mol scale and purified as outlined above. A second sample for use in the NMR studies was synthesized on a Pharmacia Gene Assembler Plus automated synthesizer on the 10 μ mol scale. Purification and sample preparation were as outlined above.

Thermal Denaturation of Oligonucleotide Duplexes

The oligonucleotides in 0.1 M NaCl, 0.01 M sodium cacodylate (pH 7.0) were melted and reannealed by heating to 95°C for 2 minutes and slow cooling to 4°C over 4 hours. The absorbance at 260 nm ($A \sim 0.6$ for both samples) was recorded as a function of temperature between 20°C and 70°C at a rate of 7°C/min. on a Hewlett-Packard 8450A UV/VIS spectrometer.

NMR Experiments

¹H NMR experiments were performed using Bruker AM-500 (¹H₂O) and AM-600 (²H₂O) spectrometers. All spectra were acquired and plotted in the phase-sensitive mode. 2QF-COSY and 3QF-COSY spectra were recorded utilizing standard pulse sequences and phase cycling (14,15). 2Q and 3Q spectra were acquired using the pulse sequence and phase cycling described in (16), with a composite 180° pulse (17). TOCSY (18,19) experiments were acquired using the modifications described by Rance (20). Pure absorption NOESY spectra were acquired using the standard pulse sequence (21). For the ²H₂O spectrum, a short Hahn-echo period was included to improve the quality of the baseline (22). The ¹H₂O NOESY spectrum was acquired with the observation pulse replaced by a jump-and-return composite pulse (23). For the 2QF-COSY, 3QF-COSY, 2Q and 3Q spectra, the carrier was placed to the low field side of the C1' protons and the base proton correlations were folded in the ω_1 dimension. For the NOESY and TOCSY spectra, the carrier was placed on the solvent resonance and time-proportional phase incrementation (24) was used to achieve quadrature detection in the ω_1 dimension.

¹H₂O NOESY spectra were acquired at 1°C, 7°C, and 28°C with $\tau_m = 190$ or 200 ms, 96 or 128 scans/ t_1 value, $t_{1max} = 25.6$ –49.6 ms. An experiment at 1°C was also acquired for the parent duplex. The selective excitation sequence was

adjusted to give two maxima, in the middle of the imino and aromatic proton regions. All other experiments were performed at 28°C. The 2QF-COSY (64 scans/ t_1 value) and 3QF-COSY (48 scans/ t_1 value) were acquired with $t_{1max} = 128$ ms. After appropriate scaling, these two spectra were co-added to generate an E-COSY spectrum (25). A P. COSY spectrum (26) with a 35° observe pulse was recorded for the parent duplex. The 2Q (32 scans scans/ t_1 value) and the 3Q (48 scans scans/ t_1 value) spectra were acquired with t_{1max} of 25.6 ms. The TOCSY spectrum was acquired with WALTZ-16 mixing for 81 ms, 32 scans/ t_1 value and a $t_{1max} = 51$ ms. The Hahn-echo NOESY spectrum ($\tau_m = 50$ ms) was acquired with 64 scans/ t_1 value and $t_{1max} = 61$ ms. An identical experiment was acquired for the parent duplex. The data were processed using an Aspect 3000 data station with Bruker software or using a CONVEX C240 with FTNMR software kindly provided by Dr. D. Hare.

RESULTS AND DISCUSSION

Thermal stability

The melting temperature (T_m) of the self-complementary oligonucleotide duplexes d-(GCATTAATGC)₂ and d-(GCA'T-A'A'GC)₂ have been measured spectrophotometrically at 260 nm under identical experimental conditions (see Materials and Methods). The resulting curves for both decanucleotides exhibit the expected sigmoidal shape and show a distinct reversible transition with an increase in absorbance of 13% upon denaturation. The derived T_m values are 39.5°C for the parent decamer and 49.5°C for the modified decamer. This increase of 10°C in T_m upon substitution of six dAs by six dA's is in accordance with other published data (e.g. 3,27,28) and can be explained by stronger base pairing due to the introduction of six additional hydrogen bonds into the duplex.

¹H NMR Resonance Assignments for d-(GCA'TA'A'GC)₂

The complete ¹H sequential resonance assignments for the parent duplex d-(GCATTAATGC)₂ have been reported previously (11). The methods used to obtain the assignments for the modified duplex with adenines replaced by A' were essentially the same. The assignments for all observed ¹H NMR resonances are given in Table 1. We note that in the years since the earlier paper, new techniques and more sophisticated applications for NMR experiments have been developed. While the assignments will be only briefly summarized here, some attempt will be made to describe the application of these newer techniques.

The ten 1'H-2'H-2''H spin subsystems were fully resolved in the 2QF-COSY (Fig. 1) and 3QF-COSY (not shown), thereby providing a basis for the complete assignment for all sugar rings. For each spin system, cross peak intensities and multiplet structure were fully consistent with the characteristic observations for C2'-endo sugar conformations as found in B-type DNA, eg. with $J_{1'2'} > J_{1'2''}$ (29). All ten 2'H-3'H cross peaks were observed in the 2QF-COSY and 3QF-COSY spectra. Although no 2''H-3'H connectivities were observed in 2QF-COSY, seven low intensity cross peaks were observed in the 3QF-COSY spectrum. Cross peak multiplet structures clearly indicated that $J_{2'3'}$ was small (<4 Hz) in all cases. The ability to observe these cross peaks in the 3QF-COSY spectrum is a direct result of the weaker dependence of cross peak intensity on the magnitude of the coupling constants in the 3QF experiment, relative to the 2QF-COSY experiment (30,31). All ten 3'H-4'H cross peaks were observed in the 2QF-COSY. Although a number

Table 1. Proton Chemical Shifts of d-(GCA' TTA' A' TGC)₂ at pH 7.0, 28°C

Res.	N1H/N3H NH ₂	Chemical shifts (p.p.m.) ^a							
		5-H/CH ₃	6H/8H	1'H	2'H	2''H	3'H	4'H	5'H/5''H
G1	13.08 ^b	—	7.94	6.00	2.63	2.81	4.86	4.25	3.70,3.70
C2	—	6.46,8.44	5.44	5.84	2.22	2.52	4.90	4.25	4.10,4.16
A'3	—	5.64,5.85 ^{b,c}	7.87	6.08	2.66	2.84	4.97	4.41	4.13,4.21
		7.78,7.85 ^{b,c}							
T4	12.89	—	1.39	5.90	2.00	2.50	4.77	4.23	4.05,4.15
T5	12.74	—	1.64	5.71	2.20	2.51	4.89	4.25	4.03,4.12
A'6	—	5.62 ^{b,c}	7.79	5.75	2.64	2.75	4.99	4.33	4.01,4.17
		7.82,7.97 ^{b,c}							
A'7	—	5.65,5.93 ^{b,c}	7.63	5.98	2.52	2.78	4.89	4.41	4.09,4.18
		7.51,7.77 ^{b,c}							
T8	12.54	—	1.37	5.70	2.08	2.43	4.81	4.19	4.04,4.12
G9	12.87	—	7.80	5.95	2.55	2.72	4.95	4.32	4.03,4.18
C10	—	6.62,8.19	5.42	6.22	2.18	2.22	4.51	4.05	4.02,4.21

^a Indirectly referenced to 2,2-dimethyl-2-silapentane-5-sulphonate, sodium salt, using the H²HO resonance previously calibrated in stock buffer solution.

^b Not observed at 301K due to rapid exchange with the solvent, values given are measured at 1°C.

^c Assignment as 2-NH₂ or 4-NH₂ cannot be made with the presently available data.

of 4'H-5'H and 4'H-5''H cross peaks were observed in the MQF-filtered COSY spectra, progress in analyzing this region of the spectra was extremely limited. However, the MQ spectra proved to be particularly useful for obtaining the 5'H/5''H assignments, as discussed in more detail below.

The tracing of the 1'H-2'H-2''H-3'H-4'H spin subsystem for each of the sugar rings was also independently possible in the TOCSY and 2Q spectra. Coherence transfer by isotropic mixing in deoxyribose sugars has been discussed by Drobny and co-workers (32). Multiple quantum spectroscopy has proven to be particularly valuable in this study. Remote peaks at $\omega_1 = (2'H + 2''H)$, $\omega_2 = (1'H)$ and $\omega_2 = (3'H)$ in the 2Q spectrum provide unique connectivities for verifying these assignments (12). In addition, 3'H-4'H connectivities that are often weak in COSY spectra are uniformly and efficiently excited in 2Q (and TOCSY) spectra. The most unique feature of 2Q (and especially 3Q, *vide infra*) spectra is the identification of scalar connectivities for assigning the 5'H and 5''H resonances. In the 2Q spectrum with a 30 ms excitation period, it was possible to locate a number of direct peaks at $\omega_1 = (4'H + 5'H)$, $\omega_2 = (4'H)$ and some weak remote peaks at $\omega_1 = (5'H + 5''H)$, $\omega_2 = (4'H)$. Examples are shown in Fig. 2. The 3Q spectrum proved to be even more informative. The complete set of sugar ring scalar connectivities was observed in the 3Q spectrum acquired with a 22 ms excitation period (Fig. 3). The (4'H + 5'H + 5''H) direct peaks (box B) proved to be particularly useful because some of the assignments of these protons are not readily obtained from any of the other scalar correlated experiments. The 5'H and 5''H assignments represent an important and to our knowledge novel application of 3Q spectroscopy. Based on the connectivities observed in the 2Q and 3Q spectra, it has been possible to identify all of the 5'H and 5''H resonances, thereby providing the first example of complete sugar ring assignments for a DNA duplex of this size.

The sequential resonance assignment was obtained by the standard method, based on the observation of characteristic cross peaks in NOESY spectra that arise as a result of the short distances between sugar ring protons and base protons of the same residue and of the sequentially adjacent residue. We use the shorthand notation of Wüthrich and co-workers (29) to specify

Figure 1. Spectral region of a 600 MHz 2QF-COSY spectrum of d-(GC-A' TTA' A' TGC)₂ showing the 1'H-2'H, 1'H-2''H and 2'H-3'H cross peaks. For each sugar spin system, the two 1'H cross peaks are connected by a horizontal line at the 1'H resonance frequency. The asterisk indicates that the 3'H resonance of C10 is folded in the ω_1 dimension.

short distances and the corresponding NOEs. The region of the Hahn-echo NOESY with a 50 ms mixing time showing NOEs between the 6H and 8H base protons and the 2'H and 2''H sugar ring protons is shown in Fig. 4. There is a good correspondence between the magnitude of the NOEs and the inverse sixth power of the inter-proton distances in this spectrum, which indicates the absence of significant spin diffusion at this relatively 'short' 50 ms mixing time (11,33). In particular, note that $d_i(2';6,8) >$

Figure 2. Spectral region of a 600 MHz 2Q spectrum of d-(GCA' TTA' A' TGC)₂ showing some of the (3'H+4'H), (4'H+5'H), (4'H+5''H), and (5'H+5''H) direct peaks, and the (5'H+5''H) remote peaks. The remote peaks are highlighted with boxes.

Figure 3. Survey of the 600 MHz 3Q spectrum of d-(GCA' TTA' A' TGC)₂. Some of the characteristic spectral region are boxed: A) $\omega_1 = (1'H+2'H+2''H)$, $\omega_2 = (1'H)$; B) $\omega_1 = (4'H+5'H+5''H)$, $\omega_2 = (4'H, 5'H, 5''H)$; C) $\omega_1 = (1'H+2'H+2''H)$, $\omega_2 = (2'H, 2''H)$; D) $\omega_1 = (1'H+2'H+3'H)$, $\omega_2 = (2'H)$.

$d_i(2'';6,8)$ as expected for a standard B-form DNA duplex (29), and that both $d_i(2'';6,8)$ and $d_s(2';6,8)$ only show up very weakly, if at all. Furthermore, although these very weak cross peaks correspond to inter-proton distances that are very similar to $d_i(6,8;1')$ and $d_s(1';6,8)$, the absence of these latter cross peaks in the spectrum provides clear evidence that the intensity of the $d_i(2'';6,8)$ and $d_s(2';6,8)$ cross peaks are significantly enhanced by spin diffusion via their geminal partner.

The sequence-specific resonance assignments of the labile protons were obtained by the usual methods from NOESY spectra

Figure 4. Spectral region of a 600 MHz Hahn-echo NOESY spectrum of d-(GCA' TTA' A' TGC)₂ showing the cross peaks from 2'H and 2''H resonances to H6 and H8 resonances. Intra-residue connectivities are boxed and sequential connectivities are circled. The base proton resonance frequencies are labelled at the top. The strong peaks arise from short inter-proton distances $d_i(6,8;2')$ and $d_s(2'';6,8)$, whereas the weak peaks are $d_i(6,8;2'')$ and $d_s(2';6,8)$ connectivities that arise from spin diffusion.

Table 2. Comparison of NOE Intensities to the 6H/8H Resonances in the Parent and Modified Duplexes^a

$d_i(2')$	$d_i(2'')$	$d_s(2')$	$d_s(2'')$	$d_i(1')$	$d_s(1')$	$d_i(\text{Me})$	$d_s(\text{b;Me})$
pG ₁	8.0	1.0	—	—	0.5	—	1.2 ^b
mG ₁	7.4	1.3	—	—	0.9	—	1.6
pC ₂	8 ^c	0.9	0.7	2.2	0.2	0.6	—
mC ₂	9 ^c	1.7	0.7	2.2	0.6	1.0	—
pA ₃	9.1	1.2	0.7	2.6	0.5	0.9	4.3
mA ₃	7.8	1.5	1.3	2.6	0.8	1.6	4.0
pT ₄	7.7	1.1	0.4	4.9	0.4	0.8	9.4
mT ₄	6.0	1.2	1.4	4.4	0.9	1.4	9.0
pT ₅	8.3	1.4	1.4	3.8	<1	<1	6.0
mT ₅	11.3 ^c	(4.8) ^d	1.7	(4.8) ^d	<0.9	0.9	6.2
pA ₆	5.6	0.8	0.6	1.6	<0.6	<0.6	—
mA ₆	6.0	1.7	1.0	2.1	0.4	0.5	—
pA ₇	6.4	0.6	1.3	3.6	1.2	<0.6	3.5
mA ₇	6.2	(3.8) ^d	1.0	(3.8) ^d	1.0	1.9	3.6
pT ₈	8.6	1.3	1.3	4.2	0.4	0.4	7.0
mT ₈	8.3	1.4	1.8	4.0	1.5	1.0	6.8
pG ₉	11.1	2.0	1.4	2.5	0.7	0.5	—
mG ₉	9.9	2.2	2.1	2.9	1.2	1.2	—

^a Integrals given in arbitrary units after normalization of the two spectra to the 6H-5H cross peaks of C₂ and C₁₀.

^b 5'/5'' reported for G₁.

^c Cross peak not fully resolved, integral less reliable.

^d Only the sum of $d_i(2'')$ + $d_s(2'')$ can be measured.

acquired in ¹H₂O solution. Only a limited number of exchangeable imino and amino protons are observable at 28°C. As expected at this temperature, the imino proton of the first GC basepair is exchange broadened beyond detection, and that of second GC basepair is so broad that it can only be located in the 1D spectrum. It is interesting to note that at 28°C the linewidths of the cytosine amino proton resonances of the dG-dC basepairs are substantially narrower than that of the corresponding imino protons and that NOEs are observed only to the former in the NOESY spectrum. The slowly exchanging

Figure 5. Profiles of the sequence dependence of the changes in ^1H chemical shift induced by the incorporation of an amino group at position C2 of all adenines in $\text{d}-(\text{GCATTAATC})_2$. A positive value indicates a shift to higher field (smaller value in ppm) in the modified duplex.

amino protons can be assigned to the 4- NH_2 resonances of C2 and C10 by intraresidue NOEs to the corresponding 5H resonances. At 1°C all five imino protons are observed, with rapid solvent exchange affecting only the N1H of G1. It is also possible to detect additional amino proton resonances at lower temperatures and these were assigned to the appropriate basepair by intraresidue NOEs to the corresponding imino protons. At present we are not able to reliably assign the adenine amino proton resonances although the pairs of resonance frequencies listed in the table are clearly identified because NOEs from these broad signals are observed to the corresponding imino proton. The uncertainty lies only in the distribution of the four protons to the two signals observed. The relative intensities of the NOE cross peaks suggest that the broad high-field signals may comprise as many as three protons resonances. However, there are no other unambiguous NOEs that would permit specific assignments. This includes no NOEs evident between the ~ 5.6 ppm and $7.5\text{--}7.8$ ppm resonances themselves, probably because both lines are very broad. By analogy to the chemical shifts observed for the adenine amino groups in the parent duplex, the resonances at $7.5\text{--}7.8$ ppm most likely arise from the A' 6- NH_2 protons that are H-bonded and the broad resonances at ~ 5.6 ppm from the non-H-bonded 6- NH_2 protons. The A' 2- NH_2 protons are assigned to the broadest resonances at ~ 5.6 ppm.

Comparative analysis of the modified and parent duplex

The changes in chemical shift induced by the modification of the DNA duplex are plotted versus the sequence in Fig. 5. Although a direct interpretation of changes in chemical shift in terms of

Figure 6. Comparison of the $1'\text{H}\text{--}2'\text{H}$ and $1'\text{H}\text{--}2''\text{H}$ cross peaks of A3 and A7 from the same 2QF-COSY spectrum of $\text{d}-(\text{GCA}'\text{TTA}'\text{A}'\text{TGC})_2$ as shown in Figure 1. The spectrum was processed with the values of $t_{1\text{max}}$ and $t_{2\text{max}}$, window functions, and zero-filling identical to the spectrum shown in Figure 2 of (11).

structural parameters is not possible, the chemical shift is accepted as a sensitive indicator of *differences* in homologous molecules. The graphs in Fig. 5 demonstrate that the chemical shifts of nucleotides A3 to T8 are clearly influenced by amination at the C2 position of adenine. For the adenine nucleotides, all proton resonances are shifted to higher field with the largest effects observed at the 8H resonances. Substantial effects on the chemical shifts of the C1' protons are also observed. For the thymine nucleotides, a large effect is observed for the 2'H resonances of T5 and T8, but the corresponding resonance in T4 is not significantly perturbed. The general trends observed in this study are similar to that observed for poly $(\text{dA}'\text{--dT})\cdot\text{poly}(\text{dA}'\text{--dT})$ at low salt (8), poly $(\text{dA}\text{--dT})\cdot\text{poly}(\text{dA}\text{--dT})$ (34,35), $\text{d}-(\text{TA}'\text{TA}')_2$ (10) and $\text{d}-(\text{TATA})_2$ (36).

A comparison of the chemical shifts of the labile imino and amino protons of the parent and modified duplex has also been made. NOESY experiments were acquired at 1°C to minimize the exchange of the labile protons with solvent, thereby maximizing the number of observable resonances in the spectrum. The imino and C4 amino protons of the two GC basepairs resonate at virtually identical frequencies in the two duplexes. In contrast, the imino proton resonances of the $\text{dA}'\text{--dT}$ basepairs have shifted >0.7 ppm upfield into the spectral region of the imino protons of the GC basepairs. This change in chemical shift suggests the presence of an additional H-bond in the $\text{dA}'\text{--dT}$ basepair, consistent with the increase in the stability of the modified duplex. Although specific assignments are not available for the adenine amino protons of the modified duplex, if the two discrete amino resonance frequencies observed for each dA' residue are assumed to correspond to the 6- NH_2 protons (the sole amino group in dA residues), then a general downfield shift of ~ 0.2 ppm is observed for the H-bonded amino proton and a general upfield shift of ~ 0.6 ppm is observed for the non-H-bonded amino proton. It is not clear whether these changes in chemical shift can be correlated with differences in the H-bonding of the 6- NH_2 group in the modified duplex because the additional 2- NH_2 group: (i) introduces changes in the electronic

Table 3. Spin-spin Coupling Constants in d-(GCAATTAGC)₂ and d-(GC-A'A'TTA'GC)₂ at pH 7.0, 28 °C.^a

Parent	J _{1'2'} (Hz)	J _{1'2''} (Hz)	Modified	J _{1'2'} (Hz)	J _{1'2''} (Hz)
G1	8.9	5.7	G1	8.5	5.8
C2	9.5	5.4	C2	9.3	6.0
A'3	9.5	6.2	A'3	8.9	5.8
T4	8.8	6.1	T4	7.9	7.0
T5	9.0	5.8	T5	9.2	5.9
A'6	10.3	5.6	A'6	9.9	5.6
A'7	9.2	6.6	A'7	8.3	6.2
T8	8.8	6.0	T8	8.3	6.3
G9	8.9	5.7	G9	8.2	6.2
C10	7.2	7.2	C10	7.2	7.2

^a Values measured from an E-COSY spectrum (P. Cosy for the parent duplex) to a precision of ± 1 data point (0.35 Hz).

structure of the base; (ii) creates greater geometric constraints (due to there being three rather than two mutually adjacent coplanar H-bonds).

There are several factors that can affect the chemical shift parameter and the exact nature of their relative influence is not yet fully understood (37). It is therefore not possible to determine from our *chemical shift* data whether the derivitization of adenine has introduced any significant structural effects into the DNA duplex. In fact, the observed differences in chemical shifts could conceivably be solely due to the change in the electronic structure of the base; the A' base protons affected by differences in the through-bond shielding and the other protons by differences in the inherent ring current of the modified base.

The introduction of *significant* structural perturbations of the DNA as a result of the incorporation of dA' should be identifiable by comparing the magnitude of NOEs of corresponding proton pairs in the two duplexes. Quantitative comparisons have been made by acquiring NOESY spectra under identical conditions for the parent and modified duplexes. The normalized cross peak intensities measured for d(2';6,8), d(2'';6,8), d(1';6,8) and d(CH₃;6,8) intra-residue and sequential NOEs are listed in Table 2. In the light of the estimated precision in these values, there is an extremely close correspondence in these data. Even subtleties such as the relative ratios of d_i(2';6,8) to d_s(2'';6,8) NOEs are faithfully reproduced. These results provide strong, direct evidence that the local structure of the DNA duplex is not substantially effected by the modification of the adenine bases under the low salt conditions employed in these studies. Although similar general conclusions have been drawn from the results of ¹H NMR studies of poly (dA'-dT)·poly (dA'-dT) at 0.1 M NaCl (8) and of d-(TA'TA')₂ (10), the results reported here are more conclusive because the spectral resolution is far superior to that for the large polymer and there is little possibility of the end effects that are likely to dominate for the tetranucleotide duplex.

The ensemble of scalar coupling constants in each sugar ring of the modified DNA duplex has been comparatively analyzed versus the parent duplex, first by visual inspection of the cross-peak multiplet structure observed in 2QF-COSY spectra and in some cases, quantitatively. For example, the 1'H-2'H, 1'H-2''H and 2'H-3'H cross peaks of the modified duplex shown in Fig. 1 can be compared with the left panel of Fig. 11 in (11); the cross peak multiplet structures are virtually identical. An expanded view of the 1'H-2'H and 1'H-2''H cross peaks of A3 and A7 is given in Fig. 6 to demonstrate the details of cross peak multiplet fine

structure; this can be compared to Fig. 2 in (11). Similar detailed comparisons have been made for all resolved cross peaks of the modified duplex. We find no substantial differences (> 1 Hz) in the values of the deoxyribose sugar ring coupling constants. Furthermore, the 1'H-2'H and 1'H-2''H scalar couplings of both the parent and modified DNA duplexes have been quantitatively evaluated in E-COSY spectra and compared (Table 3). The values are clearly very similar. For all residues but G1, the sum $J_{1'2'} + J_{1'2''} > 13.5$ Hz, indicating the predominance of the S-type sugar ring conformation (38) as is typical for deoxyribose sugars in B-type DNA duplexes.

Finally, ³¹P NMR spectra (data not shown) have also been acquired for the parent and modified duplexes. The chemical shifts are found to be very similar, all located in the narrow spectral range that is typical of B-type DNA duplexes. The great similarity of the spectra provides further evidence for the absence of any large structural perturbation due to amination of the C2 position of the adenine residues.

CONCLUSIONS

A detailed biophysical characterization of d-(GCA'TTA'A'TG-C)₂ has been carried out under low salt conditions, and the results compared to the unmodified duplex. UV melting curves show that the modified duplex is thermodynamically more stable than the parent duplex, as indicated by the increase of 10 °C in the melting temperature. The pucker of the deoxyribose sugar rings, as determined from measurements of spin-spin couplings and of the relative spatial disposition of protons of the bases and sugars from NOEs, have been compared and are found to be virtually identical. The data indicate the predominance of C2'-endo deoxyribose sugar ring conformations, characteristic of B-type DNA duplexes. The presence of the modified bases has no *substantial* effect on the solution conformation of the DNA duplex even though base pair stability and dynamics are affected by the addition of a third hydrogen bond to the dA-dT basepairs. However, the observed differences in the chemical shifts of certain non-labile protons (Fig. 5) may be indicative of *subtle* structural differences that are beyond the limits of sensitivity of the NOE and scalar coupling parameters. We conclude that upon the introduction of the 2-NH₂ group in dA, the factors causing the substantial local and/or global structural perturbations reported under high salt conditions (6–8) are not effective under low salt conditions. The results reported here form a basis for understanding some of the genetic properties and the functional role of the AT → A'T conversion, and for ongoing studies of the relative rates and binding affinities of drugs that bind in the minor groove of the parent and modified duplexes.

ACKNOWLEDGEMENTS

We thank Guidon Ayala for technical assistance with synthesis and purification of the derivatized decamer and Annette Gonzales for preparation of Figure 6. M.R. gratefully acknowledges support by the National Institutes of Health (grant GM40089) and the National Science Foundation (grant DMB8903777).

ABBREVIATIONS

A', 2-NH₂-deoxyadenosine; CD, circular dichroism; NMR, nuclear magnetic resonance; 1D, one-dimensional; 2D, two-dimensional; NOE, nuclear Overhauser effect; NOESY, 2D NOE spectroscopy; COSY, correlated spectroscopy; 2Q(3Q), two (three) quantum; 2QF-(3QF)-COSY, 2Q (3Q) filtered-COSY; TOCSY, total correlation spectroscopy.

REFERENCES

1. Kirnos, M.D., Khudyakov, I.Y., Alexandrushkina, N.I. and Vanyushin, B.F. (1977) *Nature*, **270**, 369–370.
2. Khudyakov, I.Y., Kirnos, M.D., Alexandrushkina, N.I. and Vanyushin, B.F. (1978) *Virology*, **88**, 8–18.
3. Chollet, A. and Kawashima, E. (1988) *Nucleic Acids Res.*, **16**, 305–317.
4. Sagi, J., Vorlickova, M., Kypr, J. and Otvos, L. (1989) *Biochem. Biophys. Res. Commun.*, **161**, 1204–1212.
5. Sagi, J., Ebinger, K., Vorlickova, M., Kypr, J. and Otvos, L. (1990) *J. Biomol. Struct. Dyn.*, **7**, 1073–1082.
6. Vorlickova, M., Sagi, J., Szaboles, A., Szemzo, A., Otvos, L. and Kypr, J. (1988) *Nucleic Acids Res.*, **16**, 279–289.
7. Vorlickova, M., Sagi, J., Szaboles, A., Szemzo, A., Otvos, L. and Kypr, J. (1988) *J. Biomol. Struct. Dyn.*, **6**, 503–510.
8. Borah, B., Cohen, J.S., Howard, F.B. and Miles, H.T. (1985) *Biochemistry*, **24**, 7456–7462.
9. Coll, M., Wang, A.H., Van der Marel, G.A., Van Boom, J.H. and Rich, A. (1986) *J. Biomol. Struct. Dyn.*, **4**, 157–172.
10. Rinkel, L.J., Mellema, J., Van der Marel, G.A., Van Boom, J.H. and Altona, C. (1986) *Eur. J. Biochem.*, **154**, 259–265.
11. Chazin, W.J., Wüthrich, K., Hyberts, S., Rance, M., Denny, W.A. and Leupin, W. (1986) *J. Mol. Biol.*, **190**, 439–453.
12. Grütter, R., Otting, G., Wüthrich, K. and Leupin, W. (1988) *Eur. Biophys. J.*, **16**, 279–286.
13. Chollet, A., Chollet-Damerius, A. and Kawashima, E. (1986) *Chemica Scripta*, **26**, 37–40.
14. Rance, M., Sorensen, O.W., Bodenhausen, G., Wagner, G., Ernst, R.R. and Wüthrich, K. (1983) *Biochem. Biophys. Res. Commun.*, **131**, 1094–1102.
15. Rance, M. and Wright, P.E. (1986) *J. Magn. Reson.*, **66**, 372–378.
16. Braunschweiler, L., Bodenhausen, G. and Ernst, R.R. (1983) *Mol. Phys.*, **48**, 535–560.
17. Levitt, M. and Freeman, R. (1979) *J. Magn. Reson.*, **33**, 473–476.
18. Braunschweiler, L. and Ernst, R.R. (1983) *J. Magn. Reson.*, **63**, 521–528.
19. Bax, A. and Davis, D.G. (1985) *J. Magn. Reson.*, **65**, 355–360.
20. Rance, M. (1987) *J. Magn. Reson.*, **74**, 557–564.
21. Macura, S. and Ernst, R.R. (1980) *Mol. Phys.*, **41**, 95–117.
22. Davis, D.G. (1989) *J. Magn. Reson.*, **81**, 603–607.
23. Plateau, P. and Guéron, M.J. (1982) *J. Amer. Chem. Soc.*, **104**, 7310–7311.
24. Marion, D. and Wüthrich, K. (1983) *Biochem. Biophys. Res. Commun.*, **113**, 967–974.
25. Griesinger, C., Sorensen, O.W. and Ernst, R.R. (1987) *J. Magn. Reson.*, **75**, 474–492.
26. Marion, D. and Bax, A. (1988) *J. Magn. Reson.*, **80**, 528–533.
27. Cheong, C., Tinoco, Jr., I. and Chollet, A. (1988) *Nucleic Acids Research*, **16**, 5115–5122.
28. Hoheisel, J.D. and Lehrach, H. (1990) *FEBS Lett.*, **274**, 103–106.
29. Wüthrich, K. (1986) 'MR of Proteins and Nucleic Acids', Wiley, New York.
30. Müller, N., Ernst, R.R. and Wüthrich, K. (1986) *J. Amer. Chem. Soc.*, **108**, 6482–6492.
31. Rance, M., Chazin, W.J., Dalvit, C. and Wright, P.E. (1989) *Meth. in Enzymology*, **176**, 114–134.
32. Flynn, P.F., Kinatar, A., Reid, B.R. and Drobny, G. (1988) *Biochemistry*, **27**, 1191–1197.
33. Lefèvre, J.-F., Lane, A.N. and Jardetzky, O. (1987) *Biochemistry*, **26**, 5076–5090.
34. Assa-Munt, N. and Kearns, D.R. (1984) *Biochemistry*, **23**, 791–796.
35. Borah, B., Cohen, J.S. and Bax, A. (1985) *Biochemistry*, **24**, 747–765.
36. Mellema, J.-R., Pieters, J.M.L., van der Marel, G.A., van Boom, J.H., Haasnoot, C.A.G. and Altona, C. (1984) *Eur. J. Biochem.*, **143**, 285–301.
37. Shindo, H., Okhubo, S., Matsumoto, U., Giessner-Prettre, C. and Zon, G. (1988) *J. Biomol. Struct. Dyn.*, **5**, 913–931.
38. Rinkel, L.J. and Altona, C. (1987) *J. Biomol. Struct. Dynam.*, **4**, 621–649.