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# Molecular dynamics simulation of the hydration shell of a B-DNA decamer reveals two main types of minor-groove hydration depending on groove width

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ABSTRACT **The conformation of the self-complementary B-DNA decamer C-C-A-A-C-G-T-T-G-G is known from a high-resolution x-ray crystal structure analysis. Molecular dynamics simulation of the hydration shell of the decamer has revealed two main types of minor-groove hydration, depending on groove width. The narrow part of the minor groove has a spine of hydration analogous to that described for the A+T-rich center of the minor groove in the dodecamer C-G-C-G-A-A-T-T-C-G-C-G [Drew, H. R. & Dickerson, R. E. (1981) *J. Mol. Biol.* 151, 535–556], the first hydration layer of which contains one water molecule per base pair. In contrast, in the wide part of the minor groove, each base is hydrated individually, water molecules lying predominantly in the base plane. In intermediate-width regions, preferred water-molecule sites are shifted away from the base plane in a 3′-to-5′ direction. This shift becomes more pronounced as the minor groove narrows, until the two water molecules lie approximately midway between base pairs. If the minor groove is narrowed still further, it accommodates only one water molecule, and the hydration transforms to the well-known water spine. The observed pattern agrees with available crystallographic data and with our earlier calculations. The results confirm the assumption that preferred positions of water oxygens in the minor groove depend predominantly on groove width rather than on base sequence. However, the location of water hydrogens, and the network of hydrogen bonding, can depend on base sequence. We suggest a simple explanation of water-spine formation in the narrow minor groove of a random DNA sequence. The spine of hydration may be a property of the minor groove of overwound variants of B-DNA, the C and D forms, for which the middle part of the decamer C-C-A-A-C-G-T-T-G-G can serve as a model.**

The hydration of synthetic DNA oligomers has been the subject of several recent detailed x-ray crystallography studies (1–4). The structure of the hydration shell depends on the sequence and conformation of the DNA molecule and can play an important role in the stabilization of its three-dimensional structure. A well-known example is the spine of hydration, which originally was observed in the A+T-rich center of the DNA minor groove in the Drew dodecamer, C-G-C-G-A-A-T-T-C-G-C-G (5, 6), and which has been suggested (5–9) as an important factor in the stabilization of the B conformation of DNA. In contrast to the notion that the amino group of guanine disrupts the spine of hydration in the minor groove, further x-ray studies have shown that the spine of hydration also occurs in narrow regions of the minor groove containing G·C pairs (10), whereas a wider minor groove containing A·T pairs shows a different type of hydration (11–13).

In general, localization of water molecules hydrating the DNA is difficult at the 1.9- to 2.6-Å resolution of the various dodecamer structures (3), but even the 1.3- to 1.6-Å resolution of the decamers (10–13) cannot localize the water protons that are necessary to determine the hydrogen bonding pattern that stabilizes the water shell. Computational methods can contribute much to the solution of this problem. Several attempts at computer simulation of the structure of DNA hydration shells have been undertaken using atom–atom force fields (14–19). In principle, such computations can yield complete information on the structure and energetics of the DNA hydration shell at the atomic level.

Monte Carlo simulation (14) has shown that the spine of hydration with the hydrogen bonding suggested previously (6, 9) is formed in the narrow minor groove of the B′ form of poly(dA)·poly(dT). In the classic B form with a wide minor groove, each base pair is hydrated by two water molecules (14). However, Monte Carlo calculations using a different potential yield an alternative structure for the hydration shell of C-G-C-G-A-A-T-T-C-G-C-G in the classic wide-groove B-form, in which each base pair is hydrated by only a single water molecule lying in the base plane (15, 16). This pattern is at variance with computations published earlier (14) and with the available x-ray data (6, 11). Thus the dependence of the structure of the minor-groove hydration shell on groove width and on base sequence remains to be determined.

Here we present the results of a molecular dynamics simulation of the hydration shell of the decamer C-C-A-A-C-G-T-T-G-G, which contains a narrow central groove region with C·G base pairs, and wide minor-groove regions toward the ends of the helix (10).

## METHODS

Molecular dynamics simulation of the hydration shell of the C-C-A-A-C-G-T-T-G-G decamer was carried out, with the DNA conformation held fixed to that of the crystal structure (10) during the simulation process. Hydrogen atom positions were generated with BIOSYM's MOLEDT program (20), and BIOSYM's INSIGHT program (20) surrounded the decamer with 491 water molecules forming a layer with a minimal thickness of 4.8 Å (Fig. 1). We used the AMBER force field (21) supported by the BIOSYM programs (20) and decreased the residue charge from $-1.0\ e$ to $-0.3\ e$ by simple reduction of the two phosphate oxygen charges, in order to mimic the effect of counterions. At the first step, the BIOSYM program DISCOVER (20) minimized the potential energy until the energy gradient decreased to 0.5 kcal/mol·Å. This was followed

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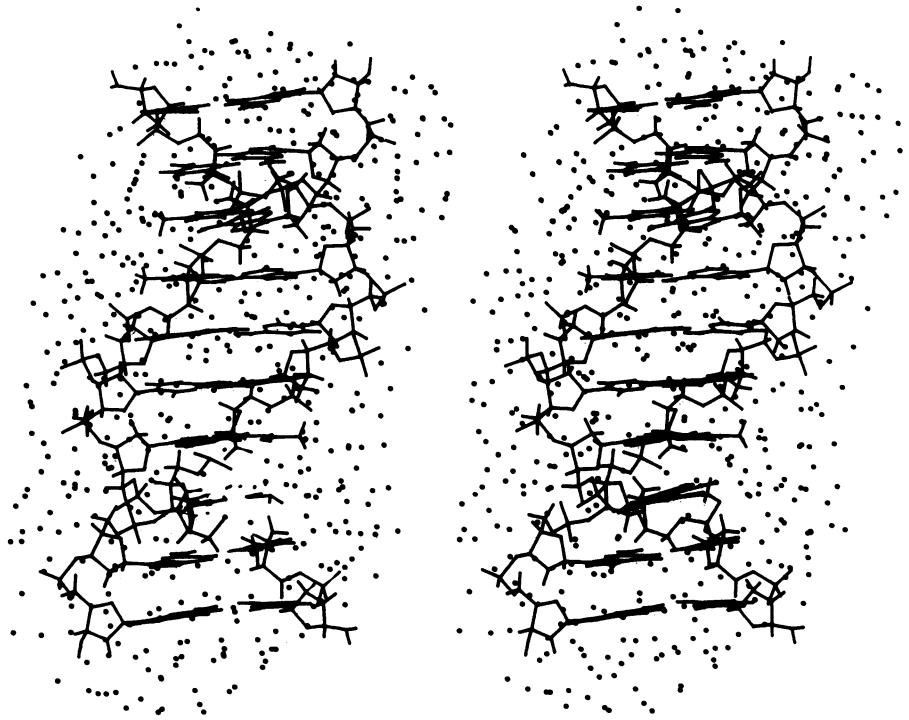

FIG. 1. Stereo view of the C-C-A-A-C-G-T-T-G-G double helix as determined by x-ray crystallography (10). Dots indicate water oxygens in positions that they occupied after the equilibration period of molecular dynamics.

by a molecular dynamics simulation of the system (also with DISCOVER). The system was equilibrated initially for 12 ps, followed by 28-ps simulation at constant temperature of 300 K. Computations were carried out with a nonbonded cutoff distance of 8.5 Å. In both the energy minimization and dynamics simulation procedures, the DNA molecule was kept rigid, and only water molecules were allowed to move. All computations were performed in vacuum and, in accordance with ref. 17, no periodic boundary conditions were imposed. Not a single water molecule "evaporated" during the computations.

No information about crystallographically determined water positions was used in the placement of water molecules in the hydration shell. The narrow part of the minor groove had no water molecules, either in the initial model or in the energy-minimized structures. But during the molecular dynamics calculations, the basic features of minor-groove hydration formed within the 12-ps equilibration period and did not change significantly during subsequent simulation. For analysis, we have collected 54 snapshots of our system at equally spaced intervals along the last 28 ps of the trajectory.

## RESULTS AND DISCUSSION

Our main goal was to use molecular dynamics to study the hydration of the minor groove of the B-DNA decamer C-C-A-A-C-G-T-T-G-G, in the conformation observed in the x-ray structure (10). The central region of this double helix has a rather narrow minor groove containing G·C base pairs, with a shortest P–P distance across the groove of 10.2 Å. The minor groove widens to 12.6 Å toward the ends of the helix. Here we describe the most prominent features of minor-groove hydration. A full analysis of DNA hydration, including water binding to major-groove and phosphate atoms, will be published elsewhere.

For analysis we have considered first and second hydration layers around the minor-groove atoms: N9, C4, N3, C2, and O4′ of adenine, N9, C4, N3, C2, N2, and O4′ of guanine, and N1, O2, C2, and O4′ of thymine or cytosine. The first coordination layer is defined as those water oxygens in direct van der Waals contact (3.2 Å or less) with these minor-groove atoms. The second layer is made up of water molecules lying within 3.2 Å of water molecules in the first layer. Hence the first and second coordination layers contain water molecules linked to DNA or to the previous layer either by hydrogen bonds or by van der Waals contacts. (The type of contact will always be stated explicitly in the following analysis.) A hydrogen bond is assumed to exist between a water and a DNA oxygen or nitrogen whenever the hydrogen–acceptor distance is less than 2.5 Å.

Two different patterns of minor-groove hydration are observed in narrow and wide regions (Fig. 2). During virtually the entire dynamics trajectory, the narrow minor groove is occupied by a zigzag spine of hydration whose water oxygen positions correspond to the spine revealed by x-ray analysis of the same decamer sequence (10). An analogous spine of hydration was observed in the narrow A+T-rich region of the minor groove of C-G-C-G-A-A-T-T-C-G-C-G (5). The existence of such a spine was supported both by Monte Carlo simulation in the poly(dA)·poly(dT) structure in the B′ conformation (14) and by calculation of water accessibilities in the fiber diffraction structure of D-form DNA (22).

In the flanking regions of the C-C-A-A-C-G-T-T-G-G decamer, where the minor-groove width opens up to 12.6 Å, this spine of hydration is absent. Instead, each base is hydrated individually, the water molecule lying predominantly in the base plane. An analogous hydration pattern was observed in several crystal structures (11–13) and was predicted by Monte Carlo simulation for poly(dA)·poly(dT) in the classic B conformation (14). In the intermediate-width region of the C-C-A-A-C-G-T-T-G-G minor groove, the preferred water positions are shifted systematically away from the base plane in a 3′-to-5′ direction. The magnitude of the shift correlates with groove narrowing, and the hydration transforms smoothly to a classic spine in the narrow central part of the helix.

Let us consider in more detail the fine structure of the spine of hydration. Fig. 3*a* represents a typical hydration pattern observed during the trajectory, in the narrow region of the minor groove. The spine is represented by a zigzag string of water molecules in which oxygen atoms of more buried water molecules are situated approximately midway between the base pairs, and oxygen atoms of the less buried water molecules are approximately in the base planes. Fig. 3*b* shows a view down the helix axis of the central C-G step of the decamer. It indicates that no steric clash need arise

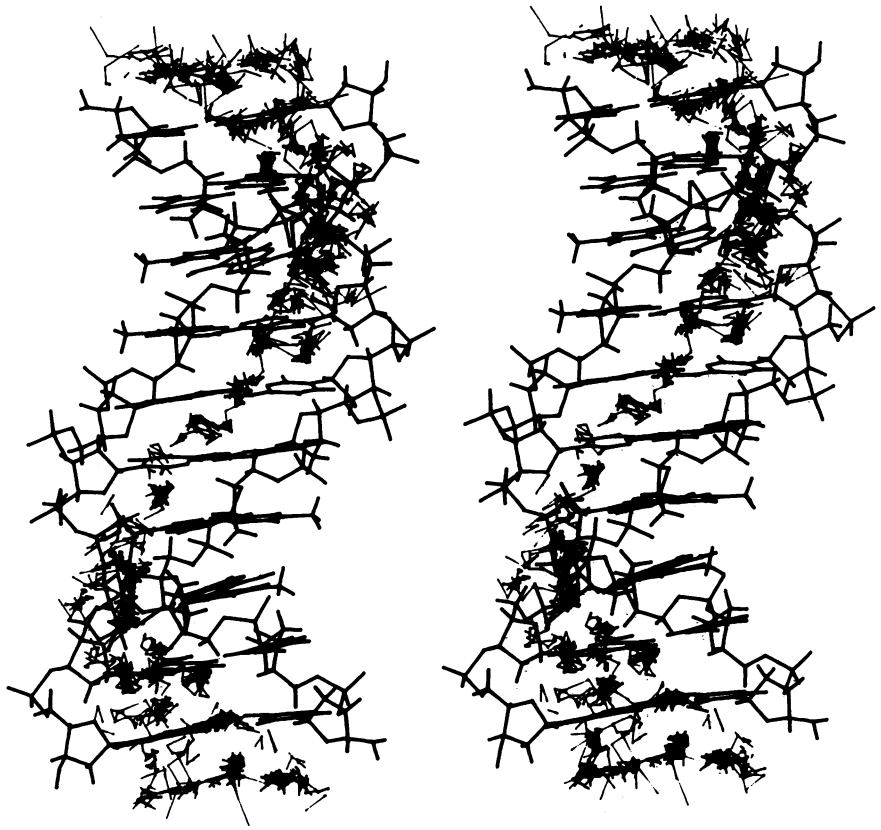

FIG. 2. Traces of first-hydration-layer water oxygens over the entire 28-ps simulation period. Each trajectory is marked by a zigzag line (see text for details). Waters of the second and higher hydration layers are not shown.

between the water molecules located between the base pairs and the N2 amino group of guanines. This lack of clash may be a consequence of the large winding angle at this step and the absence of large propeller. However, as Fig. 3*b* shows, the guanine amines do prevent a single water molecule from bridging the N3 acceptor atoms of G6 and G16, as would be the case in an A+T-containing region.

The water molecule between the central pairs C·G and G·C fluctuates rapidly during dynamic simulation, and hydrogen bonds occur with different acceptors and donors of G6 and G16. In particular, hydrogen bonds are formed with N3 of G16 (50%), N3 of G6 (40%), and N2 of G6 (40%). Several hydrogen bonding bridges occur, of which that between N3 of G16 and NH2 of G6 (25%) is the most probable. Contrary to conclusions from the x-ray analysis (10), this water molecule never bridges the N3 atoms of G6 and G16 at any time during the simulation. It is conceivable that the total absence of these bridges in the calculations along the trajectory may arise in part from the shortness of the computed trajectory.

The situation looks somewhat different when one evaluates water bridges by the criterion of van der Waals contacts. The bridging probabilities of water molecules then increase slightly—for instance, to 30% for N3(G16)–N2(G6). New water bridges also are formed such as O4′(G16)–N2(G6) (20%). There is no bridge N3(G6)–N3(G16) with nitrogen–oxygen distances less than 3.2 Å. But the probability of this bridge within 3.5 Å is 30%, and 90% probability within 3.8 Å. This signifies, in particular, that water does not always realize the hydrogen bonding possibilities suggested by crystallographic studies. In agreement with x-ray analysis (6, 7), less buried water molecules can form bridges between neighboring waters of the first layer, thus becoming a part of the second coordination layer. But these molecules also can contribute to the first coordination shell by hydrogen bonding with guanine amino groups (see Fig. 3).

Fig. 4*a* represents one of the most typical structures of the minor-groove hydration shell in the wider flanking region. In comparison with the narrow part of the minor groove, hydration of the wide region is significantly more irregular because of the greater mobility of water molecules. Each base is hydrated by its own water molecule and, in some cases, guanine is hydrated by two water molecules. As was observed also in Monte Carlo simulations (14), there are different hydrogen bonding bridges involving a single water molecule. However, the probability of even the most stable bridge is no more than 20%. Hydrogen bonding bridges between the base N3 (purine) and O2 (pyrimidine) atoms and the furanose oxygen of the next sugar [the so-called strings (11)] are the most preferred according to x-ray analysis, but in our study they occur with a probability of less than 20%. Again, if we consider van der Waals-contact water bridges, the probabilities for the formation of well-defined strings increase to 20–50%. It is possible that the low probability of these strings is related in part to the absence of acceptor status for O4′ atoms in the AMBER force field (20, 21).

Where the minor groove has intermediate width (Fig. 2), peaks of water-molecule probabilities shift from the base plane into the space between base pairs in the 3′-to-5′ direction. This shift becomes more pronounced as the groove narrows. For guanine, additional peaks occur, which is caused by independent hydration of the N3 and N2 atoms. The N2 amine-linked waters lie predominantly in the base plane, while the N3-linked waters are shifted from the base plane toward the 5′ end of the nucleotide. This intermediate type of hydration also appears in the central part of the decamer, where the minor groove is rather narrow (10.2 Å). This was observed in our simulation at 17.5 to 22 ps (Fig. 4*b*), comprising only 18% of the whole trajectory. In the x-ray study, these two water molecules between pairs G16·C5 and C15·G6 could be identified as one and perceived as a spine of hydration.

All these results confirm our assumption based on x-ray analysis (6, 10–12) and Monte Carlo computations (14) that the structure of the minor-groove hydration shell, when only the positions of water oxygen atoms are considered, depends more on groove width than on base sequence. But comparison of our data with the computations for poly(dA)·poly(dT) (14) suggests that, in spite of the similarity in water oxygen

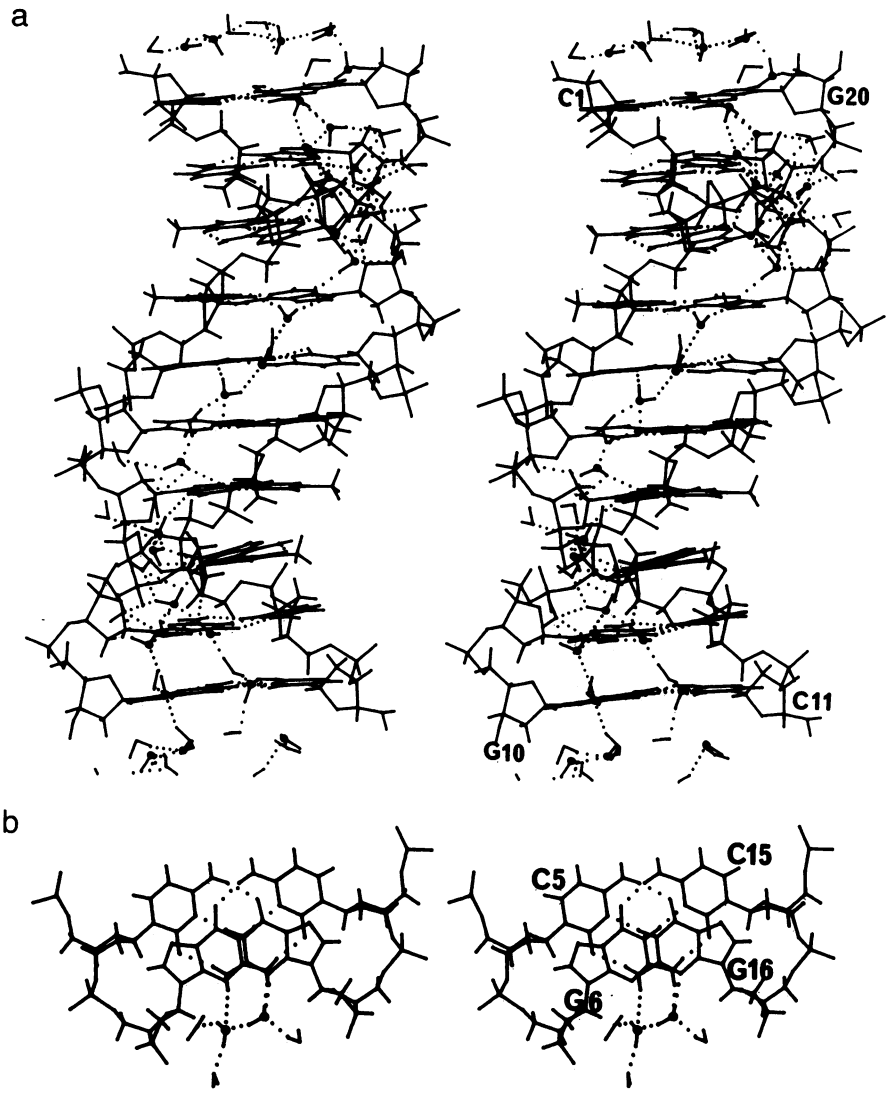


FIG. 3. Snapshot of the first point along the trajectory (after equilibration) showing the minor-groove hydration in detail. Water molecules of the first and second hydration layers are shown in skeleton representation, and first-layer water oxygens are further identified as dots. Hydrogen bonds are shown as dotted lines, but those with second-layer water are omitted for clarity. (*a*) Entire structure. The absence of water molecules around base C5 only means that in this snapshot the closest water oxygens were more than 3.2 Å from minor-groove atoms (see text for details). (*b*) Central dinucleotide step viewed down the helix axis.

arrangement in the narrowed minor groove of DNA of different sequences, the hydrogen-bond network formed in the first and the second coordination layers can depend on base sequence. In particular, guanine can prevent formation of a water bridge connecting two acceptors of neighboring bases belonging to different chains, and also between a base acceptor atom and a sugar O4′ from the other chain. Bridges of this type occur frequently in poly(dA)·poly(dT) (14).

Our results and those reported in ref. 14 are at variance with a Monte Carlo simulation (15, 16), which indicated a single spine of hydration in the minor groove of the dodecamer C-G-C-G-A-A-T-T-C-G-C-G even with the classic wide-groove B form. In the minor groove of poly(dA)·poly(dT) in the B form (14) and in the minor groove of the decamer studied here, where the minimum P–P separation is more than 11.5 A, the spine of hydration is absent and each base is hydrated individually. Moreover, in the Monte Carlo simulation (15, 16) the water molecules of the first coordination layer lie in the base-pair planes, whereas x-ray analyses and our computations show that first-shell waters in the narrow-groove spine prefer to lie between base pairs. More recent Monte Carlo calculations (23) are in better agreement with our results.

Proceeding from the available data on the hydration of B-like DNA, we shall try to give a simple explanation of the formation of the water spine. As mentioned earlier, in a structure with an intermediate minor-groove width, a water molecule shifts in a 3′-to-5′ direction from the base that it predominantly hydrates. In other words, two water molecules lying between two neighboring base pairs usually hydrate the same two cross-strand noncomplementary bases (Fig. 4*b*) that are hydrated by a single water in the spine (6). Repulsion of the water molecule by the O4′ sugar atom on the next residue seems to be responsible for this 3′-to-5′ shift (Fig. 4*b*). In a structure with a narrower minor groove, there is room for only a single water molecule, and it tends to hydrate the same two cross-strand bases. It seems that the presence of a guanine amine in real structures having a narrow minor groove should be compatible with this arrangement of a water molecule between the bases. This could be because cross-strand base acceptors are sterically accessible to water molecules only if the water molecules are positioned between the bases. The preferable position of a second-layer water molecule, in turn, is in the base-pair plane. This position is sterically favorable, ensures tetrahedral coordination of water molecules of the first layer, and is stabilized by van der Waals interactions with sugars on both strands. With C·G base pairs this water molecule can belong to either the first or the second layer because it hydrogen bonds with the guanine amine. In either case the location of the water oxygen atom is similar, although differing slightly in the depth from the bottom of the minor groove (Fig. 3*b*).

Thus, in the narrow minor groove of a random DNA sequence, a regular water structure like the spine of hydration (5) can be formed that sterically fills the groove, ensures hydration of nearly all acceptors and donors of bases, and

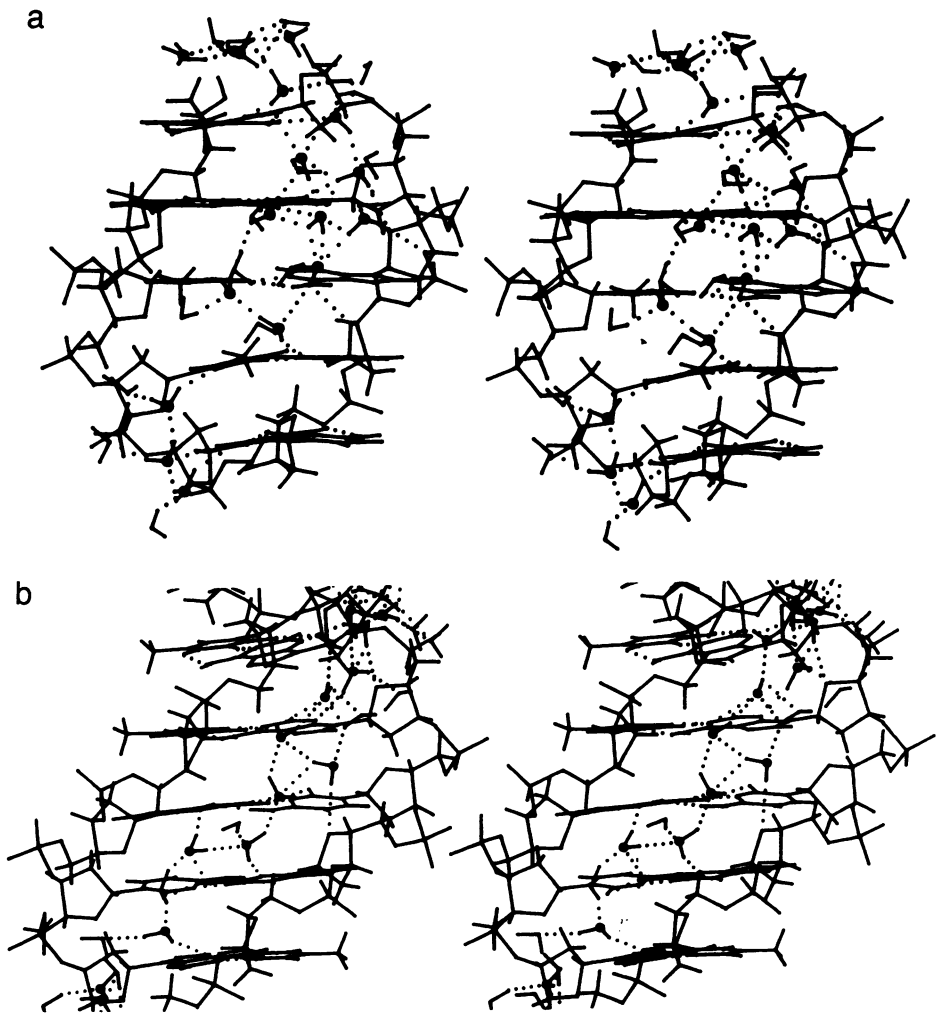


FIG. 4. Snapshots of minor-groove hydration in C-C-A-A-C-G-T-T-G-G. Water molecules and their interaction with the DNA are represented as in Fig. 3. (*a*) Typical hydration pattern in the wide part of the minor groove 2.5 ps after the end of the equilibration period. (*b*) Unusual hydration pattern in the narrow part of the minor groove 19.5 ps after the end of the equilibration period. When a wide minor groove is compressed, steric clash with sugars may shift water molecules from their initial positions in the base plane toward the intermediate positions shown here.

forms a tetrahedral network of water–water hydrogen bonding. The important consideration for formation of a spine of hydration is not the particular base sequence *per se*, but the width of the minor groove. In contiguous wider regions of the minor groove, two ribbons or strings of hydration are to be expected, as was observed in the x-ray crystal structure analyses of C-C-A-A-G-A-T-T-G-G (11, 12) and C-C-A-A-C-G-T-T-G-G (10).

A special property of the C-C-A-A-C-G-T-T-G-G duplex is the increased winding angle of 44.8° at the central C5·G16/G6·C15 base-pair step, which effectively hides the guanine amino groups underneath the neighboring purine base. This may favor formation of the spine of hydration (see Fig. 3*b*). This local conformation is reminiscent of the overwound variants of B-DNA that occur in a condensed state, the C- and D-DNA forms (24), and supports the idea that the spine of hydration evidently is a very common feature and can exist in B′, C, and D variants of the B helix with different sequences.

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1. Westhof, E. (1988) *Annu. Rev. Biophys. Chem.* **17**, 125–144.
2. Saenger, W. (1987) *Annu. Rev. Biophys. Chem.* **16**, 93–114.
3. Dickerson, R. E. (1990) in *Structure and Methods*, eds. Sarma, R. H. & Sarma, M. H. (Adenine, Schenectady, NY), Vol. 3, pp. 1–38.
4. Kennard, O. & Hunter, W. (1989) *Q. Rev. Biophys.* **22**, 327–379.
5. Drew, H. R. & Dickerson, R. E. (1981) *J. Mol. Biol.* **151**, 535–556.
6. Kopka, M. L., Fratini, A. V., Drew, H. R. & Dickerson, R. E. (1983) *J. Mol. Biol.* **163**, 129–146.
7. Fratini, A. V., Kopka, M. L., Drew, H. R. & Dickerson, R. E. (1982) *J. Biol. Chem.* **257**, 14686–14707.
8. Chuprina, V. P. (1985) *FEBS Lett.* **186**, 98–102, and erratum (1986) **195**, 363.
9. Chuprina, V. P. (1987) *Nucleic Acids Res.* **15**, 293–311.
10. Privé, G. G., Yanagi, K. & Dickerson, R. E. (1991) *J. Mol. Biol.*, in press.
11. Privé, G .G., Heinemann, U., Chandrasegaran, S., Kan, L.-S., Kopka, M. L. & Dickerson, R. E. (1987) *Science* **238**, 498–504.
12. Privé, G. G., Heinemann, U., Chandrasegaran, S., Kan, L.-S., Kopka, M. L. & Dickerson, R. E. (1988) in *Structure and Expression*, eds. Sarma, R. H. & Sarma, M. H. (Adenine, Schenectady, NY), Vol. 2, pp. 27–47.
13. Heinemann, U. & Alings, C. (1989) *J. Mol. Biol.* **210**, 369–381.
14. Poltev, V. I., Teplukhin, A. V. & Chuprina, V. P. (1988) *J. Biomol. Struct. Dyn.* **6**, 575–586.
15. Subramanian, P. S., Ravishanker, G. & Beveridge, D. L. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 1836–1840.
16. Subramanian, P. S. & Beveridge, D. L. (1989) *J. Biomol. Struct. Dyn.* **6**, 1093–1122.
17. Seibel, G. L., Singh, U. C. & Kollman, P. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6537–6540.
18. van Gunsteren, W. F., Berendsen, H. J. C., Geurtsen, R. C. & Zwinderman, H. R. (1986) *Ann. N.Y. Acad. Sci.* **482**, 287–303.
19. Swamy, K. & Clementi, E. (1987) *Biopolymers* **26**, 1901–1927.
20. BIOSYM Technologies (1988) *DISCOVER, User Manual, Version 2.5* (BIOSYM Technologies, San Diego, CA).
21. Weiner, S. J., Kollman, P. A., Nguyen, D. T. & Case, D. A. (1986) *J. Comp. Chem.* **7**, 230–252.
22. Lipanov, A. A., Beglov, D. B. & Chuprina, V. P. (1989) *J. Mol. Biol.* **210**, 399–409.
23. Subramanian, P. S., Svaminathan, S. & Beveridge, D. L. (1990) *J. Biomol. Struct. Dyn.* **7**, 1161–1165.
24. Chandrasekaran, R. & Arnott, S. (1989) in *Landolt-Börnstein, New Series*, ed. Saenger, W. (Springer, Berlin), Group 7, Vol. 1b, pp. 31–170.