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Hydration of DNA in aqueous solution: NMR evidence for a kinetic destabilization of the minor groove hydration of d-$(TTAA)_2$ versus d-$(AATT)_2$ segments

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ABSTRACT

The residence times of the hydration water molecules near the base protons of d-$(GTGGAATTCCAC)_2$ and d-$(GTGGTTAACCAC)_2$ were investigated by nuclear magnetic resonance (NMR) spectroscopy. Nuclear Overhauser effects (NOE) were observed between base protons of the DNA and hydration water in NOESY and ROESY experiments. Large positive NOESY cross peaks observed between the resonances of the water and the adenine 2H protons of the central d-$(AATT)_2$ segment in the duplex d-$(GTGGAATTCCAC)_2$ indicate the presence of a 'spine of hydration' with water molecules exhibiting residence times on the DNA longer than 1 nanosecond. In contrast, no positive intermolecular NOESY cross peaks were detected in the d-$(TTAA)_2$ segment of the duplex d-$(GTGGTTAACCAC)_2$, indicating that no water molecules bound with similarly long residence times occur in the minor groove of this fragment. These results can be correlated with the larger width of the minor groove in d-$(TTAA)_2$ segments as compared to that in d-$(AATT)_2$ segments, as observed previously in single crystal structures of related oligonucleotide duplexes in B type conformation. The present experiments confirm earlier experimental results from single crystal studies and theoretical predictions that a 5′-dTA-3′ step in the nucleotide sequence interrupts the spine of hydration in the minor groove.

INTRODUCTION

Distinct hydration patterns are observed in DNA around the sugar–phosphate backbone and in the major and minor grooves (e.g. 1–5). It is believed that the hydration of the backbone is related to the global conformation of DNA, whereas the hydration of the grooves is far more sequence dependent than the hydration of the backbone. Furthermore, hydration water molecules located in the grooves of the DNA are in a position to mediate specific contacts to DNA binding ligands and may play a special role in protein–DNA recognition (6–8).

Considerable sequence dependent variations of the local structure have been observed within the B-type family of DNA conformations ever since Dickerson and coworkers solved the single crystal structure of the self-complementary dodecamer d-$(CGCGAATTCGCG)_2$ (1–4). For this particular nucleotide sequence, the minor groove was found to be significantly narrower in the central d-$(AATT)_2$ region than for the two flanking segments containing GC base pairs. At the same time, a well ordered zig-zag array of water molecules was detected in the minor groove. The water molecules in this 'spine of hydration' bridge N3 of adenine and O2 of thymidine in consecutive base pairs and contribute significantly to the stability of this particular conformation (5).

Recent ^1H NMR experiments showed that the spine of hydration in the minor groove of d-$(CGCGAATTCGCG)_2$ is also particularly stable in aqueous solution (9, 10). Thereby, the residence times of the hydration water molecules were assessed from the sign of the NOEs detected between the water signal and the resonances of the DNA in NOESY (NOE spectroscopy in the laboratory frame of reference) and ROESY (NOE spectroscopy in the rotating frame of reference) experiments. Most of the water–DNA NOEs displayed negative or vanishing cross peak intensities in NOESY, which indicates that the residence times of the corresponding hydration water molecules must be much shorter than 1 nanosecond (9, 10). However, positive NOESY cross peaks were observed with the adenine 2H protons in the minor groove indicating significantly longer residence times for the hydration water molecules of the spine of hydration which are in close vicinity of the A2H protons. Since all hydration water molecules are accessible to bulk water, the water molecules of the spine of hydration are thus surprisingly inert against chemical exchange which emphasizes their structural role in stabilizing the DNA conformation.

The proposal that d-$(A_nT_n)_2$ segments in duplexes of B type DNA ($n \geq 2$) are generally associated with a small minor groove

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width of about 5Å is corroborated by at least two further experimental observations. First, a narrow minor groove has been observed in the single crystal structures of the dodecamer, d-(CGCAAATTTGCG)$_2$, both free (11, 12) and complexed with the minor groove binding ligand berenil (13). Second, hydration water with long residence times were detected by ^{1}H NMR in aqueous solution in the minor groove of d-(AAAAATTTTT)$_2$ (10) and other DNA fragments containing d-(A)$_n$·d-(T)$_n$ segments (14).

In contrast to the DNA fragments mentioned above, DNA duplexes containing a d-(T$_n$A$_n$)$_2$ segment, i.e. a 5′-d-TA-3′ step, exhibit a wider minor groove and a disordered hydration pattern in their minor grooves. Such a behaviour was suggested by Chuprina (15) based on theoretical calculations and has recently been confirmed by the results of the single crystal X-ray structure determination of the decanucleotide d-(CGATTAATCG)$_2$ (16). The central d-(TTAA)$_2$ segment in this duplex exhibits the largest minor groove width in this duplex and no regular spine

Table 1. Chemical shifts for the assigned protons of d-(GTGGAATTCCAC)$_2$ at 4°C and pH 7.0[a]

base	1′H	2′,2″H	3′H	4′H	C5H	TCH$_3$	6H	8H	A2H	C4NH$_2$	G1NH/T3NH	A6NH$_2$/G2NH$_2$	OH
G$_1$	5.91	2.57 2.70	4.73	4.13				7.90			12.80	nd	6.06
T$_2$	5.65	1.93 2.26	4.76	4.08		1.25	7.22				13.93		
G$_3$	5.37	2.50 2.55	4.87	4.21				7.70			12.84	7.9 5.3	
G$_4$	5.38	2.49 2.62	4.91	4.27				7.67			12.49	nd	
A$_5$	5.90	2.47 2.85	4.96	4.38				8.00	7.06			7.33 5.68	
A$_6$	6.07	2.45 2.86	4.92	4.37				8.04	7.52			7.03 6.02	
T$_7$	5.84	1.91 2.50	4.75	4.11		1.13	7.06				13.73		
T$_8$	6.03	2.08 2.49	4.82	4.14		1.41	7.28				13.84		
C$_9$	5.85	2.02 2.38	4.87	4.13	5.47		7.44			8.12 6.69			
C$_{10}$	5.27	1.99 2.27	4.76	4.03	5.53		7.42			8.51 6.91			
A$_{11}$	6.18	2.64 2.81	4.93	4.33				8.22	7.83			8.10 6.34	
C$_{12}$	5.97	2.00 2.00	4.39	3.91	5.27		7.28			8.15 6.85			6.45

[a]In ppm using a water chemical shift of 5.0 ppm. The 2′ and 2″ protons were not stereospecifically assigned. The 5′ and 5″ protons were not assigned.
nd = not determined.

Table 2. Chemical shifts for the assigned protons of d-(GTGGTTAACCAC)$_2$ at 4°C and pH 7.0[a]

base	1′H	2′,2″H	3′H	4′H	C5H	TCH$_3$	6H	8H	A2H	C4NH$_2$	G1NH/T3NH	A6NH$_2$/G2NH$_2$	OH
G$_1$	5.91	2.57 2.72	4.73	4.15				7.90			12.81	nd	6.09
T$_2$	5.79	2.07 2.44	4.81	4.16		1.25	7.28				13.81		
G$_3$	5.61	2.60 2.64	4.91	4.28				7.79			12.74	7.9 5.3	
G$_4$	5.87	2.48 2.66	4.84	4.35				7.63			12.72	nd	
T$_5$	5.87	1.95 2.43	4.74	4.12		1.19	7.17				13.87		
T$_6$	5.55	1.96 2.33	4.78	4.01		1.52	7.28				13.53		
A$_7$	5.78	2.66 2.80	4.96	4.33				8.20	6.70			7.54 6.27	
A$_8$	5.97	2.48 2.73	4.90	4.37				8.03	7.47			7.46 5.90	
C$_9$	5.63	1.87 2.23	4.63	4.05	5.02		7.11			7.87 6.42			
C$_{10}$	5.34	1.98 2.28	4.70	3.99	5.42		7.38			8.42 6.82			
A$_{11}$	6.17	2.59 2.81	4.91	4.32				8.19	7.78			8.11 6.30	
C$_{12}$	5.97	2.00 2.00	4.40	3.91	5.27		7.28			8.14 6.85			6.45

[a]In ppm using a water chemical shift of 5.0 ppm. The 2′ and 2″ protons were not stereospecifically assigned. The 5′ and 5″ protons were not assigned.
nd = not determined.

of hydration was observed in the minor groove. NMR data of related DNA duplexes support the view that d-$(TTAA)_2$ tracts are associated with wider minor grooves than d-$(AATT)_2$ segments in aqueous solution, too (17, 18).

The results mentioned above suggest that the widening of the minor groove at 5′-d-TA-3′ steps and the less ordered arrangement of the hydration water molecules in the minor groove might also correlate with shortened residence times of the water molecules in the minor groove. To test this hypothesis, we have performed the present ^1H NMR study, in which we have searched for hydration water molecules with long residence times in the self-complementary duplex d-$(GTGGAATTCCAC)_2$ and the corresponding DNA duplex where the central d-$(AATT)_2$ segment was replaced by the sequence d-$(TTAA)_2$.

MATERIALS AND METHODS

NMR detection of hydration water

Individual hydration water molecules can be detected by the observation of NOEs between the ^1H NMR signal of the water and the DNA protons. In two-dimensional NOESY and ROESY experiments, the NOEs are detected as cross peaks between the water signal and the DNA resonances. Because of the degeneracy of the chemical shifts of all water protons, the water−DNA cross peaks are found in the same cross section taken through the diagonal peak of the water resonance. The sign of the NOESY cross peaks is sensitive to the residence times of the hydration water molecules: the NOE is negative or positive, respectively, if the intermolecular dipole−dipole interaction pertains for longer or shorter than approximately 500 picoseconds (19). Note that negative NOEs lead to positive NOESY cross peaks, i.e. with the same sign as the diagonal peaks, and vice versa. In contrast, the NOEs are always positive in ROESY. Only very weak NOEs are expected in either NOESY or ROESY, if the residence times of the water molecules become much shorter than 100 ps (19). However, the intermolecular NOEs are more easily detected in ROESY than in NOESY, if the modulation of the intermolecular dipole−dipole interaction occurs in the time range where the NOE inverts its sign in the NOESY experiment. In the following we will always refer to the sign of the cross peaks rather than the sign of the NOEs.

NMR sample preparation

The self-complementary DNA sequences 5′-d-$G_1T_2G_3G_4A_5A_6$-$T_7T_8C_9C_{10}A_{11}C_{12}$-3′ and 5′-d-$G_1T_2G_3G_4T_5T_6A_7A_8C_9C_{10}A_{11}C_{12}$-3′ were synthesized on a Pharmacia DNA-synthesizer. After cleavage of the protecting groups by heating in concentrated ammonium hydroxide, the samples were purified by anion-exchange chromatography on Q-Sepharose columns with a sodium cloride gradient (0.5 M NaCl to 2.0 M NaCl) as eluting solvent and finally desalted by repeated dialysis against water. The lyophilized DNA samples were dissolved in a mixture of 90% H_2O/10% D_2O and the pH was adjusted to 7.0 by the addition of NaOH. The final concentration of the samples was about 1 mM in DNA duplex. No further salts or buffers were added to avoid the catalysis of the chemical exchange of labile DNA protons with water (e.g. 20, 21). The low concentration of exchange catalysts was verified by the observation of relatively narrow hydroxyl proton resonances from the 3′ and 5′ terminal desoxyriboses of the duplexes (see below).

NMR measurements

For each DNA duplex two-dimensional NOESY and ROESY spectra were recorded under identical conditions on a Bruker AMX2-600 NMR spectrometer: temperature 4°C, mixing time τ_m = 50 ms, t_{1max} = 71.2 ms, t_{2max} = 167.9 ms, time domain data size 800×096 points. The total recording time was about 9 hours per spectrum. Water suppression was achieved by appending a delay τ and a spin-lock pulse of 2.5 ms duration to the conventional NOESY and ROESY pulse schemes [Figure 1 in ref. (22) and Figure 1B in ref. (23)]. The delay τ was set to 156 μs which resulted in a spectral excitation profile described by $\sin[0.59(\delta_2-5.0)]$, where δ_2 is the chemical shift in ppm along the F_2 frequency axis. Four homospoil pulses of 2 ms duration each were applied at regular intervals during the NOESY mixing time to prevent radiation damping (24) and for improved water suppression (22). After Fourier transformation, the spectra were baseline corrected in both dimensions.

RESULTS AND DISCUSSION

Complete resonance assignments were obtained for virtually all labile and non-labile base protons and most of the sugar protons (Tables 1 and 2) using standard methods (25). These assignments present the basis for the observation of the intermolecular NOESY cross-peaks between the resonances of the water and the DNA duplexes. Under the conditions used, no DNA resonances are at the same chemical shift as the water signal which facilitates the assignment of the NOEs with the hydration water.

Intermolecular NOE cross-peaks between H_2O and d-$(GTGGAATTCCAC)_2$

Figure 1 shows the one-dimensional ^1H NMR spectrum and cross sections through the two-dimensional ROESY and NOESY spectra of d-$(GTGGAATTCCAC)_2$ taken along the F_2 frequency axis at the F_1 chemical shift of the water resonance. Positive cross-peaks in the cross section of the ROESY spectrum indicate the presence of chemical exchange between the water and the imino protons of the terminal base pairs and the hydroxyl protons G1 5′OH and C12 3′OH of the terminal deoxyriboses. The broad positive ROESY cross peak at about 7.2 ppm may be attributed to chemical exchange between protons of the water and G1 NH_2. Like in the earlier hydration studies of DNA (10), positive ROESY cross peaks are observed also with the 5′ and 5' protons of G1 which originate from a homonuclear Hartmann−Hahn effect with the intense exchange cross-peak of the G1 5′OH resonance.

With two exceptions, all negative ROESY cross peaks represent intermolecular NOEs between protons of hydration water molecules and the DNA. The exceptions are the cross peaks with the protons 2′H, 2″H and 4′H of C12 which are spatially close to the hydroxyl proton of C12; they represent NOEs with the C12 3′OH proton which appears at the chemical shift of the water resonance because the chemical exchange of the hydroxyl proton with the water is sufficiently rapid to provide an exchange relayed magnetization transfer mechanism (10).

Of all the water−DNA NOEs observed in the ROESY cross section (Figure 1B), only the NOEs with A5 2H and A6 2H are also represented by positive cross peaks in the corresponding NOESY cross section (Figure 1C). These base protons are directed into the minor groove and are the only base protons of

the d-$(AATT)_2$ segment which would be expected to be close to the hydration water molecules of a spine of hydration. The positive sign of the NOESY cross peaks with these resonances is clear evidence for the presence of a spine of hydration with hydration water molecules characterised by residence times of longer than about 1 nanosecond (19), in complete analogy to previous hydration studies of DNA duplexes containing d-$(AATT)_2$ segments (9, 10). No positive NOESY cross peak is observable in Figure 1C involving A11 2H which is sandwiched between two GC base pairs. This indicates that at least two consecutive AT base pairs in a DNA sequence are required for the occurrence of kinetically stable hydration water molecules in the minor groove.

Most intermolecular water–DNA NOE cross peaks observed in the ROESY cross section involve the base protons of the purine bases and the thymine methyl protons T $5CH_3$. In contrast, the cytidine base protons C 5H and C 6H as well as the thymidine base protons T 6H which are also located in the major groove of the DNA duplex, are not involved in cross-peaks of similar intensity. The corresponding cross peaks in the NOESY cross-section are either negative or missing. The simple diffusion model (19), where the modulation of the interproton vector between a water proton and a DNA proton is described by the free rotational and translational diffusion of spheres, would attribute residence times of the order of about 0.5 ns to the hydration water molecules which show sizeable NOEs in ROESY but not in NOESY. Within the diffusion model, it is expected that the NOESY cross-peaks for these intermolecular interactions should become increasingly negative with increasing temperature and concomitantly increased diffusion rates. However, spectra recorded at 15°C did not show this effect. Instead, the NOEs became less intense both in the ROESY and the NOESY experiment (data not shown). Previous model calculations have shown that a rapid reorientation of the interproton vector connecting a water proton with a proton of the macromolecule can quench the intermolecular NOE more strongly in NOESY than in ROESY, also if the water molecule does not leave the hydration site (19). This quenching effect is most pronounced, if the correlation times of the local reorientation are of the order of 1 nanosecond. Therefore, the combination of intermolecular diffusion with increased local reorientation rates could explain why the NOEs are rather well represented in the ROESY spectrum but only poorly in the NOESY experiment. Yet, the absence of any positive NOESY cross peaks with the major groove protons in the cross section of Figure 1C could not be interpreted with the same long residence times which must prevail for the hydration water molecules near A5 2H and A6 2H (9, 10, 19).

Intermolecular NOE cross-peaks between H_2O and d-$(GTGGTTAACCAC)_2$

Figure 2 shows the one-dimensional ^1H NMR spectrum of the DNA fragment d-$(GTGGTTAACCAC)_2$ and cross sections through the ROESY and NOESY spectra taken along the F_2 frequency axis at the F_1 chemical shift of the water resonance. A comparison of the corresponding cross sections in Figures 1

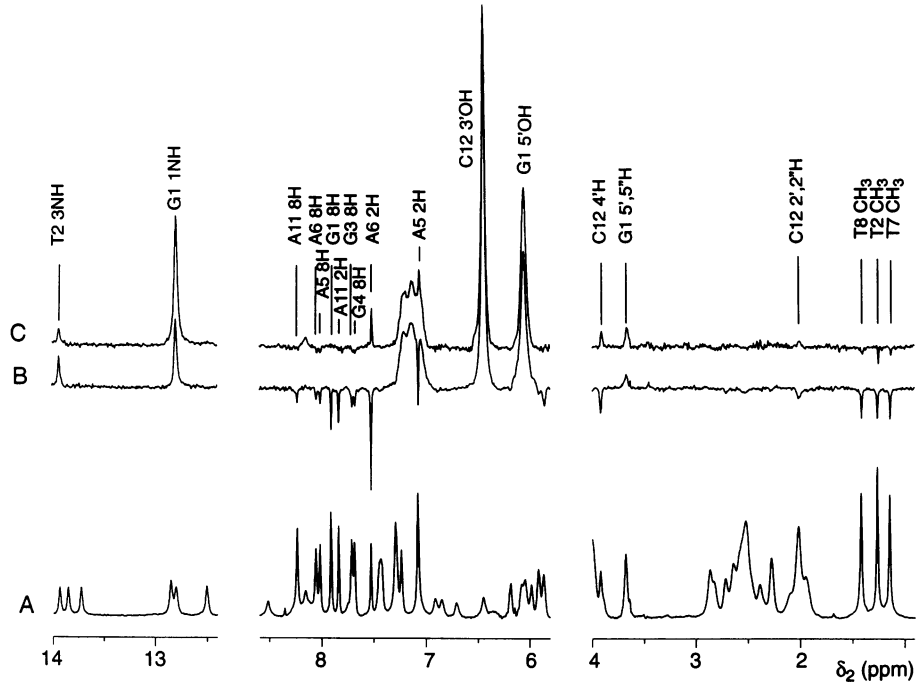

Figure 1. ^1H NMR spectra showing NOEs between water protons and protons of d-$(GTGGAATTCCAC)_2$ at 4°C, pH 7.0. The DNA concentration was 1 mM in duplex in 90% H_2O/10% D_2O. The spectra were recorded with mixing times of 50 ms using a spin-lock pulse for water suppression before the detection period. The water suppression scheme resulted in a sinusoidal excitation profile with maxima near 2.3, 7.7 and 13.1 ppm (see Materials and Methods). The spectral regions from 1 to 4 and 12 to 14 ppm were inverted to revert the sign inversions in the excitation profile. **(A)** One-dimensional ^1H NMR spectrum obtained by Fourier transformation of the first FID (free induction decay) of the NOESY experiment. **(B)** Cross section through the ROESY spectrum taken along F_2 at the F_1 frequency of the water line ($\delta(H_2O)$ = 5.0 ppm). **(C)** Cross section through the NOESY spectrum taken along F_2 at the F_1 frequency of the water line. The intermolecular cross-peaks involving water and DNA protons are assigned at the top of the figure (Table 1).

and 2, respectively, reveals that a virtually identical set of intermolecular water−DNA NOEs is observed for d-(GTGGAATTCCAC)$_2$ and d-(GTGGTTAACCAC)$_2$. In particular, the cross peaks due to chemical exchange are of similar intensities for both DNA fragments and negative ROESY cross peaks are observed between the water signal and the DNA resonances of the purine protons 8H, 2H and the thymidine protons T 5CH$_3$. This indicates that the residence times of the hydration water molecules near these major groove protons must be similarly short in both DNA fragments, i.e. shorter than about 500 picoseconds.

A clearly different behaviour is observed for the minor groove protons A7 2H and A8 2H which do not show positive NOESY cross-peaks with the water signal in d-(GTGGTTAACCAC)$_2$ (Figure 2C). In addition, a comparison of the cross sections through the ROESY spectra of the two dodecanucleotide duplexes shows that the negative ROESY cross-peaks involving the A7 2H and A8 2H protons in d-(GTGGTTAACCAC)$_2$ are much weaker than the ROESY cross peaks involving the A5 2H and A6 2H protons in d-(GTGGAATTCCAC)$_2$ (Figures 1B and 2B). These differences in ROESY cross peak intensities and the concomitant absence of positive NOESY cross peaks involving A7 2H and A8 2H protons clearly indicate that there is no spine of hydration in the minor groove of the d-(TTAA)$_2$ segment of the duplex d-(GTGGTTAACCAC)$_2$ that is kinetically nearly as stable as the spine of hydration in d-(GTGGAATTCCAC)$_2$.

CONCLUSION

The present experimental results demonstrate that (i) a spine of hydration containing long lived hydration water molecules can be observed in aqueous solutions of DNA duplexes of B type conformation containing a d-(AATT)$_2$ segment but not in duplexes containing a d-(TTAA)$_2$ segment. (ii) The residence times of the hydration water molecules in the major groove of B type DNA duplexes are shorter than about 500 picoseconds independent of the residence times of the hydration water in the minor groove.

The present results on the kinetics of minor groove hydration water correlates with the structurally disordered hydration pattern observed in the minor groove of d-(TTAA)$_2$ but not d-(AATT)$_2$ segments in single crystal X-ray structures of related DNA duplexes, which also show a consistently larger width of the minor groove for d-(TTAA)$_2$ segments compared to d-(AATT)$_2$ segments (11−13, 16). This suggests that the occurrence of hydration water molecules with relatively long residence times might depend on the presence of a narrow minor groove.

It will be of interest to investigate, to which extent the presence of a relatively long-lived spine of hydration in the minor groove of B type DNA in solution correlates with the binding affinity and binding specificity of simple minor groove ligands or ligands that bind to DNA by simultaneously contacting the major as well as the minor groove (26 and references therein). In a recent report of the single crystal X-ray study of the 1:1 complex between d-(CGCGAATTCGCG)$_2$ and the minor groove binding drug propamidine, a short-chain homologue of the drug pentamidine, the spine of hydration in the minor groove of the central d-(AATT)$_2$ segment was replaced by propamidine, but a continuous spine of hydration water molecules was found along the outer edge of the minor groove instead (27). It will be of great interest to determine whether this 'secondary' spine of hydration would also be characterized by relatively long residence times.

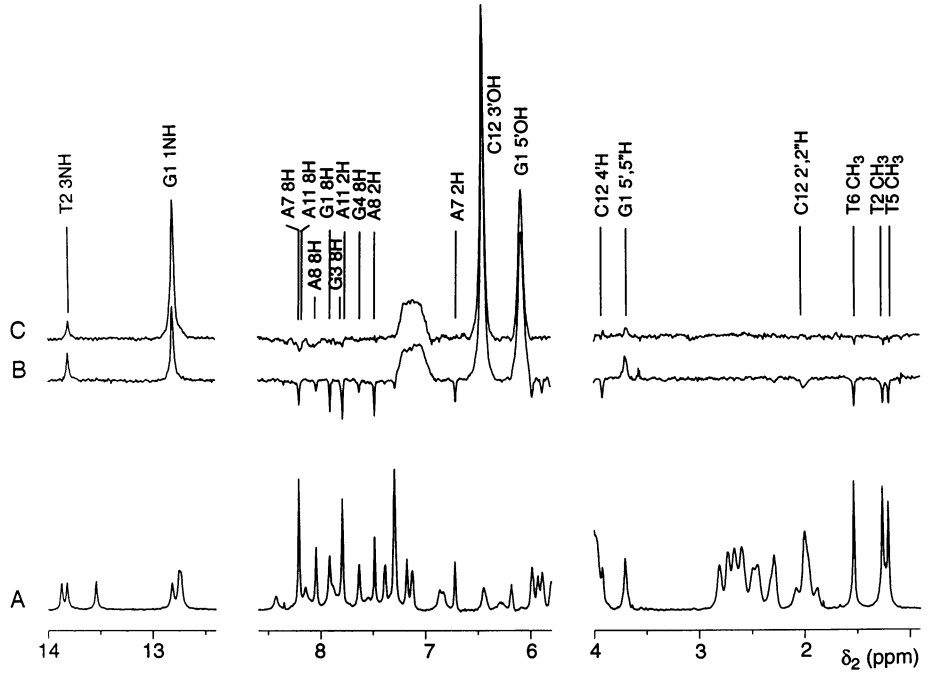

Figure 2. ^{1}H NMR spectra showing NOEs between water protons and protons of d-(GTGGTTAACCAC)$_2$. ROESY and NOESY spectra were recorded using the same experimental parameters, conditions and presentation as for the spectra of Figure 1. **(A)** One-dimensional ^{1}H NMR spectrum obtained by Fourier transformation of the first FID of the NOESY experiment. **(B)** Cross section through the ROESY spectrum taken along F_2 at the F_1 frequency of the water line. **(C)** Cross section through the NOESY spectrum taken along F_2 at the F_1 frequency of the water line. The intermolecular cross peaks involving water and DNA protons are assigned at the top of the figure (Table 2).

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