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NMR observation of individual molecules of hydration water bound to DNA duplexes: direct evidence for a spine of hydration water present in aqueous solution

Edvards Liepinsh, Gottfried Otting and Kurt Wüthrich*
Institut für Molekularbiologie und Biophysik, ETH-Hönggerberg, CH-8093 Zürich, Switzerland

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ABSTRACT

The residence times of individual hydration water molecules in the major and minor grooves of DNA were measured by nuclear magnetic resonance (NMR) spectroscopy in aqueous solutions of d-(CGCGAATTCGCG)$_2$ and d-(AAAAATTTTT)$_2$. The experimental observations were nuclear Overhauser effects (NOE) between water protons and the protons of the DNA. The positive sign of NOEs with the thymine methyl groups shows that the residence times of the hydration water molecules near these protons in the major groove of the DNA must be shorter than about 500 ps, which coincides with the behavior of surface hydration water in peptides and proteins. Negative NOEs were observed with the hydrogen atoms in position 2 of adenine in both duplexes studied. This indicates that a 'spine of hydration' in the minor groove, as observed by X-ray diffraction in DNA crystals, is present also in solution, with residence times significantly longer than 1 ns. Such residence times are reminiscent of 'interior' hydration water molecules in globular proteins, which are an integral part of the molecular architecture both in solution and in crystals.

INTRODUCTION

The hydration of DNA duplexes has been the subject of great interest in context with attempts to rationalize the sequence-specific recognition of DNA by proteins and other compounds (*e.g.*, 1, 2), and with the possible role of water in stabilizing sequence-dependent conformational variations in double-helical DNA (3–9). Dickerson and coworkers noted the importance of hydration water in the crystal structure of the self-complimentary dodecamer duplex d-(CGCGAATTCGCG)$_2$, where the minor groove of the central base pairs is filled with an ordered zig-zag array of water molecules, the 'spine of hydration' (3, 4). Subsequent conformational energy calculations suggested that the presence of the spine of hydration is a prime reason for the significant narrowing of the minor groove of poly(dA)·poly(dT) tracts in B-type DNA conformations (7), while other theoretical studies indicated that a similar spine of hydration may also be present in the minor groove of G·C rich DNA sequences (8, 9). Studies of biological macromolecules in aqueous solution have recently added an important facet to rationalizing the important structural role of the hydration water, by demonstrating conclusively that the residence times at the hydration sites are usually very short. Thus, the residence times of surface hydration water molecules in proteins were shown by nuclear magnetic resonance (NMR) experiments to be shorter than about 500 ps, while the residence times observed for hydration water molecules in the protein interior, where they represent an integral part of the protein architecture, were found to be in the range of about 10^{-3} to 10^{-8} s (10). The present paper describes NMR evidence that the water molecules of the spine of hydration in DNA duplexes have residence times longer than about one nanosecond, which is comparable to the behavior of interior waters in globular proteins. This emphasises the important role of these water molecules in the molecular architecture of B-DNA.

MATERIAL AND METHODS

NMR detection of hydration water

Hydration water near DNA protons can be detected by the observation of nuclear Overhauser effects (NOE) between the protons of the DNA and the protons of the water. Because of water exchange between the hydration water sites and the bulk water, all water protons appear at the chemical shift of the dominant signal of the bulk water (11). Therefore, in two-dimensional (2D) [^{1}H, ^{1}H]-NMR experiments the water–DNA NOEs are detected in a single cross section along ω_2 taken at the ω_1 chemical shift of the water line (12).

In assigning water–DNA NOE cross peaks, care has to be taken to discriminate between these chemical exchange peaks, and NOEs of non-labile protons of the DNA with labile protons of the DNA which exchange sufficiently rapidly with the water to appear at the bulk water chemical shift (10–13). In the present work the assignment of direct water–DNA NOE cross peaks was based on the following two criteria: (i) the DNA protons involved in the cross peaks do not exchange rapidly with the water; (ii) the DNA protons with NOEs to the water resonance are spatially well separated from potentially labile and rapidly exchanging DNA protons in the B-DNA type conformations. Rapidly exchanging, labile DNA protons were identified by their

* To whom correspondence should be addressed

chemical exchange cross peaks with the water signal, which have positive sign in NOESY (NOE spectroscopy in the laboratory frame of reference) *and* ROESY (NOE spectroscopy in the rotating frame of reference) spectra, while NOE cross peaks are negative in ROESY (12, 14). The aforementioned criterion (ii) is applied to eliminate potential errors in the assignment of direct water−DNA NOEs for water molecules in the slow motional regime (*i.e.*, water molecules bound with residence times exceeding 1 ns), which cannot *a priori* be distinguished from NOEs between non-labile and labile DNA protons with magnetization transfer to the bulk water signal by rapid chemical exchange.

NMR sample preparation

The self-complementary DNA sequences d-(CGCGAATTCGCG)$_2$ (*dodecamer*) and d-(A$_5$T$_5$)$_2$ (*decamer*) were investigated. To slow down the chemical exchange of the labile protons in the DNA duplexes with the solvent, the samples were desalted by extensive ultrafiltration to remove exchange catalysts such as phosphate ions (*e.g.*, 15). The lyophilized DNA samples were dissolved in a mixture of 90% H_2O/10% D_2O and the pH adjusted by the addition of minute amounts of HCl or NaOH. The final sample concentrations were about 1.0 mM and 1.2 mM in duplex for the *decamer* and the *dodecamer*, respectively, with a pH value of 6.0 for the *decamer* and 7.0 for the *dodecamer*.

NMR measurements

All NMR measurements were performed at low temperatures, *i.e.*, 10°C and 4°C, to slow down the exchange of the imino and amino protons of the DNA. Two-dimensional homonuclear ^{1}H NOESY and ROESY spectra were recorded under identical conditions on a Bruker AMX 600 NMR spectrometer. Adequate water suppression was achieved with the use of the SL$_x$-τ-SL$_y$ element before the acquisition period, where SL$_x$ and SL$_y$ denote spin-lock pulses of 0.5 and 2 ms duration, respectively (16), or with a modified scheme where the first spin-lock pulse was replaced by a homospoil pulse applied at the beginning of the NOESY mixing period (13). The delay τ was set to 156 μs, which results in optimum spectral excitation near 2.3, 7.7 and 13.1 ppm (16). After Fourier transformation, all spectra were baseline corrected in both dimensions to avoid interference of baseline artefacts with the one-dimensional cross section through the water line.

RESULTS

Intermolecular NOEs of d-(CGCGAATTCGCG)$_2$, with hydration water molecules

Figure 1 shows the one-dimensional ^{1}H NMR spectrum and cross sections through the two-dimensional NOESY and ROESY spectra of d-(CGCGAATTCGCG)$_2$ taken along ω_2 at the ω_1 chemical shift of the water resonance. Intermolecular water-DNA NOEs are observed for the protons in position 2 of A5 and A6 and for the methyl groups of T7 and T8. All these protons show negative cross peaks in ROESY (Figure 1B). Since they are far from any rapidly exchanging labile protons of the DNA, they must represent direct NOEs between the DNA and hydration water molecules. Most important, the cross peaks of A5 2H and A6 2H are positive in the NOESY cross section, with the cross peak of A5 2H appearing as a shoulder of the more intense cross peak of C 1 4NH^b (Figure 1C). In the same spectrum, negative cross peaks are observed with the methyl groups of T7 and T8, the protons in position 8 of guanine, the proton in position 3 of C3, and some of the deoxyribose 2′ protons. All these protons are located in the major groove of the DNA. As shown previously (10), negative NOESY cross peaks indicate rapid modulation of the internuclear vector connecting the protons of the macromolecule with those of the hydration water molecules, showing that the hydration water residence times are shorter than about 500 ps (10). (Note that in the presentation of Figure 1, negative NOESY cross peaks correspond to positive cross relaxation rates, σ^{NOE}, and *vice versa*.) In this way, the different signs observed for the NOEs with adenine 2H and with different protons in the major groove present a direct experimental criterion to distinguish highly mobile hydration water molecules in the major groove of the DNA, which have residence times shorter than about 0.5 ns, from water molecules near A5 2H and A6 2H in the minor groove of the DNA, which must be bound with residence times longer than 1 ns.

The identification of the aforementioned DNA−H_2O NOEs was dependent on a detailed analysis of the origin of the other

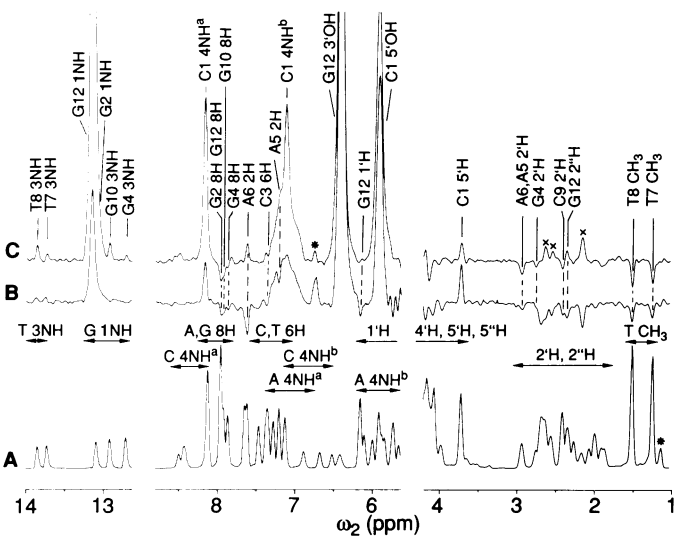

Figure 1. Proton NMR spectra showing NOEs between protons of d-(CGCGAATTCGCG)$_2$ and water protons (*dodecamer* concentration = 1.2 mM in duplex, solvent 90% H_2O/10% D_2O, T = 10°C, pH = 7.0, ^{1}H frequency = 600 MHz; the experimental schemes of Figure 1, A and B, in ref. (16) were used, with mixing times τ_m = 60 ms for NOESY and τ_m = 30 ms for ROESY, SL$_{\phi 4}$ = 0 and SL$_{\phi 5}$ = 2 ms; a homospoil pulse of 4 ms duration was applied at the beginning of the mixing time in the NOESY experiment (13); time domain data size = 330×2048 points, t_{1max} = 33 ms and t_{2max} = 84 ms, total experimental time about 20 h per spectrum; spectral excitation profile sin[0.59(δ-5.0)], where δ is the chemical shift relative to TSP ([2,2,3,3-D$_4$]-trimethyl-silylpropionate) in ppm, with excitation maxima near 7.7 and 2.3 ppm; the spectral region between 5.8 and 8.7 ppm has been inverted for improved readability). (A) Conventional one-dimensional ^{1}H NMR spectrum obtained by projecting the NOESY spectrum along ω_1 onto the ω_2 frequency axis. (B) Cross section through the ROESY spectrum along ω_2 at the ω_1 frequency of the water line. (C) Cross section through the NOESY spectrum along ω_2 at the ω_1 frequency of the water line. In (A) the double arrows indicate the chemical shift ranges for the different hydrogen positions in the *dodecamer*. In (C), resonance assignments for individual peaks are indicated with the one-letter symbol for the nucleotide and the sequence position in d-(C$_1$G$_2$C$_3$G$_4$A$_5$A$_6$T$_7$T$_8$C$_9$G$_{10}$C$_{11}$G$_{12}$), where the same numeration is used for both strands to account for the two-fold symmetry in the NMR spectrum. For amino groups the two protons are distinguished by the superscripts a and b. Crosses (×) in (C) identify cross peaks corresponding to intramolecular NOEs with those 3′ deoxyribose protons that have their chemical shifts at or near the water frequency, and asterisks in (A) and (C) identify peaks arising from impurities.

peaks in Figure 1. The largest signals in both the NOESY and ROESY cross sections are the positive exchange cross-peaks from the imino proton of the terminal base pair, G12 1NH, at 13.1 ppm, and the hydroxyl protons G12 3'OH at 6.4 ppm and C1 5'OH at 5.95 ppm. Note that with the single exceptions of G12 1NH and possibly G2 1NH, all imino protons exchange too slowly with the solvent to lead to strong exchange cross peaks at 10°C. Intense exchange-relayed cross peaks (17) are observed with the $4NH_2$ group of C1, which arise from the following sequence of magnetization transfers during the mixing time: $H_2O \rightarrow$ G12 1NH by chemical exchange, G12 1NH $\rightarrow$ C1 $4NH^a$ by NOE, and C1 $4NH^a \rightarrow$ C1 $4NH^b$ by exchange due to the rotation of the $4NH_2$ group about the 4C-4N bond. As one would expect from this cascade of transfers, the cross peak intensities with the water resonance decrease in the same order (Figure 1, B and C). (The corresponding cross peaks in the ROESY cross section (Figure 1B) have much smaller intensities than in NOESY, because the carner frequency of the spin-lock was placed at the water frequency. This caused strong off-resonance effects for the imino proton region in the ROESY experiment (18), which hindered efficient magnetization transfer from the water resonance to the imino protons, and from the imino protons to the C1 $4NH_2$ signals.) The broad exchange cross peak undemeath the C1 $4NH^b$ cross peak was attributed to the NH_2 group of G12, which is broadened by the rotation about the 2C-2N bond. The weak cross peaks of G12 2''H and G12 1'H arise from intramolecular NOEs with the labile chain-terminal hydroxyl proton G12 3'OH, which appear at the water frequency due to chemical exchange of this hydroxyl proton with the water. Similarly, the cross peak with C1 5'H at about 3.7 ppm arises from interaction with C1 5'OH. The fact that the cross peak is positive in the ROESY cross section (Figure 1B) is explained by a homonuclear Hartmann–Hahn effect with the hydroxyl proton. While this magnetization transfer mechanism between scalar coupled protons may occur in ROESY experiments (19), it is not present in NOESY, where the corresponding cross peak represents a NOE with the rapidly exchanging proton of the C1 5'OH group. Further intense cross peaks are from intramolecular NOEs with the 3'-protons of G2, T8 and G10, which have virtually the same chemical shift as the water resonance (crosses in Figure 1).

A further interesting aspect is revealed by a comparison of the cross peak intensities in the NOESY and ROESY cross sections of Figure 1. Because a two-fold longer mixing time was used in the NOESY experiment and because of the off-resonance effect in ROESY which decreases the signal intensities for the resonance frequencies far from the water frequency (see above), the exchange cross peaks and NOE cross peaks are more intense in NOESY than in ROESY. An important exception is presented by the water–DNA NOE of A6 2H, which is almost twice as intense in ROESY. Considering the two-fold longer mixing time used in the NOESY experiment of Figure 1C, the cross relaxation rate, σ, between this proton and the protons of the hydration water molecules must be about four times faster in ROESY (σ^{ROE}) than in NOESY (σ^{NOE}), although σ^{ROE} would be expected to be at most two times larger than σ^{NOE} for hydration water molecules that are stably bound with a lifetime $\geq$ 1 ns. Therefore, the reduced NOE intensity in NOESY indicates either local reorientation of the hydration water during the residence time, or exchange with the bulk water on a time scale shorter than 1 ns (compare Figure 2 of ref. 10).

The aforementioned result is supported by corresponding experiments recorded at 4°C. Figure 2, B and C, shows the cross sections taken at the ω_1 chemical shift of the water line through the NOESY and ROESY experiments. Most notably, the NOE cross peak between the water signal and the A6 2H resonance is more intense in NOESY (Figure 2C) than in ROESY (Figure 2B). This shows that at this temperature σ^{NOE} has reached its maximum value attainable in the slow motional regime, where σ^{NOE} is half as big as σ^{ROE} (14). The more intense NOESY cross peak in Figure 2C is then explained by the two-fold longer mixing time used in the NOESY experiment and by the faster auto-relaxation rate during the ROESY mixing time. While the hydration water near A6 2H is thus shown to be immobile at 4°C on a time scale of about 1 ns, the NOESY cross peaks between the water and the thymine methyl goups in the major groove are still negative, which indicates that the residence times of the hydration water molecules near these groups are still shorter than about 500 ps at 4°C. From the spectrum of Figure 2C it cannot be decided whether the other negative NOESY cross peaks observed at 10°C (Figure 1C) are also present at 4°C, because the water resonance is shifted to lower field by about 0.07 ppm when going from 10°C to 4°C, so that there is more pronounced overlap with some intramolecular NOEs with the 3'H resonances of the DNA (crosses in Figure 2).

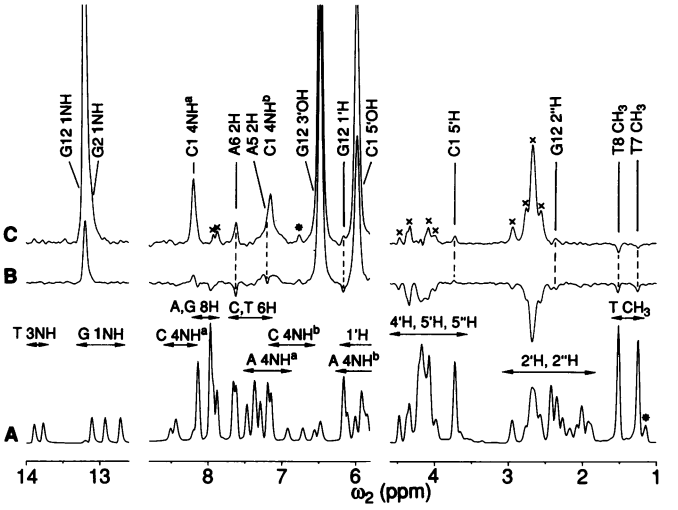

Figure 2. Proton NMR spectra showing NOEs between protons of d-(CGCGAATTCGCG)$_2$ and water protons (*dodecamer* concentration = 1.2 mM in duplex, solvent 90% H_2O/10% D_2O, T = 4°C, pH 7.0, ^1H frequency = 600 MHz; same experimental schemes as in Figure 1 with identical parameters, except that the homospoil pulse was of 5 ms duration, the time domain data size was 580×2048 points, t_{1max} was 37 ms and the total experimental time about 45 h per spectrum). (A) One-dimensional ^1H NMR spectrum obtained by projecting the NOESY spectrum along ω_1 onto the ω_2 frequency axis. (B) Cross section through the ROESY spectrum along ω_2 at the ω_1 frequency of the water line. (C) Cross section through the NOESY spectrum along ω_2 at the ω_1 frequency of the water line. Peak identification as in Figure 1.

Intermolecular NOEs of d-$(A_5T_5)_2$ with hydration water molecules

Hydration water molecules that are stably bound in the DNA minor groove are also evidenced by experiments corresponding to those of Figures 1 and 2 performed with d-$(A_5T_5)_2$ (Figure 3). A detailed NMR investigation of this decamer was previously reported (20), and here we limit the discussion to the spectral

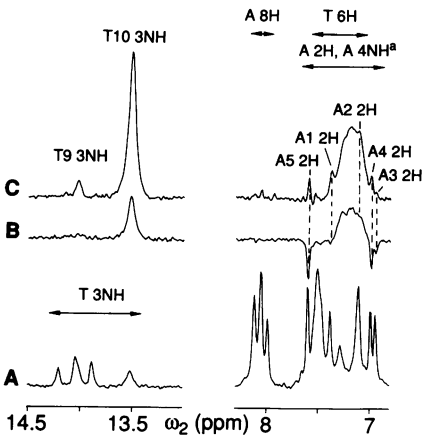

Figure 3. Spectral regions (6.8–8.3 ppm and 13.0–14.5 ppm) from NMR spectra showing NOEs between protons of d-$(A_5T_5)_2$ and water protons (*decamer* concentration 1.0 mM in duplex, solvent 90% H_2O/10% D_2O, T = 4°C, pH = 6.0, ^1H frequency = 600 MHz; same experimental schemes as in Figure 1, except that $SL_{\phi 4}$ = 0.5 ms and no homospoil pulse was used; time domain data size = 440×2048 points, t_{1max} = 30 ms, $t2_{2max}$ = 84 ms τ_m = 30 ms in both NOESY and ROESY, total experimental time about 46 h; same spectral excitation profile as in Figure 1; the spectral region between 6.8 and 8.3 ppm was inverted before plotting). (A) One-dimensional ^1H NMR spectrum obtained by projecting the NOESY spectrum along ω_1 onto the ω_2 frequency axis. (B) Cross section through the ROESY spectrum along ω_2 at the ω_1 frequency of the water line. (C) Cross section through the NOESY spectrum along ω_2 at the ω_1 frequency of the water line. Peak identification as in Figure 1.

regions containing the resonances of the imino protons, amino protons and base protons of A and T. The most important observation is that positive NOESY cross peaks are observed with the protons in position 2 of A3, A4 and A5 (Figure 3C). The cross peak with A2 2H is very weak and the cross peak with A1 2H is probably due to an exchange-relayed NOE with the labile imino proton of T10 rather than a direct NOE with hydration water. We conclude that in the non-terminal base pairs of the DNA duplex, the hydration water near the A 2H protons is characterized by residence times longer than 1 ns, while the weaker NOE cross peak intensities observed towards the chain ends in the duplex indicate that the fraying of the ends also reduces the residence lifetimes of the hydration water molecules. No evidence was obtained for stably bound water molecules in the major groove. The weak peaks observed for the protons in position 8 of adenine (Figure 3C) come from A 3'H – A 8H cross peaks which have their maximal intensities in neighboring cross sections. Relatively broad lineshapes were observed for the methyl resonances of T (not shown), so that no cross peaks could be discerned between these signals and the water line in the NOESY spectrum. However, hydration with residence times longer than 1 ns near these methyl goups, near the protons in position 8 of adenine, or near position 6 in thymine would be expected to result in strong, positive NOE cross peaks, which were definitely not observed.

As with the *dodecamer* (Figures 1 and 2), these conclusions are based on tracing the origins of all other peaks in Figure 3, B and C. Comparison of Figure 3, A and C, shows that only the imino protons of T10 and T9 give rise to observable chemical exchange cross peaks. The exchange cross peak at 7.2 ppm was assigned to the adenine $4NH_2$ protons of the terminal base pairs, with a magnetization transfer pathway similar to that described above for the cytosine $4NH_2$ resonances of the *dodecamer*. In Figure 2 the same mixing time was used for NOESY and ROESY, which results in comparable size of the exchange cross peaks in the two spectra, except for the imino proton region where off-resonance effects (18) are dominant in the ROESY experiment (see above), while the NOE cross peaks are about two times more intense in the ROESY cross section (Figure 2B) than in the NOESY cross section (Figure 2C), as expected for hydration water molecules bound with residence times exceeding 1 ns.

DISCUSSION

The key implication from the present work is that hydration water molecules in the minor groove of A_nT_n tracts in DNA duplexes have residence times exceeding 1 ns, although these hydration sites are accessible to the bulk solvent (3–6). This observation supports the notion that these water molecules have an important structural role in the duplex architecture. Unlike interior water in protein structures, which is typically completely inaccessible to the solvent, all hydration water molecules form hydrogen bonds to the bulk water in both B- and A-type DNA, and are in this sense 'surface hydration waters'. Since surface hydration water in proteins has very short residence times in the hydration sites (10), the long residence times of water molecules in the minor groove of the DNA are a rather unexpected result. Two stably bound water molecules with access to the bulk water have recently also been reported for the active site of the *Lactobacillus casei* dihydrofolate reductase–methotrexate–NADPH complex (21). However, these water molecules are located at the end of a channel in the complex and the extent of exposure to the bulk solvent is through hydrogen bonds with a single water molecule at a time (22), and is thus much more restricted than for the hydration water of the DNA.

The hydration water molecules detected by the NOEs with the adenine 2H signals must be part of the 'spine of hydration' that has first been observed in the X-ray crystal structure of the *dodecamer* (3, 4). In this crystal structure, the spine of hydration contains two different types of hydration sites. The innermost hydration water molecules form hydrogen bonds with the thymine 2O and adenine 3N atoms of adjacent base pairs on opposite strands of the DNA duplex, while the second type of hydration water is located further away from the bases and connects two adjacent water molecules of the inner hydration layer. Based on the crystal coordinates of the *dodecamer* (3–6), only the inner type of hydration water molecules would be expected to give observable NOEs with the adenine 2H protons. The water molecules which are hydrogen-bonded to A6 3N and T8 2O of the opposite strand have a proton–proton distance of about 2.5 Å to A6 2H and of about 3.0 Å to A5 2H. The NOEs with A6 2H are expected to be further enhanced by the water molecule at the central A–T step of the *dodecamer*, which is hydrogen bonded to the thymine 2O atoms of base pairs 6 and 7, with its protons at a distance of about 3.0 Å from A6 2H. No conclusive experimental evidence could be obtained for the outer water molecules of the spine of hydration. Their protons are about 4.0 Å away from the adenine 2H protons and even farther from any other non-labile proton of the DNA that could be resolved in the present experiments.

It should be noted, however, that the detailed locations of the water molecules representing the spine of hydration in the single crystal structure of the *dodecamer* may be different in aqueous solution. A recent single crystal X-ray analysis of d-(CGC-AAATTTGCG)$_2$ showed a somewhat different arrangement of

the water molecules in the spine of hydration (23). In this structure, fewer water molecules were found that bridge the two DNA strands. Because there was no clear distinction between an inner and an outer hydration layer, the authors referred to the hydration water in the minor groove of this crystal structure as a 'ribbon of hydration' (23). Interestingly, the X-ray analysis resulted in a much better definition of the water molecules in the minor groove than of those in the major groove. The NMR studies of d-(CGCGAATTGCGC)$_2$ and d-(A$_5$T$_5$)$_2$ now show that the increased order of the hydration water in the minor groove of the DNA in the single crystal is also reflected by significantly longer residence times in aqueous solution.

Quite generally, the detection of hydration water molecules in DNA is limited by the small number of DNA proton resonances that can be used as reporter signals. For example, most of the NOEs between the water signal and deoxyribose protons are obscured by overlap with intraresidual NOEs with 3'H signals near the water frequency (Figure 1), and the 1'H region is further obscured by its proximity to the water resonance and the strong exchange cross peaks of the chain terminal 3' and 5' hydroxyl protons. Furthermore, the rotation of NH_2 groups about the C-N bond broadens the signals of these groups and thus interferes with the observation of their NOEs with the water signal. In particular, the NH_2 resonances of G are broadened beyond detection by this exchange process, so that no conclusive experimental evidence could presently be obtained relating to the possible presence of a stable spine of hydration also in the minor groove of DNA segments with G·C base pairs (8, 9). In contrast to the minor groove, the hydration water in the major groove of the DNA is highly mobile, with residence times shorter than 500 ps evidenced by negative NOESY cross peaks with the methyl goups, several of the 8H and 6H resonances of the bases, and some 2'H signals of the sugar moieties. Additional details on DNA hydration, including studies of possible long-lived hydration water in the minor groove of G_nC_n tracts, may in the future emerge from similar NMR measurements with more concentrated DNA samples, which will allow the use of three-dimensional experiments to assign those water–DNA NOEs which are obscured by overlap with intraresidual cross peaks in the cross sections of Figures 1–3 (16), or by applying heteronuclear NMR experiments with ^{13}C-labeled DNA.

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REFERENCES

1. Zhang,R.G., Joachimiak,A., Lawson,C.L., Schevitz,R.W., Otwinowski,Z. and Sigler,P.B.(1987) *Nature* **327**, 591–597.
2. Aggarwal,A.K., Rodgers,D.W., Drottar,M., Ptashne,M. and Harrison,S.C. (1988) *Science* **242**, 899–907.
3. Drew,H.R. and Dickerson,R.E. (1981) *J. Mol. Biol.* **151**, 535–556.
4. Kopka,M.L., Fratini,A.V., Drew,H.R. and Dickerson,R.E. (1983) *J. Mol. Biol.* **163**, 129–146.
5. Westhof,E., Prange,T., Chevrier,B. and Moras,D. (1985) *Biochimie* **67**, 811–817.
6. Westhof,E. (1987) *J. Biomol. Struct. Dyn.* **5**, 581–600.
7. Chuprina,V.P. (1987) *Nucleic Acids Res.* **15**, 293–311.
8. Subramanian,P.S. and Beveridge,D.L. (1989) *J. Biomol. Struct. Dyn.* **6**, 1093–1122.
9. Subramanian,P.S., Swaminathan,S. and Beveridge,D.L. (1990) *J. Biomol. Struct. Dyn.* **7**, 1161–1165.
10. Otting,G., Liepinsh,E. and Wüthrich,K. (1991) *Science* **254**, 974–980.
11. Otting,G., Liepinsh,E. and Wüthrich,K. (1991) *J. Am. Chem. Soc.* **113**, 4363–4364.
12. Otting,G. and Wüthrich,K. (1989) *J. Am. Chem. Soc.* **111**, 1871–1875.
13. Otting,G., Liepinsh,E. and Wüthrich,K. (1992) *J. Am. Chem. Soc.* **114**, 7093–7095.
14. Bothner-By,A.A., Stephens,R.L., Lee,J., Warren,C.D. and Jeanloz,R.W. (1984) *J. Am. Chem. Soc.* **106**, 811–813.
15. Guéron,M., Kochoyan,M. and Leroy,J. (1987) *Nature* **328**, 89–92.
16. Otting,G., Liepinsh,E., Farmer,B.T.II and Wüthrich,K. (1991) *J. Biomol. NMR* **1**, 209–215.
17. Van de Ven,F.J.M., Janssen,H.G.J.M., Gräslund,A. and Hilbers,C.W. (1988) *J. Magn. Reson.* **79**, 221–235.
18. Griesinger,C. and Ernst,R.R. (1987) *J. Magn. Reson.* **75**, 261–271.
19. Neuhaus,D. and Keeler,J. (1986) *J. Magn. Reson.* **68**, 568–574.
20. Celda,B., Widmer,H., Leupin,W., Chazin,W.J., Denny,W.A. and Wüthrich,K. (1989) *Biochemistry* **28**, 1462–1471.
21. Gerothanassis,I.P., Birdsall,B., Bauer,C.J., Frenkiel,T.A. and Feeney,J. (1992) *J. Mol. Biol.* **226**, 549–554.
22. Bolin,J.T., Filman,D.J., Matthews,D.A., Hamlin,R.C. and Kraut,J. (1982) *J. Biol. Chem.* **257**, 13650–13662.
23. Edwards,K.J., Brown,D.G., Spink,N., Skelly,J.V. and Neidle,S. (1992) *J. Mol. Biol.* **226**, 1161–1173.